INHERITED CHANGES INDUCED BY THE ENVIRONMENT

Alan Durrant

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Aberystwyth University (AU), Institute of Biological, Environmental and Rural Sciences, IBERS,
Edward Llwyd Building, Penglais Campus, Aberystwyth, Ceredigion SY23 3DA, UK

PREFACE

Experiments which began in 1953 in the Department of Agricultural Botany, Aberystwyth University (AU), have shown large differences are induced in the size of flax plants when their parents are grown in some environments. Many plants are three or four times the size of others and these and other induced changes are clearly recognisable after 50 generations, 50 years later. This is a summary of published and unpublished results obtained at Aberystwyth and by researchers at other institutions. They have taken some time to obtain and verify because inherited responses to the environment cannot be seen as an orderly process, as revealed at least by flax, and the effects of environments of previous generations, which may not have been visible or recognised at the time, can determine the effect the present environment has on the next generation.

The experiments were initially part of biometrical studies on variation in fruit flies, and on responses of flax varieties to fertilisers. A closer look at the causes of variation in flax prompted an assessment of how much could be due to the parental environment, whether by its effect on the quality of the seed, or perhaps by mediating transient genetic change. These led to larger and more specific experiments on the influence of parental environments on later generations.

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1.1 Reasons why we would not expect environmentally induced inherited changes in plants.

It is useful to look first at arguments for and against the occurrence of induced inherited changes, and at some of the problems, as the situation was in the early 1950s when these investigations began. There are several reasons why we would not expect environmentally induced inherited changes to occur. The first is that genetic theory apparently precludes it. Mendelian segregations, cytological and linkage studies, observations on sex chromosomes, have all demonstrated the mechanism of heredity whereby genes carried on the chromosomes divide equally at each cell division and are transmitted unchanged to the next generation, though they segregate, assort and recombine. The genes are interdependent and switch one another on, leading to the synthesis of proteins during development and the characteristic appearance of individuals of that genotype. The environment, e.g., temperature, nutrition, day length, also affects the activity of the genes, modifying growth and development. But the genes themselves, transmitted to the next generation, are held to be unchanged, undifferentiated, where they once again switch on according to the same developmental pattern as that of their parents, though this may be modified again by the new environment.

A second reason is there appears to be no evidence for it. Despite the thousands of experiments of all kinds with plants, observations in plant nurseries, in the general practice of commercial agriculture and horticulture, there appears to have been no kindling of belief in it.

A third reason concerns the kind of result we should expect if we attempted to look for inherited changes. Here we are not speaking of some gross chemically induced anatomical change which could easily be seen and recorded if it occurred but, say, an increase in weight in a good environment. If the induced increase in weight is inherited, presumably the progeny of such plants would be larger than the progeny of parental plants grown in a poorer environment. But the progeny from both kinds of parents would be compared in a common environment, so if in turn we grew the progeny of these progeny they should all be the same regardless of the environments of their grandparents. If indeed the changes lasted for more than one generation eventually a multiplicity of environments and induced changes over generations would generate variation which would probably be ascribed to, and appear no different from, differences due to the environment of the ordinary acceptable kind, or to the accumulation over time of random mutations having small effects. The Lamarckian theory of the inheritance of acquired characters is centred on the use and disuse of

organs over many generations. Jean Baptiste Lamarck said it was perfectly self-evident that it occurred through the use or disuse or modification of organs due to change in the environment persisting over a long period of time. After Charles Darwin 50 years later, Gregor Mendel rediscovery 100 years later and from our present knowledge of chromosomes, mutations and genetic variation, this is not evident.

A fourth reason is the climate of opinion, and the schism between east and west genetics, especially in the 1950s and 1960s, in the shadow of Trofim Denisovich Lysenko, a latter day Lamarckist. There was the impression, or an atmosphere, that studies on the possible inheritance of environmental effects or on similar studies would be unacceptable. Lysenko was dismissed in 1964 from the experimental farm he directed after a commission of the Soviet Academy of Sciences judged his methods a failure.

A fifth reason is experiments can take several, perhaps initially fruitless, years and there are problems, mentioned later, in demonstrating unequivocally environmentally induced changes.

1.2 Reasons for investigating the occurrence of environmentally induced inherited changes in plants.

An experimenter is unlikely to turn to investigating inherited induced changes unless he has some notion as to whether such studies would be rewarding, whether they could occur, and how they could occur. First, it could be argued that considering the great variety of mechanisms and ploys in nature it would seem at least possible that such changes could sometimes occur. Second, since genetic changes occur and are passed on from one cell to another during mitotic cell division during development and formation of tissues (epigenetic changes), it is feasible that such genetic changes perhaps influenced by the environment might sometimes pass through meiotic divisions during the formation of gametes, influencing individuals of the next generation.

Thirdly, if they do occur they may not be recognised. Differences in some characters are clear cut; flower colour in sweet peas for example. We can distinguish absolutely between red flowered and white flowered varieties because the contrast is so striking, and the environment has virtually no effect on the colour. They are determined by so-called major genes. But if these genes had an effect on plant height instead, the tall may not be so easily separated from the short because the environment influences plant height, and the height difference due to the genes may merge with the height differences due to the environment and be undetected. We might believe there were no

genetic differences affecting the heights of the plants, only differences due to environment differences. But separating out genetic differences from environmental differences may be even more difficult than this.

Many characters such as plant height, weight, flowering time, branching, and so on, are not determined by a single major gene difference but by several, or many, genes. Each gene individually may have a small effect on the plant but acting together they can have a large effect. They may occur anywhere on the chromosomes and different combinations of the genes in different plants can give a range of variation among plants in, say, plant height which may be indistinguishable from the range of variation in height due to the environment. Genetic, i.e., breeding experiments are necessary to determine how much of the variation is due to genetic differences and how much to environmental differences. The separation of small amounts of genetic differences is difficult; it would be more difficult to separate out a third, more elusive variable like environmentally induced inherited changes.

Unusual patterns of genetic variation are sometimes found in plants. This variation is due to genetic changes occurring at frequencies well above those normally associated with gene mutations, and are primarily allocated to a particular chromosome region for any one character showing the variation. They are called highly mutable genes, or unstable genes. They are not due to classical mutations but to changes in the regulation of the activity of the genes, which may be maintained for indefinite periods.

The presence of an unstable gene is often revealed by the occurrence of a mosaic of cells, or of colour flecking or spotting of a tissue. Taking one of many examples, in *Antirrhinum major* (Harrison and Fincham, 1964) an allele, pal^{rec}, at the anthocyanin locus gives white petals but it is unstable, becoming activated to *Pal* in some cells. Whenever this occurs, red spots appear on the white petals so the instability of this gene is easily recognised and the frequency of activation recognised. The more red spots the greater the instability and the more cells possessing the activation.

Such changes could be due to normal processes of differentiation during growth (colour patterns are frequently seen in flowers) whereby genes are switched on or off in a controlled manner to give the pattern of development characteristic of the plant or species, except for two observations. The first is that some of the plants with red spots on their petals give rise to progeny with pure red flowers instead of white. So the activation of pal^{rec} to *Pal* is transmitted to the progeny. Normally any developmental changes in the activity of genes (epigenetic changes) are not transmitted to the

progeny. The embryo in a seed is essentially in an undifferentiated state capable of developing into another plant like its parent (apart from differences due to normal segregation of genes and direct effects of the environment on the plant itself). But here, in the antirrhinum example, the changes are transmitted by the gametes, i.e., through the pollen and egg cells, to the seeds from which new types develop. Other examples of unstable genes are those which cause yellow patches on soybean leaves (Peterson and Weber, 1969) and coloured spots on maize grains (Brink, 1960; Peterson, 1976). These and other kinds of instabilities are perceived to be due to the movement of controlling elements (McClintock, 1956), increased chromosome coiling (heterochromatisation) or mechanisms associated with cellular differentiation. They are not due to changes in the genes themselves (mutations) but to changes in their expression.

The second observation is that the instability of the genes is markedly influenced by the environment, particularly temperature. The higher the temperature the more red spots on the petals and the more progeny with red flowers. Since the changes in activity are affected by the environment and the changes can be inherited we have a situation where environmentally induced changes are inherited, albeit sporadic and not uniform.

But in these examples the expression of the genes, i.e. their effect on the appearance of the plant (on its phenotype) is readily observed. In the antirrhinum example, red petals and white petals are clearly distinguished. If unstable genes were to affect characters like plant weight or height they are unlikely to be noticed, either in changes in their activity in any one generation, or in the inheritance of the induced change to the next, because they could be lost amongst the environmental variation and perhaps other genetic variation, and because of the sporadic nature of their instability. These speculations are unlikely to attract much support without evidence for their occurrence, or some indication of how they could occur, although in the intervening years Darwinian mechanisms have been questioned by some as the sole explanation for biological evolution.

1.3 Problems

(i) *Environments used in induction experiments*

Studies on environmentally induced inherited effects do not involve simply growing a few plants in different environments and comparing their progeny. There are a number of problems to address. The first is, what environments should be used? The main interest is on the possible inherited response to naturally occurring environments which rules out organic and inorganic substances which plants would not normally experience. This leaves naturally occurring differences in soils,

temperature, light and day length as permissible environmental factors. Some levels or combinations of these factors not normally experienced by a subject species could be used on the grounds that if the species migrated or were introduced elsewhere it could experience them.

Some treatments can be specified, applied and replicated precisely, others cannot. Temperature, sunlight, day length, rainfall even soils and soil based compost, vary daily, weekly and differ hugely from year to year, so a designated overall environment is only a label for approximate average conditions. Drought occurs for several weeks in some years; wet conditions may delay ripening for a month. Therefore the aim is to apply treatments which have an obvious and significant effect on the treated parent plants over and above that of the uncontrolled variation, so any induced changes can be reasonably attributed to them. The consequences of any untoward excess of any one factor can be clarified in subsequent experiments. Culture solutions applied in growth control rooms are not necessarily entirely constant nor always available.

(ii) *Environments in which the progeny of the treated plants are grown*

To determine whether any induced changes have occurred the progeny would be grown initially in an environment promoting good growth to enable the plants to realise their full potential. But differences may appear in stress or other environments, so ideally they should be tested in a range of environments, substantially increasing the numbers of plants to be grown. A distinction must be made between an inherited non-adaptive change induced by an environment on the one hand and, on the other, an inherited adaptive change mediated by some unknown mechanism to produce fitter individuals in that environment. The latter has not been seen and is not central to these studies. It is however appropriate to examine the progeny in similar and dissimilar environments to that of the parents.

(iii) *Magnitude of induced changes*

Inherited changes in quantitative characters giving a range of variation, such as plant weight and seed yield for example, may be small and hidden among the environmental variation affecting these characters. In a plant breeding context, a 10 per cent increase would be welcome and perhaps induced changes of this order should be looked for in the progeny of treated plants. A large number of plants and fairly uniform conditions would be required to establish convincingly an induced difference such as this with no guarantee of success. But much larger differences could occur. Also smaller numbers of plants can be vetted and more grown if suggestive differences emerge. Initial

experiments probing for environmentally induced changes can be combined with main experiments and analysed as though the latter were the sole aim and without detriment to them, so that there would be no waste of time or resources if no induced changes occurred.

(iv) *Choice of species and plants*

This is one of the big problems in studies of this kind. Apart from the obvious as to whether plants or species chosen in initial experiments might respond to inducing environments, it is desirable they should be true breeding, ie, they should be genetically alike, produce progeny all alike and like their parents, when all are grown in a similar environment. Such plants are said to be homogeneous, and also homozygous because the two chromosomes of each homologous pair carry exactly the same set of genes so that when the paired chromosomes separate in the production of gametes all the gametes will carry a similar set of genes. At fertilisation these fuse to produce the plants of the nest generation which therefore have exactly the same genes on their homologous chromosomes as their parents. Hence any environmentally induced inherited changes can more readily be picked out among the progeny. On the other hand if they are not homozygous, i.e. if the plants do not breed true, this in itself is no real problem because the only penalty would be the lower sensitivity of the experiment. This means more parent plants, and a lot more progeny, would have to be grown to regain the sensitivity given by pure breeding plants in detecting real induced changes.

The real problem is that if the plants are genetically different, or the paired genes on homologous chromosomes are not the same, then if the plants are grown in different environments there is the possibility that the different environments may favour different genes, chromosomes or gametes. In other words selection may occur so that different genes are inherited by the progeny according to the different environments of the parents, giving the impression that the environments induced inherited genetic changes when they had not.

This is a particularly difficult problem because one can never be one hundred per cent certain that the plants are pure breeding, whether they are entirely homogeneous and homozygous. The best choice therefore are inbred varieties of a species which is normally self-fertilised, which has been maintained by self-fertilisation and observed for many generations. Even so, heterozygosity could remain at some loci (especially if the plants naturally outcrossed and had inbreeding subsequently imposed on them) by selection for heterozygotes. If the genes on a homologous pair of chromosomes are different these may impart an advantage to the plants carrying them compared with plants carrying either one or the other on both homologous chromosomes, so they could be

maintained in a heterozygous association for generation after generation. If then they are grown in different environments the selective pressures may change destroying the selective advantage of the previously favoured heterozygosity so that new plants emerge. Or a recent mutation may be present and be favoured in different environments. Perhaps these situations are unlikely but their possibility has to be weighed against the nature of suspected induced change and other features.

It is also desirable that the species can be conveniently grown inside and outside a greenhouse, will self easily without incompatibilities, be technically easy to cross and have very little dormancy, i.e. the seeds should germinate within a few weeks of ripening.

(v) *Past environments*

Even less tangible are the effects of environments in previous generations. These may be unknown and influence the capacity of plants to respond to experimentally applied inducing environments. If environmentally induced inherited changes occur then either (a) they are maintained more or less permanently in later generations, (b) they decline slowly over years or (c) they disappear after one generation. If the induced changes disappear they are ephemeral and are not of great interest here. If they are maintained over generations and no further changes are observed when grown in different environments, including those of their original parents, then they have apparently lost their capacity for environmentally induced inherited change, i.e. the environment has also induced an inherited change to an unresponsive, or stable, type. In which case the response, or lack of response, to environments in one generation additionally depends on the environments of previous generations. Previous environments may stabilise some plants; others may have the opposite effect. Varieties of crop plants selected and grown in agricultural soils with fertilisers, or grown in nurseries for laboratory use, may have been stabilised by the environment, in contrast to plants in natural environments. An alternative view is that cultural procedures may produce plants in a state of stress so they are more likely to be unstable.

(vi) *Maternal effects*

Maternal and cytoplasmic effects are less of a problem but need to be mentioned. Because the embryo and seed develop on maternal tissue, and the cytoplasm of the ovule (which develops into the seed on fertilisation by pollen) is essentially maternal cytoplasm (the pollen contributing little or none) a plant may resemble its maternal more than its paternal parent. If plants are different in size because they have been grown in different environments then the larger plants may produce larger

plants and larger seeds, or better seeds, giving larger seedlings and larger plants in the next generation. Hence an inherited change is produced but it is ephemeral, usually disappearing and have little effect on the mature plants. It is possible that under some conditions small seedling differences could be magnified during logarithmic growth, or there could be a knock-on effect to another generation, but this can be tested by making reciprocal crosses. Pollen (male) is transferred from plants (or from their progeny) grown in one environment to the styles (female) of those grown in the other environment. This is repeated with male and female interchanged with respect to the two environments. If the progeny of the reciprocal crosses are different, and more like their respective maternal parents, the inheritance is judged to be maternal. If the reciprocal difference is maintained, it is more likely to be an induced change in the cytoplasm. If the reciprocals are the same but different from one or both parents, then the parental nuclei are different, i.e. there is a genetic difference.

(vii) *Repeating the results*

Mendelian inheritance and the chromosome theory of inheritance permits experimenters to expect the units of inheritance, the genes, for the most part, to be transmitted unchanged from one generation to the next. This cannot be if inherited changes are induced. We may not know what the genetic constitution is of a sample of plants in a particular environment in a particular generation. Repeating the experiment using fresh material multiplied up from a previous generation may yield different results, straining credibility.

1.4 Variation among inbred fruit flies

Experiments began with looking at the causes of variation among inbred fruit flies, *Drosophila melanogaster*, and inbred flax plants, *Linum usitatissimum*. One reason why the variation among inbred lines is of interest, apart from its importance here in assessing environmentally induced changes, is because it is used as an estimate of environmental variation, which subtracted from the total variation in a heterogeneous population gives a rough estimate of the genetic variation in that population. The percentage genetic variation is usually called the heritability of the particular character measured. But inbred lines which are genetically different can also differ in the amount of environmental variation. The environmental variation among large plants is generally larger than among small plants. It is also argued that if individuals of an inbred line are fit they will control their

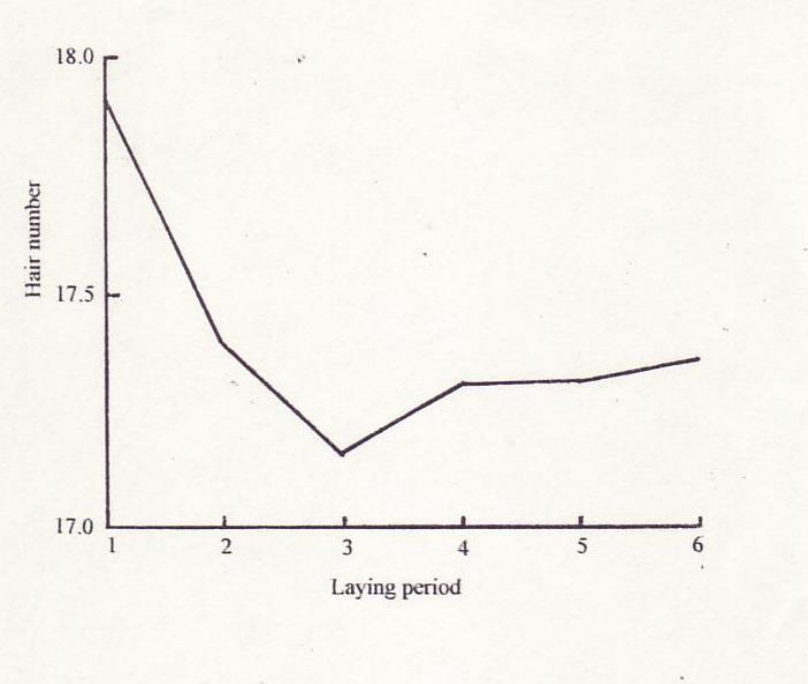
development better, they will be pushed around less by the environment and they will be more uniform. Inbreeding individuals of a naturally outbreeding species like *Drosophila* for example results in decreasing fitness. On the other hand a particular inbred line may be more variable because it is able to respond and adapt to fluctuations in the environment and thus be considered to have greater fitness. One way of resolving this paradox is to measure characters that obviously would be disadvantageous were they to vary overmuch, such as asymmetry in flies. Flies with asymmetric wings in length or shape, or with unequal hairs in size or number, on the two sides of a fly, might be judged less fit in that they were less able to control their development. They would be said to have developmental instability.

Two stocks of fruit flies, Oregon and Samarkand, held in the Genetics Department, Birmingham University, were markedly variable although they had been inbred by brother/sister mating for several hundreds of generations. This rules out residual genetic variation, and chromosome assays (Durrant and Mather, 1954; Paxman, 1957) were unable to pick up mutations of sufficient magnitude or frequency, so presumably the cause was a highly variable environment and/or developmental instability. In cultures, one or more pairs, male and female, lay eggs over several days on nutrient media, the adult flies emerging after ten days or more depending on the temperature, usually 18^{o}, 20^{o} or 25^{o}. These conditions can be controlled but larvae continue to emerge, crowding and churning up the media so the environment of larvae of later flies hatching is different from those hatching earlier. Hence this alone would explain the variation among flies. In fact by deliberately causing excessive crowding, adult flies can be obtained less than a quarter the size of normally cultured flies.

Previously there had been several reports of the effect of maternal age on the offspring of guinea pigs (Wright, 1926), mice (Holt, 1948) and humans (Penrose, 1939). Bridges (1929) found that the recombination frequency in *Drosophila* (i.e. between paired chromosomes at the meiotic division prior to the formation of the eggs) varied with the age of the female parent. The Samarkand stock was chosen at Aberystwyth, because of its lower fecundity giving less crowding, to see whether maternal age had an effect on the number of hairs (also variously referred to elsewhere as bristles or chaetae) on the sternopleural region, a character extensively used by geneticists studying quantitative variation. Pairs of flies were transferred at 12 hour intervals through a relay of six vials, to avoid effects of staling media and to separate flies from eggs laid in each sequence of 12 hour periods. Several relays were run at 25^{o}C (1955) and more data were added later giving the curve in Fig. 1.1 which shows a drop in hair number from the first to the third period followed by a rather

erratic recovery. The maximum difference in hair number is less than one bristle on average per fly so here maternal age contributes only a small amount of variation to the inbred line, but it is highly significant. There was no overall correlation with the number of flies in each relay. Other experiments showed that changing the environment (altering the temperature, removing the male temporarily, selecting parents from different hatching periods before commencing the relay, reducing the media) also influences the next generation. The changes were not permanent, being over-ridden successively by more maternal or cytoplasmic effects each generation, and the experiments were discontinued.

Fig. 1.1 Mean sternopleural hair numbers of flies hatching from eggs laid at 12 hour intervals.



A few years later Parsons (1962), Genetics Department, Cambridge University, transferred inbred flies at 24 hour intervals and recorded several characteristics. Both inbred lines used were longer lived and more fecund that the one described above but they gave similar curves stretched out over the longer life span. Developmental instability with maternal age was recorded in egg size and hatchability, and also in asymmetry of sternopleural hairs in the following generation.

Twenty years later interest rose again when Beardmore, Lints and Al-Baldawi (1975), Genetics Department, University of Swansea, and Laboratoire de Genetique, University of Louvain, found that in *Drosophila* populations heritability estimates of sternopleural hair number increased

with maternal age. In other words, genetic variation apparently increased. Caligari and Baban (1981), Genetics Department, Birmingham University, set up a large experiment to reproduce and explore these effects at maternal ages of 3, 10 and 17 days in two inbred lines and their reciprocal crosses. Being mindful of the effects of crowding the experiment was set up in two parts, one where normal crowding associated with increase in maternal age was permitted, and the other where a constant number of eggs were transferred and allowed to hatch. The results were quite clear. There was no effect of maternal age when the egg numbers were kept constant but there was when crowding was allowed in the vials. Therefore what appeared to be due to the effects of maternal age were in fact due to concomitant crowding.

How can two sets of experiments apparently of the same kind, the earlier at Aberystwyth and the later at Birmingham, give different results? The aims were different. In the later experiment the aim appropriately was to distinguish between the effects of crowding and maternal age using cultural routines similar to those used in genetic population experiments. Batches of flies, 14 or more, were taken at each of the three ages and allowed to lay at 18^{o} C. They showed that under these conditions crowding was the prime cause. In the earlier experiments the sole aim was to provide conditions where maternal age effects would most likely be seen, if present. The same pair of flies was transferred from vial to vial in each replicate; the average number of flies hatching per vial was only 14 and there was no correlation between hair and fly number (r = -0.085, virtually zero). Nevertheless the maternal effects were small and the outcome not particularly interesting. The results were different with flax. Here when experiments were designed to pick up any environmentally induced inherited changes, large unequivocal differences were obtained.

SUMMARY: CHAPTER 1

This introductory chapter gives some of the views held in the early 1950s on environmentally induced inherited changes Unstable genes had been known for some time and their instability can be influenced by the environment. The main problem is establishing that changes have been induced by the environment of the previous generation and are not due to selection of residual variation in the plants used. Some remarks on variation are followed by a description of experiments on fruit flies which show that the main cause of variation in long inbred lines is environmental but specifically designed experiments show maternal age has a small but significant effect.

CHAPTER 2

INDUCED CHANGES IN FLAX IN 1954

2.1 Preliminary observations on flax varieties

In agricultural research a number of varieties of a crop species may be grown in several environments or localities, or with different fertiliser combinations to see which is the best under each of these conditions. These interactions between genotypes and environments are interesting genetically and in 1953 studies began on the genetic differences between means, and variances, of flax and linseed varieties especially with regard to their responses to different environments.

The species *Linum usitatissum* was chosen for several reasons, Firstly, it is an inbreeding species. Selfing is virtually complete so it is expected to be homozygous and breed true, though precautions are still taken to ensure the plants self. Secondly, crops have been grown for centuries from ancient Egyptian and Roman times and there are many varieties. Thirdly, there are two main types, flax and linseed, which cross easily, providing additional comparisons and genetic variation. Linseed, a short, branching type with numerous capsules is bred for seed yield; flax, with its greater height and fewer capsules is bred for its fibre for linen. There is much ready material for studies on interactions between varieties, within and between the two types, and the environment. Furthermore unlike linseed and many other important crop plants, flax has not been bred primarily for seed yield or plant size, but for a long, relatively unbranched stem with few capsules and sown at a much higher seed rate.

There have been a number of comments over the years on the quality of flax seed. Percival (1935), for example, reports that the best yield of flax, in yield and quality, is obtained from well dried seed kept in barrels for two or three years; or better still, from fully ripened seed harvested from “barrel” flax. It is believed some localities produce poor seed, and more importance is attached to weight of flax seed, small seed giving a poor crop. On the other hand it is generally reckoned that plants tend to grow out of maternal effects due to poor seed, being at maturity not much different, it is claimed, from plants grown from what is perceived to be good seed. In preliminary experiments at Aberystwyth flax seed collected in a good harvest year gave plants nearly twice as large as from seed collected in the following poor year when grown together the year after, but there was no carry over to the subsequent year. In another experiment plants were grown from seed from capsules at varying stages of ripeness down to the point where there was no germination. Not surprisingly the plants decreased in size as seed weight and ripeness decreased. Tyson (1959, AU) harvested seed

from green unripe capsules, and from heavily shaded plants, of several flax and linseed varieties and compared the weights of plants grown from them with plants from first class seed. Although the poor seed was one third the weight of the best seed there was no overall significant difference in plant weight, only small though significantly different varietal responses. The plants were however well spaced and grown under good cultural conditions.

Seed of twelve flax varieties and four linseed varieties were kindly sent to us by staff at the Plant Breeding Station at Stormont in Northern Ireland and preliminary studies began in 1953. The seed was sown in boxes outside the greenhouse and the plants transplanted into observation plots. Each variety was in a single long row of well spaced plants for an initial assessment of their appearance, growth and distinguishing characteristics. The plants within each variety were very uniform. The linseed varieties were obviously different from the flax but there were also clear differences between many of the flax varieties, in height, branching and flower colour. The boxes contained the same soil, which was not made up into compost, as in the plots and no fertiliser was added, nor had been added recently, to the soil.

Flower buds were bagged on several plants of each variety to get selfed seed and also for making reciprocal crosses. The seed was sown the following year (1954) in the greenhouse with different combinations of fertilisers and the plants then grown to maturity in Latin Square layouts in the field where they received the same fertilisers respectively as they received in the greenhouse.

In addition, to check the quality of the seed from the plants in 1953, some seed was collected from branches which had been pruned to allow only two capsules on each branch to develop, as may be the case in a crossing programme, and from unpruned branches, of ten flax and two linseed varieties and sown at the same time as the rest of the experiment in 1954. At maturity flax plants from seed from the pruned branches were significantly ten per cent taller than those from unpruned branches whereas the linseed varieties showed no significant difference due to pruning. Though these may be maternal effects, probably due to seed size, possibly larger maternal effects could occur if plants were grown with different fertilisers, as they were in the main experiment in 1954, and affect estimates of genetic and environmental variation. A subsidiary experiment was grown in 1955 solely to find out how much effect the fertilisers applied in 1954 had on their progeny in 1955.

As mentioned, many plants, varieties and crosses had been grown in 1954 with all combinations of fertilisers, and seed had been taken from most of them so there was ample choice. A single flax variety was chosen, Stormont Cirrus, being a good average example of the flax varieties grown, in height, branching, flowering and responses to the environment. There were large

differences in plant weight among the progeny grown in 1955 from parents which received the different fertilisers in 1954, which initiated the further studies described here.

2.2 The environment

The environments and general conditions of the subsidiary experiment which began in 1954 is described here in some detail because they are broadly the same as those used in later experiments though changes were made according to circumstances and results over the years. The seed was sown during the last week of March in boxes in a heated greenhouse where they received solutions of fertilizers in different combinations. The plants were removed from the greenhouse about the 5th week from sowing and transplanted at about the 6th or 7th week into field plots where they were grown in the same combinations of fertilizers as in the boxes. Each individual was recorded and traced from seed to mature plant.

It is convenient to use upper case letters for general reference to fertilizers, to their main effects and interactions, namely N (sulphate of ammonia), P (granular triple superphosphate), K (potassium chloride) and G (lime as calcium hydrate or carbonate), and their interactions, eg, N x P (the effect of N in the presence of P compared with its effect in the absence of P, or *vice versa*). Lower case letters and italics, for example *npk, nk, p,* etc*,* denote combinations of fertilizers.

(i) *Greenhouse*

The greenhouse compost was made from 7 parts sieved soil from the field where the plants were transplanted, 3 parts Irish granulated moss peat and two parts granite chippings. Fertiliser solutions providing the different environments were made by dissolving 15 grams of N, P or K in a litre of water or a combination of these so that *npk* for example was applied as a 4.5 per cent solution. To obtain the phosphate in solution the granular triple superphosphate was left in water for 24 hours, shaken at intervals and siphoned off. 250 cc of the appropriate solution was applied at sowing to each box measuring 23 x 36 x 10 cm deep, and lightly rinsed with water afterwards. These are strong solutions but most drained through, and the boxes were watered daily thereafter except for one further application 10 days later when the same solutions were applied at one/sixth strength. Lower concentrations were applied in later years and applied differently. Where required ground limestone was mixed with the compost at about 30 grams per 50 kilos. Just before transplanting the boxes were rinsed with an insecticide for control of wireworm in the field. Temperatures in the greenhouse were extremely variable from week to week and year to year in the erratic weather

conditions of April but the average maximum and minimum values were about 27° C and 10° C. After several years a new greenhouse complex came into use, easing pressure on the existing one but introducing more environmental differences, possibly averaging higher temperatures which could have been responsible for modifying results, such as increasing dominance in some crosses.

(ii) *The field*

The field was part of the University farm up to and including 1953. It was grazing land in 1949, 1950 and 1951, cropped with a feeding mixture in 1952 and with oats undersown with a grass/clover mixture in 1953. In 1952 and 1953 it received a total of 200 kilos of compound fertilizer, 250 kilos of basic slag and 150 kilos of ground limestone per acre. The soil is a light to medium loam has a low available phosphate content and had been maintained "in good heart".

In 1954 semi-permanent plots of the sixteen combinations of N, P, K and G each at two levels, applied and not applied, were set out on the field, and replicated. The 16 combinations are listed down the side in Table 2.1. Each fertiliser was applied at 300 kilos per acre except for lime which was at 800 kilos per acre. These quantities were much reduced, or none applied, in later years. The field is on a hillside overlooking the sea about a mile away. South-westerlies temporarily flattened the plants. Rainfall is relatively high, averaging about 97 cm per year. Temperatures are about 9° C in April and November climbing to about 16° C in July and August. The greenhouse environment during he first 5 to 6 weeks from sowing is generally more important than the field environment for inducing inherited changes but it is in the field where conditions can greatly affect the development of the plants and seed, for example drought at planting or excessive rain at ripening time. In one year it was so wet seeds germinated in the capsules on the plants. In later years the plants were grown in a more sheltered area about 3 miles inland, but more prone to frost.

(ii) *General environment*

This refers to an environment used for the general propagation of plants and for comparing them under good cultural conditions, though not for maintaining all stocks. The seed was sown at the end of March in boxes, pots or cups and the plants grown in the greenhouse, usually with additional heating, for the first five weeks in compost described above with the addition of John Innes Base fertilizer and sometimes with the addition of a weak *npk* solution. A compound fertilizer and sometimes lime were applied to the field where they were transplanted about six weeks from

sowing. They were spaced in the field at 30 cm intervals in rows 60 cm apart (Plate 1). As detailed in later chapters, for some purposes the plants were grown entirely in pots, or entirely out of doors.

Sometimes seed was sown in September and the plants were grown over winter in a heated greenhouse with additional lighting to utilize this period of the year It is not very satisfactory and the seed, if taken, is not used for maintaining varieties or induced types over generations, for which other environments described later are used, nor for on-going experiments over years.

Plate 1 Young flax plants in the field. They are well spaced for observation and measurement. In a normal flax crop they are grown much closer together

Table 2.1 Plant weights (oz) of the first generation plants, *$C_1$54*, of parents grown with eight combinations of fertilisers in 1954 . The first generation plants were grown with sixteen combinations of fertilisers, *$C_0$55*, in 1955. Means of five plants. Totals on the right hand side are due to fertilisers applied directly to the plants in 1955. Totals at the bottom of the table are the totals in 1955 due to fertilisers applied to plants in the previous generation in 1954. (Durrant 1962a).

				$C_1$54						
$C_0$55	*npk*	*np*	*nk*	*n*	*pk*	*p*	*k*	*nil*	Total (A)	Total (B)
npkg	4.0	1 .5	1.2	3.5	3.3	3.7	2.4	1.5	21.1	
										36.6
npk	2.8	2.8	0.5	2.3	2.0	2.0	2.1	1.0	15.5	
npg	3.7	3.3	1.2	3.6	3.0	4.2	2.9	1.7	23.6	
										38.0
np	2.3	1.9	0.7	2.3	2.2	1.6	2.5	0.9	14.4	
nkg	1.8	0.8	0.8	2.0	1.2	0.8	1.7	1.3	10.4	
										14.3
nk	0.8	0.2	0.4	0.5	1.0	0.4	0.3	0.3	3.9	
ng	1.6	1.8	1.0	0.9	1.7	1.5	1.2	1.1	10.8	
										19.4
n	1.0	1.0	0.5	1.2	2.1	0.8	1.2	0.8	8.6	
pkg	2.2	2.1	0.7	3.3	3.0	2.4	2.5	1.4	17.6	
										30.7
pk	2.7	2.2	0.3	2.9	2.8	0.6	1.1	0.5	13.1	
pg	2 .3	1.7	0.7	3.1	2.5	2.6	0.8	1.4	15.1	
										33.2
p	2.5	2.9	0.8	3.8	3.0	1.3	1.0	2.8	18.1	
kg	2.6	2.2	0.7	3.0	1.8	1.6	1.6	1.0	14.5	
										28.5
k	1.9	2.3	0.8	2.3	2.3	1.0	2.1	1.3	14.0	
g	1.5	2.0	0.6	2.4	1.6	1.0	0.9	1.3	11.3	
										23.4
nil	2.1	1.8	0.8	1.6	2.7	1.3	0.9	0.9	12.1	
Total	35.8	30.5	11.7	38.7	36.2	26.8	25.2	19.2	224.1	224.1

Least significant differences for $P = 5$ per cent

$C_0$55 totals (A) 8.3; *$C_0$55* totals (B) 12.0; *$C_1$54* totals 4.78

2.3 Parents *C 0 54*

The subsidiary experiment mentioned in section 2.1, which had now become the main investigation and the first induction experiment, began with taking seed from plants of the flax variety Stormont Cirrus grown out of doors in unfertilised soil in 1953. The flower buds had been bagged and the ripe seed was sown in a heated greenhouse in 1954 in all eight combinations of N, P and K listed at the column heads in Table 2.1. No lime was added. Five seeds were sown in each of the eight combinations on 1st April and the plants transplanted into their respective fertilised plots in the field on 15th May. Seed was collected in early September from the five plants in each of the eight plots, giving eight seed samples. The flower buds had been bagged and the five plants of each plot contributed approximately the same to the sample. This is the parental generation, designated $C_0 54$ because the plants received the inducing treatments in 1954. There were thus eight treatments, *C 0 54npk*, *C 0 54np*….*C 0 54nil.*

2.4 The first generation, $C_1 54$, and parental generation, $C_0 55$

The immediate progeny of the treated plants were grown the following year, in 1955, and are designated $C_1 54$ because they are the first generation of plants receiving the inducing treatments in 1954. They were also grown with different fertilisers, as follows. Rows of six seeds of each of the eight $C_0 54$ seed samples were randomised in boxes to which were applied a new set of 16 combinations of N, P, K and G fertilisers, listed down the side of Table 2.1. These are parental treatments, designated $C_0 55$, for subsequent generations of plants. There were thus 8 x 16 = 128 combinations altogether comprising the eight $C_1 54$ and sixteen $C_0 55$ treatments. Out of a total 768 seed, 10 failed to germinate and two were abnormal or damaged. Five of the six plants in each case were transplanted into the field, the eight $C_1 54$ rows being randomised in each of the same fertiliser combinations as they received in the boxes.

A sample of ripe seed was taken from each of the 128 plots all five plants contributing approximately equally. The plants were cut at ground level and the mean fresh weight of the five plants in each row is given in Table 2.1 where any transmitted effects from $C_0 54$ to $C_1 54$, and the direct effects, $C_0 55$, of the fertilisers can be compared on the same plants. The weights were recorded in ounces.

The totals of the mean plant weight for the 16 plots receiving the fertilisers in 1955 are in column A, and adding the lime and no lime totals gives the $C_0 55$ totals for the 8 combinations of N,

P and K in column B, each calculated from 80 plants. As expected there are large differences due to these direct applications to the plants. The largest, *npg* plus *np* = 38 oz, is nearly three times the weight of *nkg* plus *nk* = 14.3 oz. The totals along the base are the weights of the C_1*54* plants, the progeny of the plants receiving the eight combinations of N, P and K in the previous generation, also calculated from 80 plants. We would expect these to show only small differences due to error variation, perhaps maternal effects or minor undefined and unassigned inherited differences. But the differences are huge, comparable with those in column B given by fertilisers applied directly to the plants. The largest, *n* (38.7 oz), is more than three times the weight of the smallest, *nk* (11.7oz).

2.5 Statistical significance

Could the differences among the C_1*54* plants be due to large variation in soil fertility, or large genetic variation in the variety? Such large variation was not seen among the parents in 1954, nor in their grandparents in 1953. Yet the *npk* and *nk* plants were easily distinguished in the field in every case. The most informative differences between the plant weights are separated in Table 2.2 and expressed as mean squares (variances) with their probabilities of being due to error variation. The first part refers to the fertilisers applied directly to the plants in 1955, the second part to the fertilisers applied in the previous generation in 1954, and the last to interactions between the two. The experiment was designed so that the second and third parts could be tested more sensitively than the first, i.e. they have smaller error variation.

The table is interpreted as follows. Taking the first part, the overall effect of N has a small mean square and a high probability of being due to error (only probabilities less than 5 per cent are shown). The overall effect of P on the other hand is highly significant; there is a probability of less than 1 per cent that the difference observed is due to error. The difference between the total for plots receiving P (138.5) and plots not receiving P (85.6) is 52.9, an increase in weight of 62 per cent. Lime (G) has a smaller significant effect, and the only other significant item is the N x P interaction which shows that N increases weight in the presence of P, 74.6 - 63.9 = 10.7, but decreases it 33.7 – 51.9 = -18.2 (due to initial adverse effect on seedlings) in the absence of P.

In the second part P again is the only main effect that is highly significant. Its effect on plant weight has a probability of less than one in a thousand of being due to error. The other highly significant items are interactions showing that the response to one fertiliser depends on the presence of one or more others. Whatever the reasons there are large and highly significant differences in plant weight due to fertilisers applied to the parents in the previous year.

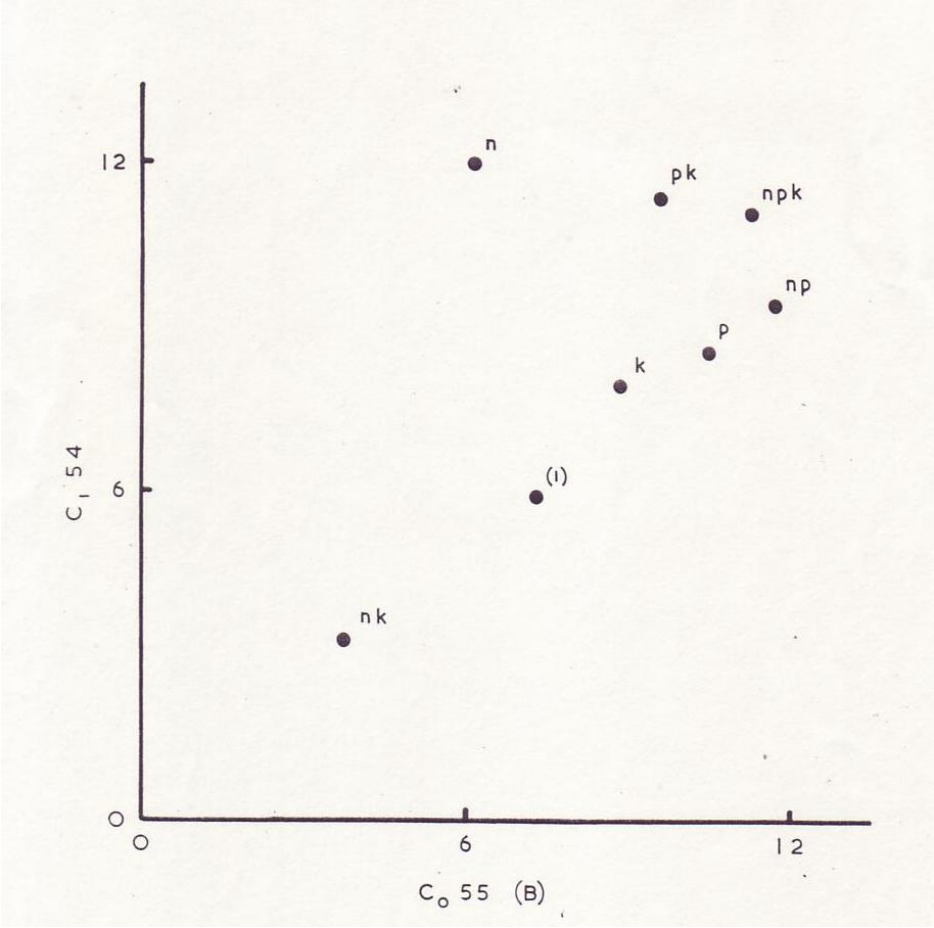
Interactions between the two sets of treatments in the third part of the analysis are grouped together and are marginally significant when tested against the highest order interactions. If true they show that some differences between the first generation plants, $C_1 54$, due to fertilisers applied to the parents are not consistent over field plots receiving different fertilisers, $C_0 55$.

The $C_1 54$ total plant weights are plotted against the $C_0 55$ total plant weights (column B, Table 1.1) in Fig. 2.1, thus comparing the effects of fertilisers transmitted from the previous generation with their effects applied directly to the plants. The two sets seem fairly well correlated except for *n* which is largely responsible for a rather low and non-significant correlation coefficient of $r = 0.52$.

Table 2.2 Analysis of variance of $C_1 54$ and $C_0 55$ plant weights in Table 1.1. (Durrant 1962a).

Item	Degrees of freedom	Mean square	Probability per cent
$C_0 55$			
N	1	0.44	-
P	1	21.86	< 1
K	1	0.12	-
G	1	4.77	< 5
N x P	1	6.53	< 5
N x K	1	0.65	-
N x G	1	3.89	-
P x K	1	0.12	-
P x G	1	0.49	-
K x G	1	0.71	-
Error	5	0.64	-
$C_1 54$			
N	1	0.68	-
P	1	9.30	< 0.1
K	1	0.31	-
N x P	1	0.06	-
N x K	1	10.75	< 0.1
P x K	1	9.96	< 0.1
N x P x K	1	6.53	< 0.1
$C_0 55$ x $C_1 54$			
1st order	12	0.38	< 5
2nd order	30	0.33	< 5
3rd order	34	0.36	< 5
Error	29	0.17	-

Fig.2.1 Weights (lb) of 80 plants. Transmitted effects of fertilisers applied in the previous generation (C_1*54*) plotted against the direct effects of the fertilisers applied directly to the plants (C_0*55*). (Durrant 1962a).

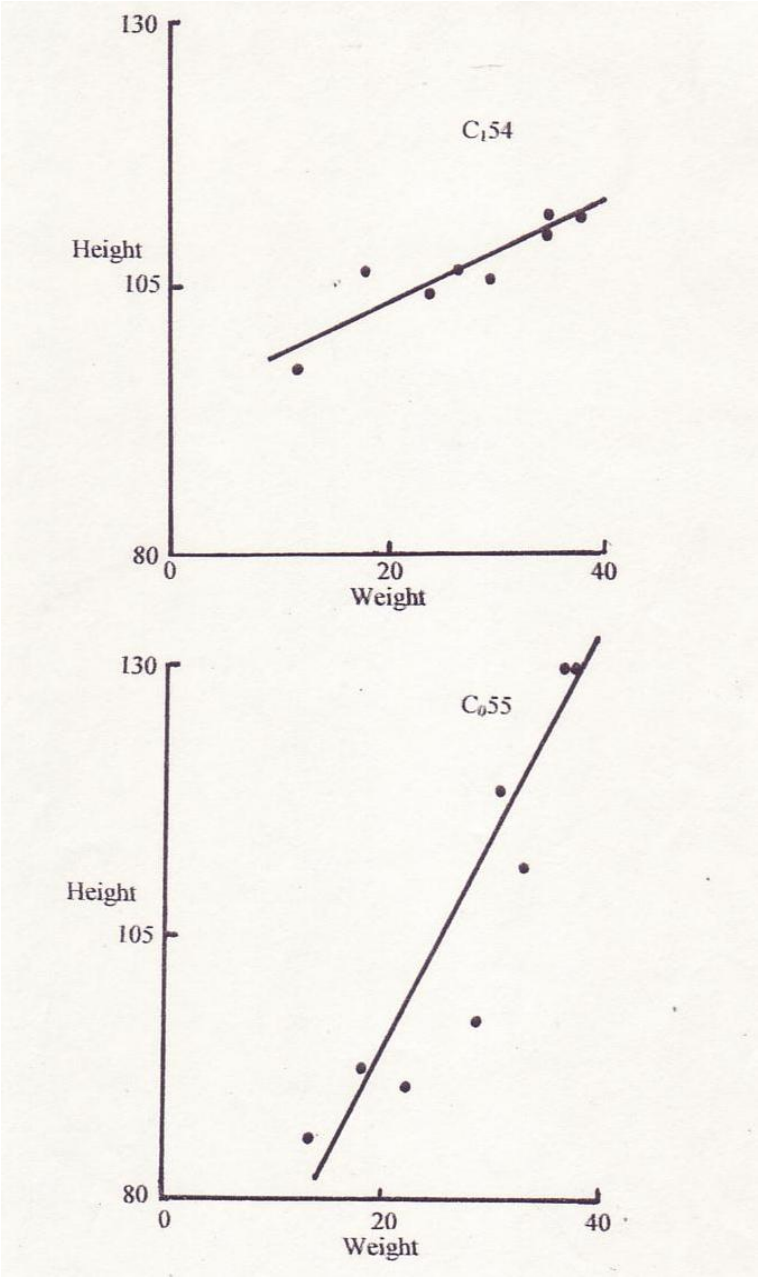


2.6 Plant height

Fertilisers applied directly to the plants produce larger differences in plant height than their induced effects from the previous generation. Final height is plotted against plant weight separately for C_1*54* and C_0*55* in Fig. 2.2 for the eight fertiliser combinations. The plant weights are the marginal totals in Table 2.1 and the heights from a similar table and therefore both graphs are calculated from the same plants. If one graph were superimposed on the other they would intersect at their common mean plant weights and heights. The regression lines, and the difference between their slopes, are highly significant. There is a fixed relationship between weight and height in both graphs over the whole range of fertiliser combinations, but $C_0$55 plant weights are associated with four times the height differences as the same weight differences in C_1*54*. Different developmental factors may be involved in the induced changes than in normal responses to fertilisers, or there are other

chromosomal factors for plant height independent of those for plant weight which are relatively unchanged by the induction process, assuming here these are environmentally induced changes.

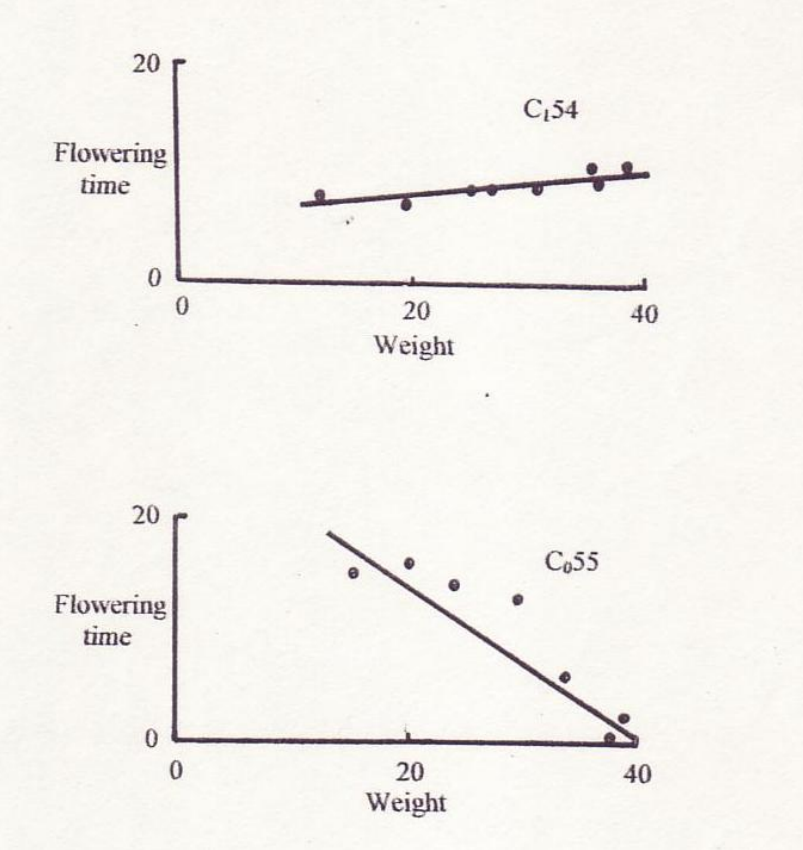
Fig. 2.2 Plant height (cm) plotted against plant weight (g) for induced effects of fertilisers applied in the previous generation, $C_1 54$, and for fertilisers applied directly to the plants, $C_0 55$.



2.7 Flowering time

Flowering time was recorded when the first flower opened on each plant, measured by the number of days after the 30th June. As with height, mean flowering time is plotted against weight separately for $C_1 54$ and $C_0 55$, the same plants contributing to both graphs (Fig.2.3). Again both regression lines and their difference in slope are highly significant. An increase in plant weight of ten grams due to fertilisers applied directly to the plants, $C_0 55$, is associated with a reduced flowering time of 3.8 days. A similar weight increase in $C_1 54$ induced by fertilisers in the previous generation is associated with an advance in flowering time of half a day. The relationship is seven times greater, and in the opposite direction, in $C_0 55$ than in $C_1 54$. So fertilisers applied directly to the plants which increase weight also increase height and earliness; fertilisers applied in the previous generation giving similar increases in weight in $C_1 54$ have minimal effect on height and flowering time.

Fig. 2.3 Flowering time (days after 30 June) plotted against plant weight for induced effect of fertilisers applied in the previous generation, $C_1 54$, and for fertilisers applied directly to the plants, $C_0 55$.



2.8 Are the induced changes adaptive?

Are the first generation plants adapted to the environments of their respective parents? Do the plants grow better in the same kinds of environments as their parents? One would hardly expect the first generation plants, C_1*54*, to be revealing but to allay doubts it is worth looking to see how they respond. The environments in the two years are not entirely equivalent but to utilise all the data the two levels of lime (presence and absence) have been summed in Table 2.3 to give an 8 x 8 table of 64 combinations of N, P and K for C_0*55* (omitting reference to lime) and for C_1*54*. The smallest of the C_1*54* plants, *nk*, is least expected to grow better in the poorest fertiliser combination, *nk*, in C_0*55*, and in fact it has one of the lowest weights. Grown with *nk* nutrient, the weight of C_1*54nk* is 1.2 oz; its mean weight grown with the other nutrients is (11.7 -1.2) ÷ 7 = 1.5 oz.

To make a more general assessment the eight cells in the leading diagonal of Table 2..3, weights 6.8, 5.2…2.2, are called "like" environments and the remaining 56 cells are "unlike" environments. The mean weight of "like" plants is 3.862, and the "unlike" plants 3.450, a difference of 0.412. On average plants are about 12 per cent heavier when grown in the same environment as their parents than in different environments. There is a lot of variation in Table 2.3, and this difference could be due to chance, or hide specific cases of larger apparent adaptations.

The data lend themselves to genetic diallel analysis one approach being to estimate the average contribution of each of the eight fertiliser combinations to apparent adaptation by calculating *alpha* values (Durrant, 1969) from differences between "like" and "unlike" cells throughout the table and averaging for each of the eight fertiliser combinations along rows or columns. For example, for *npk* and *np,* the difference is (6.8 + 5.2 - 4.3 - 6.0) ÷ 2 = - 0.85, which is in the direction of apparent adaptation and contributes to *npk* and *np alpha* values. Similar calculations throughout the table give, after adjustments for interlacing of rows and columns, the *alpha* values in Table 2.4. Their mean is the average difference, 0.412, calculated above.

Table 2.3 Plant weights after summing over the two levels of lime (applied and not applied) among the combinations of C_0*55* fertilisers in Table 2.1.

	npk	*np*	*nk*	*n*	*pk*	*p*	*k*	*nil*	Total
C_0*55*									
npk	6.8	4.3	1.7	5.8	5.3	5.7	4.5	2.5	36.6
np	6.0	5.2	1.9	5.9	5.2	5.8	5.4	2.6	38.0
nk	2.6	1.0	1.2	2.5	2.2	1.2	2.0	1.6	14.3
n	2.6	2.8	1.5	2.1	3.8	2.3	2.4	1.9	19.4
pk	4.9	4.3	1.0	6.2	5.8	3.0	3.6	1.9	30.7
p	4.8	4.6	1.5	6.9	5.5	3.9	1.8	4.2	33.2
k	4.5	4.5	1.5	5.3	4.1	2.6	3.7	2.3	28.5
nil	3.6	3.8	1.4	4.0	4.3	2.3	1.8	2.2	23.4
Total	35.8	30.5	11.7	38.7	36.2	26.8	25.2	19.2	224.1

These values separate out the anomalous treatment, *n*, referred to earlier (section 2.5) being the only one with a large positive value. The others are negative apart from the marginally positive value for *p*, indicating a general trend towards apparent adaptation. The two largest values with the same sign should indicate the two fertiliser combinations giving the largest difference between like and unlike environments. They are -1.600 and -1.866 for *npk* and *nk* respectively, summing to an estimated difference of -3.466, close to the value obtained of -3.7. The combined weight of the C_1*54npk* and C_1*54nk* plants in like environments, 8.0, is about 80 per cent more than in unlike environments, 4.3. This cannot be due to C_1*54nk* plants and the most plausible reason is that the plants whose parents received *npk*, being among the largest, take advantage of, and respond more to, the *npk* nutrients applied directly to them. This kind of interaction, common in living organisms, may be reduced or removed, when measurements are made on a different scale, usually by converting to logarithms, or here to logarithms of logarithms. These work out to loglog plant weights of -0.512 and -0.510 for “like” and “unlike” plants, reducing the percentage from 80 to a negligible amount. Similar responses are found in most of the data so there is no need here for some undefined mechanism of adaptation, at least as assessed from the plant weights.

Table 2.4 Mean contributions to differences in plant weight (oz) in $C_1 54$ between those grown with the same fertilisers as their parents and those grown with different fertilisers. Most values are negative indicating apparent adaptation.

npk	- 1.600
np	- 0.116
nk	- 1.866
n	2.284
pk	- 1.184
p	0.200
k	- 0.584
nil	- 0.434
Mean	- 0.412

SUMMARY: CHAPTER 2

Flax and linseed varieties are self fertilised and are mostly inbred but there have been comments in the past of the effect of poor environments on the quality of flax seed and on subsequent harvests. In preliminary experiments at Aberystwyth these effects were unimportant provided the progeny were grown in reasonably good conditions.

Plants of the flax variety Stormont Cirrus were grown with all eight combinations of N, P, and K fertilisers and their progeny grown the following year again in all combinations of the fertilisers with and without lime. There were large differences in plant weight due to the fertilisers applied directly to the plants but there were equally large differences due to the fertilisers applied in the previous generation.

Plants receiving *npk* were three times larger than those which received *nk* whether applied in the previous generation or to the plants themselves.

The relationships between plant weight and height, and flowering time, were different according to whether the fertilisers were applied in the previous generation or directly to the plants, suggesting different genetic factors were involved in the induced changes of weight, height and flowering time.

Plants grown in the same environments as their parents were overall 12 per cent larger than those grown in different environments, interpreted as being due to the larger types responding more to the better environments.

CHAPTER 3
INDUCED CHANGES 1955 TO 1960

3.1 The environmentally induced changes in 1954 are transmitted to later generations

The large and highly significant inherited plant weight differences in the first generation grown in 1955 of parents receiving fertilisers in 1954 are inherited by later generations. The progeny of all 128 combinations of C_1*54* and C_0*55* plants grown in 1955 (Table 2.1) were grown in a general environment in 1956. This is the second generation, C_2*54*, descended from the 1954 treated plants and the first generation, C_1*55*, from the 1955 treatments. The C_2*54* mean plant weights are plotted in Fig. 3.1 against the first generation mean plant weights, C_1*54*, calculated from the totals at the bottom of Table 2.1 converted to grams. The correlation between first and second generations is virtually complete (r = 0.97). All the original changes induced by fertilisers in 1954 which appeared in the first generation in 1955 appear almost exactly in the second generation in 1956, except the differences are larger probably because they were all grown in a general environment in 1956.

It is also clear that these and other induced changes can be transmitted over many generations. Some are present after 50 generations, grown over more than 50 years, which invites claims they are not induced changes but residual genetic differences selected by the fertilisers. Residual genetic variation of this kind and magnitude has not been seen nor established among the plants studied. More evidence for induced change is given in later chapters.

The stability in inheritance of the plant weights of two extremes, C_1*54npk* and C_1*54nk*, grown in a general environment for six generations, is shown in Table 3.1. The *npk* descendents are about three times the weight of the *nk* descendents, but larger and smaller differences occur in other environments. For example Table 3.8 shows in a particular *p* environment the *npk* type is nearly eight times larger than the *nk* type, and in another (in winter) there is no significant difference in weight. These are interactions with the environment as would be expected of any crop varieties without any suggestion they are inherited. Plate 2 shows 49th generation plants grown in a general environment in 2006. The differences between them are similar to those that might be expected from genetic factors determining quantitative variation. There are no differences in flower colour or leaf shape though the *nk* induced plants tend to be a slightly darker green. There are other differences described in later pages. It would be unrealistic to suppose that plants that are the product of environmentally induced changes would never under any circumstances show instability or undergo

further change. In fact as described later some changes have been induced in them, and latterly after several decades there appears to be some convergence in plant weight though the two types are still clearly recognisably different. It is not known whether they have inevitably a tendency to revert or because of the different location in which they have been grown in the last ten or so years. Plate 3 shows some descendents grown in 2004 for seed collection. They are 47th generation plants of those treated in 1954. The larger, *npk* type has been named L and the smaller, *nk* type, S.

Table 3.1 Mean plant weights (oz) of descendents of C_0*54npk* and $C_0$54nk plants. 80 plants were grown each year each, except 160 in 1955. (Durrant 1962a).

Generation	Year grown	*C* $_0$*54npk*	C_0*54nk*
C_1*54*	1955	2.2	0.7
C_2*54*	1956	3.5	1.3
C_3*54*	1957	2.9	0.8
C_4*54*	1958	3.5	1.2
C_5*54*	1959	2.9	0.8
C_6*54*	1960	1.7	0.6

Fig. 3.1 Mean plant weights (g) of the 2nd generation, C_2*54,* plotted against those of the 1st generation, C_1*54*, of inherited effects of fertilisers applied in 1954. (Durrant 1962a).

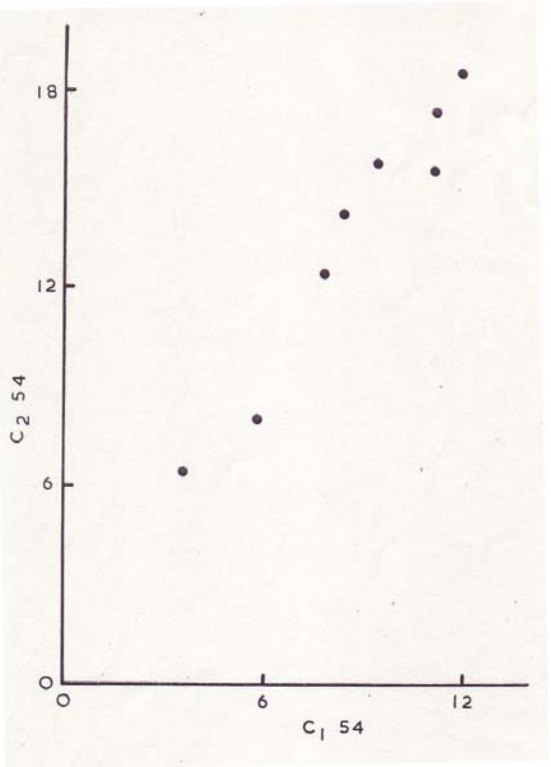


Plate 2 49th generation plants, grown in 2006, of two extreme types of flax induced by fertiliser treatments in 1954. Left, *npk*; right, *nk*.

Plate 3 47th generation plants of the two extreme types grown for seed collection in the field in 2004. On the left, *nk* type, (the S genotroph); on the right, *npk* type (the L genotroph) The plants have been caned and the flower buds routinely bagged to ensure selfing. The plants have yet to mature.

3.2 Descendents of *$C_0$54npk* and *$C_0$54nk* are uniform and selection has no effect on them

The descendents of *$C_0$54npk* and *$C_0$54nk* are very uniform. Table 3.2 gives the plants weights of 4th generation plants randomised in three blocks in the field. Seed was taken as usual from at least five plants in each generation so there was no question of uniformity being due to newly enforced relatively heterogeneous lines. When randomised in a field plot in summer in a general environment each plant is easily and unmistakably identified as either *npk* or *nk* type, a useful feature because there are no practical or statistical problems in separating out these two induced changes from environmental variation.

Table 3.2 Uniformity of descendents of *$C_0$54npk* and *$C_0$54nk* plants. Weights (g) of individual 4th generation plants all randomised in each block. (Durrant 1962a).

Block 1	$C_4$54npk	34	39	49	39	32	33	35	40
	$C_4$54nk	10	14	13	11	12	11	15	14
Block 2	*$C_4$54npk*	39	45	41	45	38	35	34	34
	$C_4$54nk	19	15	11	10	12	10	11	13
Block 3	*$C_4$54npk*	54	45	54	49	51	54	41	46
	$C_4$54nk	11	10	11	6	15	7	9	8

Could residual genetic variation be present but not expressed under the conditions of the general environment in which they were grown? There is normally more variation among *npk* induced plants than among *nk*. In Table 3.2 on average the variation of the *npk* plants is about three times that of *nk*. On a log scale, correcting for the greater plant weight of the *npk* type, the variation is about the same. Selection was imposed on the *npk* plants in an attempt to flush out any genetic variation. Two independent selection lines for large plant weight, and two for small plant weight, were begun in 1962 among the *npk* type plants and continued for four generations. In the fourth generation of selection four plots were chosen, one from each selection line, containing the most within plot variation, and seed collected from five plants in each of the four plots, to be used as parents for the next generation, giving 20 parental seed samples. They were sown the following year

in a randomised block experiment with five replications. Five plants from each of the 20 seed samples were grown in each of the five blocks, giving 500 plants in all.

Table 3.3 Weights (g) in four selection lines of *npk* type plants and mean plant weights of their progenies. (Durrant, 1971).

	Plant	Mean of plants	Progeny mean	Mean of progeny means
High selection. Line 1	24		63.6	
	48		65.0	
	43	62.0	81.0	73.7
	122		84.4	
	73		74.6	
High selection Line 2	72		82.0	
	58		83.4	
	39	51.8	73.6	74.6
	46		75.6	
	44		58.4	
Low selection Line 3	47		69.0	
	70		90.2	
	71	57.0	83.2	82.0
	47		72.4	
	50		95.2	
Low selection Line 4	45		71.6	
	68		90.0	
	74	56.4	72.6	78.2
	65		73.8	
	30		82.8	

The weights of the twenty 4th generation selected plants from which the seed samples were taken, the mean plant weights of their twenty progeny families and the means of the selected lines in Table 3.3 show that the differences between the selection lines are small and that selection over the years has been ineffective. An analysis showed there were no significant differences between the selection lines but there was within the selection lines with a significant positive correlation between parents and offspring ($P < 5$ per cent). The effect is tiny, a difference between parent plants of 10 grams giving a difference of less than 2 grams between progeny means, due presumably to maternal

or transient environmental effects since none had been picked up during the course of selection. The large variation among parents, especially in line 1, reflects the marginal soil conditions of the experimental area in which they were grown.

3.3 More inherited changes are induced when fertilisers are applied to plants from the same seed sample used in 1954.

Seed was taken from the same sample used for the 1954 fertiliser treatments and ten plants grown with each of the eight fertilisers combinations, *npk nk, p* and *nil,* with and without lime. These are C_0*56* plants. Table 3.4 shows there are large differences in the first generation, C_1*56,* which are similar to, though not identical with, those in the first generation of the respective 1954 treatments (Table 2.1). Phosphorus applied by itself now has the same effect as *nk*, inducing a small type of plants in the next generation. With lime, phosphorus induces a large plant, as also does *npk* with lime. Table 3.4 shows that the progeny are not necessarily correlated with their parents, *p* giving a large parent plant but a small first generation plant. In the three cases where the second generation was grown the effects are transmitted to this generation as well. The discrepancies between these results and those obtained following the 1954 treatments are likely to be due to differences in soil and compost, particularly in nitrogen and lime content.

Table 3.4 In 1956 fertilisers were applied again to plants of the Stormont Cirrus variety inducing more inherited changes. Mean plant weights (g). Durrant, 1962a).

	npkg	*npk*	*nkg*	*nk*	*pg*	*p*	*g*	*nil*	Number of plants each
C_0*56*	62	38	3	1	65	58	40	30	10
C_1*56*	56	42	34	23	58	20	27	31	30
C_2*56*	60			24		22			20

3.4 Induction tests on the two extreme types, *$C_1$54npk* and *C $_1$54nk*

The first generation, *$C_1$54*, of the plants receiving the eight combinations of fertilisers in 1954 were grown in all combination again in 1955 with the addition of two levels of lime (Table 2.1). These provide another set of treatments, *$C_0$55*. Their first generation, *$C_1$55*, grown in 1956, is also the second generation, *$C_2$54*, of the 1954 treatments. Would this second set of treatments revert, increase or decrease the plant weights induced by the first set of treatments?

Experiments described above showed that the large *$C_1$54npk* and small *$C_1$54nk* plants induced by the first set of treatments, in 1954, are apparently transmitted unchanged to subsequent generations when grown together in a general environment. They breed true; they are uniform and selection has no effect on them. Therefore the inherited effects, if any, of the second set of treatments, *$C_0$55* in Table 2.1, were studied on these two types first.

The 32 combinations of *$C_2$54npk* and *$C_2$54nk* with the sixteen *$C_1$55* were replicated in boxes and field with more nutrient applications, a total of 1280 plants. The main result is summarised in Table 3.5. In the second generation *$C_2$54* of the first set of treatments the *npk* induced type overall is still three times the size of the *nk* induced type, in line with previous results. In contrast there are no such large differences in the first generation *$C_1$55* of the second set of treatments, *$C_0$55*. The *$C_2$54npk* plants are very uniform and there are no significant differences. The *$C_2$54nk* plants, analysed separately because of different error variation, are more variable and showed significant differences due to the 1955 treatments but they are small, a difference of 45 per cent at the most which could reasonably be due to maternal effects.

Table 3.5 Mean plant weights (oz) in 1956 of the progeny of *$C_1$54npk* and *$C_1$54nk* plants which received fertilisers treatments in 1955 (Table 2.1, *$C_0$55*, two levels of lime summed). Means of 80 plants. The 1955 treatments have no inherited effects on these two extreme types induced by the 1954 treatments. (Durrant, 1962a).

	$C_0$55 treatments								
	npk	*np*	*nk*	*n*	*pk*	*p*	*k*	*nil*	Total
$C_2$54npk	5.6	5.9	5.7	5.7	5.7	5.6	6.1	5.7	46.0
$C_2$54nk	2.3	1.8	1.6	1.6	2.5	1.9	1.6	1.6	14.9

Therefore the results of the 1954 treatments were not repeated when similar treatments were applied in 1955 to *npk* and *nk* types induced by the 1954 treatments yet, as described above, when the plants grown from the original sample of Stormont Cirrus were treated in 1956 large inherited differences were induced in them again (section 3.3) So the environments in 1954 which induced the large *npk* and small *nk* types also induced in them an inability to undergo further induced heritable changes. They have apparently lost the capacity for responding to the inducing environments, at least to those applied here.

3.5 Induction tests on all *C* $_1$*54* types

If no inherited changes could be induced in C_1*54npk* or C_1*54nk* when more fertilisers were applied could any inherited changes be induced in any of the eight C_1*54* types? The progeny of the these eight types which had been gown in the four C_0*55* treatments of *npkg, npk, g* and *nil* (Table 2.1) were grown in 1957 in a general environment. These are therefore C_2*54* and C_1*55* plants. There were four replicates each containing four blocks consisting of the four C_1*55* types, each divided into eight plots containing the eight C_2*54* types, 640 plants in all.

The weights of C_2*54* plants after passage in the previous generation through C_0*55 npkg* and *npk* summed, and after passage through C_0*55 g* and *nil* summed, are plotted in Fig. 3.2 against the overall weights of their parents C_1*54* (column totals, Table 2.1). If all eight types were as stable as the two extremes, C_1*54npk* and C_1*54nk,* there should be a constant relationship between C_1*54* and C_2*54* irrespective of whether the eight C_1*54* types were grown in *npkg* and *npk* or in *g* and *nil*, that is like Fig. 3.1. There is a reasonable correlation in both figures, Fig. 3.2 (a,b), confirming the transmission of the C_0*54* treatment effects from the first to the second generation, but in each case there are two types, *p* and *k*, which are mainly responsible for diminishing the fit to a linear regression line. In the first (a), C_1*54 p* and *k* after passage through *npkg* and *npk*, are approximately the same weight in C_2*54* as C_2*54npk*; in the second (b), after passage through *g* and *nil*, they approach C_2*54nil* in weight. The conclusion is that C_1*54p* and C_1*54k* are the only two among the eight C_1*54* types in which inherited changes can be induced. If Figs 3.2 (a) and (b) are averaged a good relationship reappears between C_2*54* and C_1*54* as in Fig. 3.1. The movements of *p* and *k* on the graphs show that the 1955 treatments, C_0*55npk* and C_0*55nil*, albeit with and without lime, have induced changes in C_1*54p* and C_1*54k* in the same directions as they did in the original Stormont Cirrus variety.

The capacity of *p* and *k* types and none of the others to transmit environmentally induced changes to the next generation is a key observation and two more experiments were done to confirm this. The first compared the effects of four 1955 treatments, *npk, nk, p and nil,* on the four C_1*54* types, *npk, nk, p* and *nil* (Table 2.1). The progeny grown in 1957 are therefore the 16 combinations of four C_1*55* and four C_2*54* types. The mean plant weights are in Table 3.6 where the differences between the C_2*54* totals are highly significant and similar to the C_1*54* totals in Table 2.1 as expected. The differences between the C_1*55* totals are significant at the 5 per cent level only, but they are mainly in C_2*54p*, where there is a highly significant 340 per cent difference due to the C_0*55* treatments compared with 20, 40 and 50 per cent differences in the other three.

C_1*54k* also appeared to have this property which was tested again in a third experiment in 1959 in a general environment with C_1*54pk* for comparison. Table 3.7 shows that the C_2*54pk* plants have similar weights but there are large differences between the C_2*54k* plants due to the C_0*55* treatments. In these last two experiments C_0*55p* induces a small plant as well as C_0*55nk,* a pattern repeated in later experiments.

These results confirm that inherited changes are induced in C_1*54p* and C_1*54k* when they are grown with the appropriate fertilisers but not in the other C_1*54* types. They also supply additional evidence for environmentally induced inherited changes. Previously in 1956 the progeny of all C_1*54* plants receiving the combinations of fertilisers in 1955 were summed to see if overall changes had been induced in them. They gave similar results but the changes were much reduced in magnitude. The above results show why; only two amongst them were able to respond, that is, C_1*54p* and $C_1$54*k*.

Fig.3.2 (a). Mean plant weights (oz) of $C_2 54$ progeny of $C_1 54$ plants grown in *npkg* and *npk* and plotted against $C_1 54$ plant weights. Inherited increases in plant weight were induced only in $C_1 54p$ and $C_1 54k$ plants.
(b). Mean plant weights (oz) of $C_2 54$ progeny of $C_1 54$ plants grown in *g* and *nil* and plotted against $C_1 54$ plant weights. Inherited decreases in plant weight were induced only in $C_1 54p$ and $C_1 54k$ plants.(Durrant, 1962a).

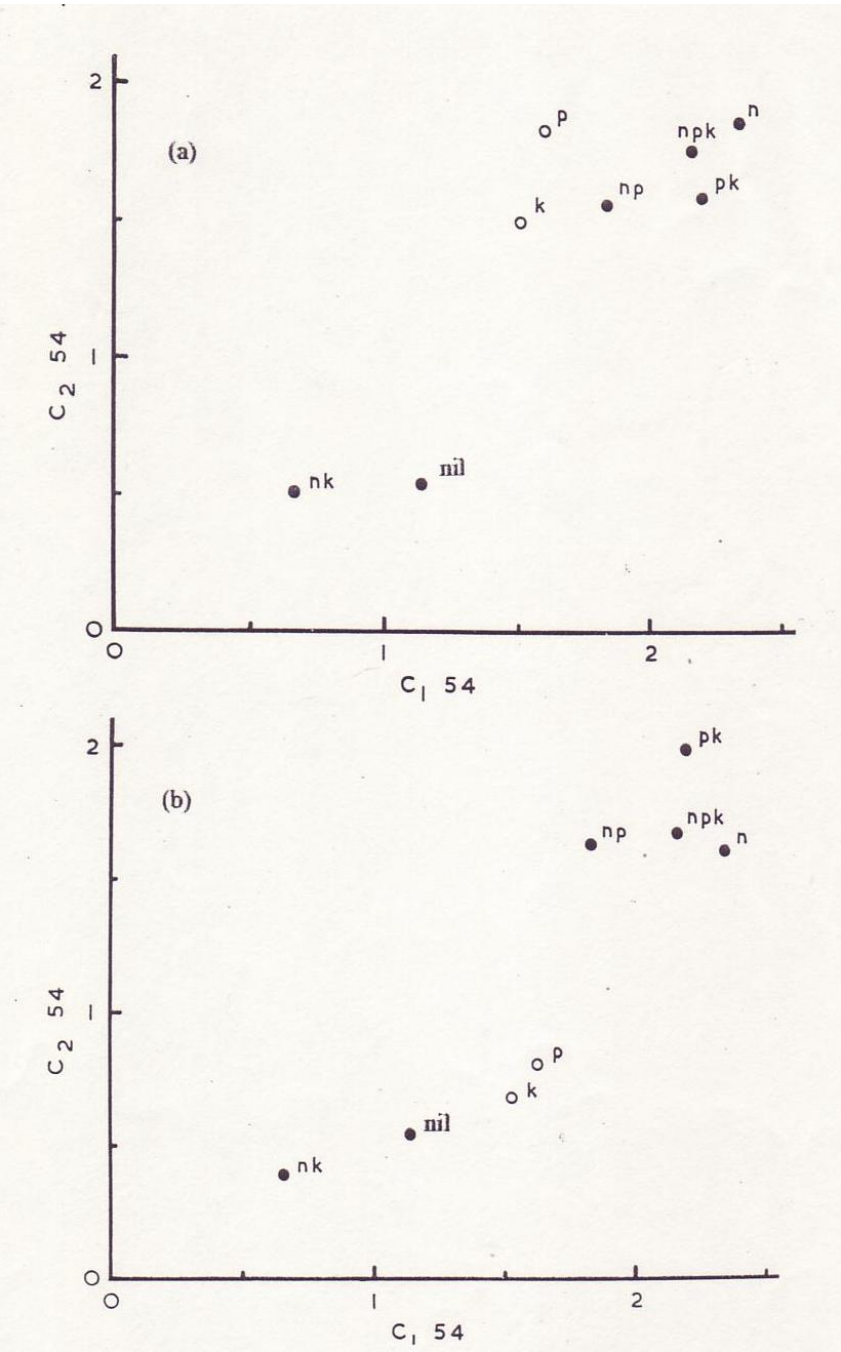


Table 3.6 Mean plant weights (g) of the progeny of C_1*54 npk*, *nk*, *p* and *nil* plants which received *npk*, *nk*, *p* and *nil* fertilisers in 1955 (Table 2.1). Means of 10 plants. Least significant difference (5 per cent) for row and column totals is 45. The differences between rows are the inherited differences induced by the original 1954 treatments. The differences between columns are the inherited differences, $C_1$55, (if any) induced by the new set of treatments in 1955. Highly significant inherited differences due to the 1955 treatments appear only among the progeny of the plants which received *p* in 1954. (Durrant, 1962).

		C_1*55*				
		npk	*nk*	*p*	*nil*	Total
C_2*54*	*npk*	65	43	61	66	235
	nk	16	11	10	9	46
	p	54	12	22	23	111
	nil	19	16	20	20	75
Total		154	82	113	118	

Table 3.7 Mean plant weights of the progeny of C_1*54pk* and C_1*54k* plants which received *npk*, *nk*, *p* and *nil* fertilisers in 1955 (Table 2.1). Means of 40 plants. Least significant difference (5 per cent) for mean plant weights in each row is 4.6. Highly significant differences due to the 1955 treatments appear among the progeny of the plants which received *k* in 1954, but not among the progeny of those which received *pk* in 1954. (Durrant 1962a).

		C_1*55*			
		npk	*nk*	*p*	*nil*
C_2*54*	*pk*	39	41	45	42
	k	49	16	16	24

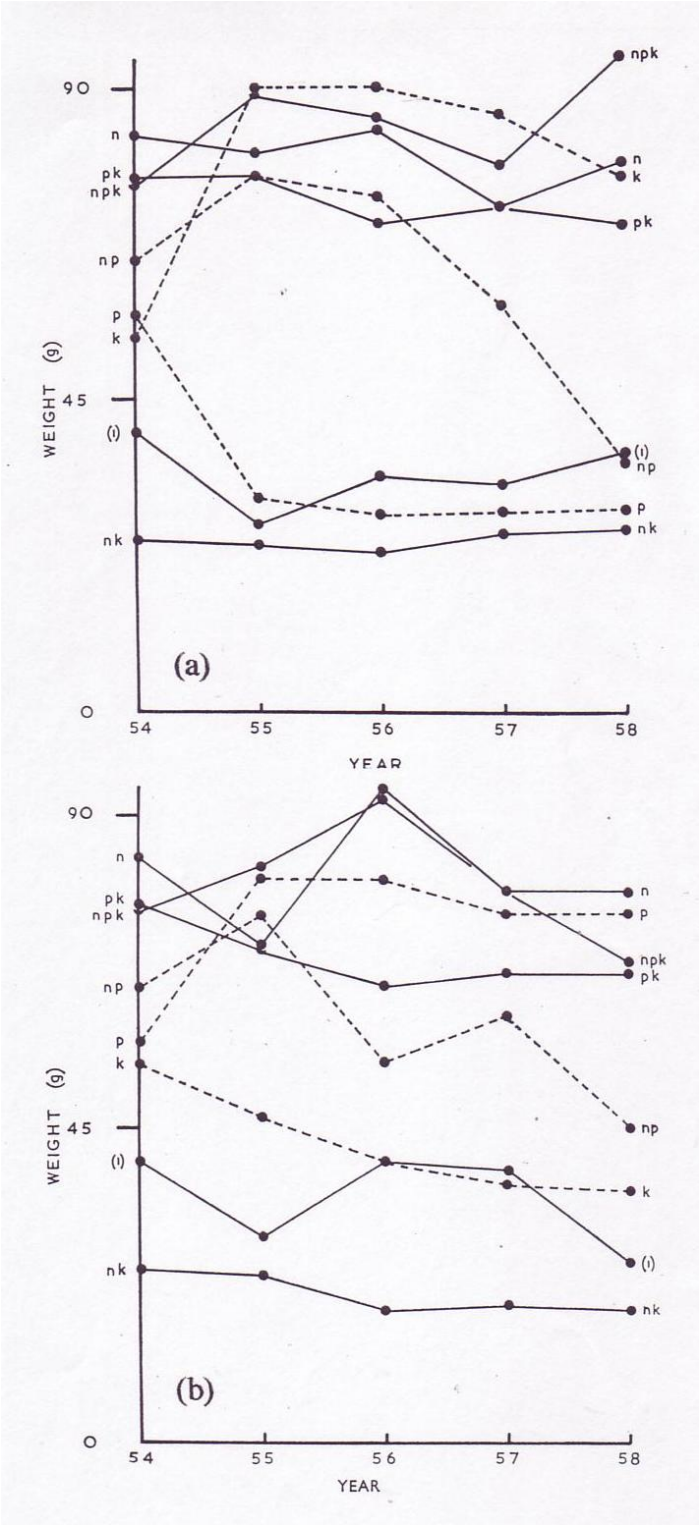
3.6 Nutritional lines

The progeny of plants receiving the eight combinations of N, P and K fertilisers in 1954 were grown in these same eight respective combinations in 1955 (along with other combinations, Table 2.1) with and without lime, giving sixteen combinations of N, P, K and G. These treatments were continued respectively for three more generations forming sixteen nutritional lines. Seed collected each year was sown in a general environment in 1959 in six replicates each divided into two blocks, fertiliser combinations with lime in one block, without lime in the other. Plants from the eight fertilisers treatments were in eight sub-blocks divided into four plots consisting of the four years, 1,920 plants.

The plants weights in Fig.3.3 include those of C_1*54* from which all the others are descended (column totals in Table 2.1), scaled to remove the overall difference in plant weight between 1955 and 1959.

Although the fertilisers may be applied routinely each year the overall environment must inevitably vary and give some erratic results. If there were adaptation with regard to plant weight then one would expect a general upward trend of the lines of which there is no evidence. In both figures at one extreme, *npk, pk,* and *n* maintain large plants throughout, and at the other extreme *nk* and *nil* maintain small plants. With lime, *p* shows a sudden increase which is maintained; *k* declines slowly. Without lime, *p* drops, *k* rises, both changes being maintained. Therefore the extreme types have remained constant but *p* and *k* have changed in agreement with previous results (section 3.5). *np* shows a decline over years, with and without lime. Though there was no previous evidence for *np* having the capacity for induced change it is in fact nearest to the intermediate types *p* and *k* among the progeny of the 1954 treated plants. Another observation is the difference lime makes to the response to *p*. With lime *p* induces a large plants comparable with *n*; without lime it induces a small plant comparable with *nk*. This was confirmed in later experiments and *n* (applied as ammonium sulphate) and *p* (applied as triple superphosphate) without lime have been used since to induce contrasting changes.

Fig. 3.3 Plant weights (g) of nutritional lines grown in a general environment in 1959. Each line was grown from 1954 to 1958 in one of the eight fertilisers combinations of N, P and K. (a) Without lime.
(b) Without lime in 1954, with lime 1955 to 1958.



3.7 Comparison of large and small induced types with the original variety Stormont Cirrus

The Stormont Cirrus plant weight is almost exactly half way between the weights of the descendents of the large type *C*$_1$*54npk,* and small type *C*$_1$*54nk*, when the plants are grown together in the field in the summer (Table 3.8, Plate 4), confirmed independently over the years at Aberystwyth by Nicholas, 1967; McLellan, 1970; and Al-Saheal, 1974. Thus the two fertiliser combinations *npk* and *nk* induced changes in plant weight in equal and opposite directions from the original variety Stormont Cirrus. In Table 3.8 the *npk* type is about three times the weight of the *nk* type as before (Table 3.1) when these two types are grown with *npk* fertiliser (roughly akin to the general environment), but grown in *p* environment it is about eight times larger, with the Stormont Cirrus plants still half way between the two types.

Table 3.8 Comparison of the plant weights(g) and heights (cm) of descendents of the large type *C*$_1$*54npk* (L) and of the small type *C*$_1$*54nk* (S) with those of the Stormont Cirrus variety from which they have been induced. Data from several experiments. (Durrant 1962a).

		Descendents of *C*$_1$*54npk* (L)	*C*$_1$*54nk* (S)	Mean of L and S	Stormont Cirrus
	Fertiliser applied to plants measured				
Weight					
Summer	*npk*	77	17	42	47
	p	78	10	44	42
	nk	1.7	0.7	1.2	1.2
Winter	*npk*	10.5	11.2	10.8	13.7
Height					
Summer	*npk*	74	68	71	89
Winter	*npk*	114	105	109	125

The plants interact strongly with other environments. When grown in pots from about the end of September with additional heating and lighting the plant weights of the *npk* and *nk* types are about the same but the Stormont Cirrus plants are significantly heavier than either (Table 3.8). These interactions with the environment are not inherited changes because the descendents are unchanged and repeat the interactions. Under these conditions there is no apparent symmetrical induction with regard to weight and the original variety now appears superior. McLellan, 1970, grew the plants from January onwards in the greenhouse and placed them outside several weeks later and found the difference between the weights of the *npk* and *nk* types much reduced at maturity with the Stormont Cirrus weight closer to that of the *npk* type than to *nk.* A similar result was obtained when the plants are kept in a greenhouse from April to August. Nicholas, 1967 and Al-Saheal, 1974 also obtained large variations in their relative plant weights in the course of other studies. So the differences in plant weight usually referred to as distinguishing the three types, *npk* type (large), *nk* type (small) and original Stormont Cirrus variety (intermediate) normally apply to plants grown in favourable conditions in the field in the summer. C_1*54p* and C_1*54k* plants have weights about half way between the extremes $C_1$54npk and C_1*54nk* and therefore have the same weights as Stormont Cirrus, and like Stormont Cirrus they have the capacity for environmentally induced inherited change.

Plant height is different. Grown in winter and summer the *npk* type is taller than *nk*, but Stormont Cirrus plants are taller than either (Plate 4; Table 3.8), so height is not symmetrically induced like weight. In fact although some of the eight combinations of fertilisers applied in 1954 induced heavier plants and some lighter plants than Stormont Cirrus all induced shorter plants. This is in line with an earlier observation (section 2.6) that changes in weight and height are not entirely the result of a single induced genetic change. Specifically, the C_1*54p* and C_1*54k* plants were intermediate in height as well as in weight between the extremes C_1*54npk* and C_1*54nk*, so *p* and *k* fertilisers applied in 1954 induced inherited changes in height like all the others, but not in weight.

Plate 4 Comparison of the two induced types, *npk* (L genotroph) left, and *nk* (S genotroph) right, with the original variety Stormont Cirrus (Pl genotroph) centre

3.8 Induced changes in the flax variety Liral Prince

Inherited changes described so far had been obtained in the variety Stormont Cirrus. Inherited changes have been obtained in another flax variety, Liral Prince. Plants of this variety were grown in the same eight combinations of fertilisers, and at the same time ($C_0 54$), as Stormont Cirrus in 1954 but they were in another block with other varieties in smaller boxes prior to transplantation so they probably experienced poorer conditions. The $C_1 54$ generation was grown in a general environment in 1958 when the seeds were four years old, and the second generation ($C_2 54$) followed in 1959. The plant weights of the progeny of plants receiving the different treatments were significantly different ($P < 1$ per cent) in both generations (Table 3.9) and the C_1/C_2 correlation was $r = 0.84$ ($P < 1$ per cent).

Table 3.9 Mean plant weights (g) in the first and second generations ($C_1 54$ and $C_2 54$) of the flax variety Liral Prince grown in 1958 and 1959 following treatments of their parents ($C_0 54$) with eights combinations of fertilisers. (Durrant, 1971).

$C_0 54$	*npk*	*np*	*nk*	*n*	*pk*	*p*	*k*	*nil*	Number of plants each
$C_1 54$	79	127	71	138	99	71	89	98	10
$C_2 54$	25	63	30	71	63	46	45	57	20

The second generation is more reliable and as with Stormont Cirrus the highest value is given by *n* and the lowest by *nk*. A high value is given by *pk* as before, *np* a little lower and *p* and *k* are again intermediate. The exceptionally low value for *npk* and somewhat higher value for *nil* are presumably due to the different general inducing environment or different characteristics of the variety.

The progeny of the two extreme types, C_2*54n* and C_2*54nk*, were used in most of the studies on this variety. They have remained stable for many generations and appear to be equivalent to the large and small types induced by *npk* and *nk* in Stormont Cirrus. Poor seed kept in store gave some conflicting results at first but later studies showed that Liral Prince plants themselves were intermediate between the largest and smallest induced types, as was the case with Stormont Cirrus.

3.9 Plastic and stable genotrophs

One conclusion from these earlier experiments with flax is that it is possible to separate out two kinds of plants, those in which inherited changes can be induced by growing them in different environments and those in which apparently no changes can be induced. The first are called *plastic* types and the second *stable* types. Both are conveniently referred to as genotrophs (genetic plus nutrition). Inherited differences between plants could be genetic or genotrophic or both, and we would not know from the outward appearance of plants whether genotrophic differences were present unless environmentally induced inherited changes had been seen to occur. It is conceivable that inherited differences between plants normally ascribed to genetic differences may have absorbed genotrophic differences in the past.

Most studies described here have been on the descendents of the large C_1*54npk* and small C_1*54nk* plants, and the original Stormont Cirrus variety, denoted

large stable genotroph	L
small stable genotroph	S
plastic genotroph	Pl

A similar notation is applied to descendents of the large C_1*54n* (L) and small C_1*54nk* (S) induced Liral Prince plants, and the Liral Prince variety (Pl) itself. An additional SC or LP is affixed as required.

A second conclusion is that the environment can induce an inherited change from a plastic type to a stable type. This means that inherited changes induced in one generation are not necessarily removed or over-ridden by environments of succeeding generations.

A third conclusion is that environmentally induced inherited changes presumably depend on the appropriate blend of genotype and inducing environment but also on environments in previous generations. For example if the 1954 treatments had been applied to Stormont Cirrus plants which, unknown to the experimenter, had been grown in a previous environment like C_0*54npk* he may have found (in the unlikely event of his doing the experiment) no inherited changes to have occurred. Other examples of environments of previous generations affecting the competence of subsequent environments to induce inherited changes are described in later chapters. There are also three practical problems; (1) If the environment has stabilised a variety how can it be de-stabilised, i.e. change a stable variety to a plastic variety? (2) If a variety is plastic how can we ensure it remains plastic when multiplied up each year? (3) In what environments should the progeny of treated plants be grown in order to reveal or demonstrate induced changes?

SUMMARY: CHAPTER 3

Plants of different weights induced by a range of eight fertiliser combinations applied to their parents in 1954 were grown with another set of combinations of fertilisers in 1955 to asses if more inherited changes could be induced in them. No further changes could be induced in the largest and smallest induced types but large changes similar in magnitude to the earlier ones appeared in the progeny of those of intermediate weight.

Plants induced by the 1954 treatments are described as large stable (L), small stable (S) and plastic genotrophs. The original Stormont Cirrus variety is a plastic genotrophs Pl. L and S breed true over generations; they are uniform and selection for high and low plant weight has no effect.

The original Stormont Cirrus variety is taller than any of the induced types, large, small or intermediate obtained from it, so induced weight and height changes are again not completely associated. The relative weights and heights of L, S and Pl change when grown in poorer winter conditions, which are non-inherited interactions with the environment.

Similar inherited changes were induced in another flax variety, Liral Prince.

CHAPTER 4
L AND S GENOTROPHS

4.1 Earlier interpretations

Several interpretations of the highly significant plant weight differences associated with the parental fertiliser treatments were considered.

1. There may have been large residual genetic variation in the variety Stormont Cirrus and chance assortment of genetic factors among the groups of parents plants receiving the different treatments produced large phenotypic differences in their progeny roughly correlated with the differences produced by the direct application of the fertilisers. It is difficult to reconcile this with the highly significant differences obtained and no noticeable large error variation in parents or progeny. Furthermore the induced changes could be repeated in another variety, Liral Prince.

2. The treatments may have had a selective effect on residual genetic variation maintained in the variety by some genetic mechanism, selection occurring at sporogenesis, fertilisation or seed setting, for genetic factors determining roughly the same plant weights as those produced directly by the respective treatments. It would seem this would require some unconvincing mechanism both for maintaining such large hidden genetic variation in the variety and, for example, permitting only those pollen grains carrying genetic factors for small plants to survive, make faster growth down the style, or take part in fertilisation in plants receiving *nk.*

3. Large maternal effects may have been responsible. Judged by its weight, Table 4.1, the seed collected in the damp 1954 season from the treated parents, C_0*54*, was of poor quality, and the seed weights varied between treatments. But seed collected in the sunny 1955 season from the first generation plants, C_1*54*, was heavier and more uniform over treatments, yet they transmitted the differences in plant weight undiminished to the second generation, C_2*54*.

Table 4.1 1000 gram seed weights of seed from the parent plants C_0*54* and from their progeny C_1*54.* (Durrant, 1962a).

	npk	*np*	*nk*	*n*	*pk*	*p*	*k*	*nil*	Total
C_0*54*	3.8	3.6	3.3	3.9	4.3	4.2	4.1	3 .8	31.0
C_1*54*	5.5	5.4	5.4	5.7	5.7	5.6	5.4	5.5	44.2

4. The parental treatments induced inherited changes in the cytoplasm, nucleus or both. This appears to be the only reasonable interpretation, implied by the foregoing account and supported by all later data. One rejected possibility is that the environments caused upsets to, or derangements of, the chromosomes. This is unlikely because of the healthy growth and high fertility of the induced types. Tyson (1959, AU) made a cytological examination of the chromosomes of the L and S genotrophs of Stormont Cirrus (descendents of C_1*54npk* and C_1*54nk*), two flax varieties (Percello and Mandarin) and two linseed varieties (Royal and Dakota) to check their chromosome numbers and for any observable nuclear differences. He compared their chromosomes at the first meiotic metaphase of pollen mother cells. Fifteen bivalents (paired chromosomes) were seen in both genotrophs and the four genotypes and there were no abnormalities during the separation of the chromosomes and the subsequent division to form tetrads. There appeared to be no difference in chromosome size between genotypes and genotrophs and the mean chiasma frequencies of L and S, as far as could be assessed, were 27.8 and 27.3, a difference well within sampling error. From these observation the two genotrophs were judged to be normal cytologically.
5. The induced changes are not due to classical gene mutations but to changes in activity or gene regulation in certain regions of the chromosomes, maybe mediated by mobile DNA elements. Later studies support this.

4.2 Reciprocal grafts between L and S genotrophs

If the differences between L and S genotrophs are nuclear then there should be no transmission through reciprocal grafts. These were made between L and S plants, and also between L plants and between S plants of Stormont Cirrus in 1957. They were the third generation of C_0*54* plants which in the 1st and 2nd generations had been grown in *npkg*, akin to a general environment. For these experiments they were grown in 13cm pots in a greenhouse, one plant to a pot, and the grafts made in the following manner. When the plants were about three weeks old the stock was prepared by removing the upper part of the plant just above the attachment of the cotelydenous leaves, and making slit downwards about half an inch long in the stem between the cotelydenous leaves, taking care not to damage these or the small axillary buds. The scion was prepared from the terminal 3 centimetres of a young plant, also about three weeks old, the lower portion of which was cut into wedge shape after removal of the leaves in this region, and inserted into the prepared stock. The join was bound with cotton and surrounded by moist peat. They were left for two weeks, during which any roots growing from the scion were rubbed off, and then the cotton and peat were removed. The

scion of the mature plants consisted solely of the central shoot and the stock included the side branches which grew from the axillary buds of the cotelydenous leaves (Durrant, 1962b).

Table 4.2 shows that the stock has no effect on the scion. The L scion weighs about 22 grams on both stocks and the S scion weighs about 9 grams on both. The stock itself is affected by the scion, but in a direction expected from the normal physiological response of side branching to terminal shoot growth. The totals show that overall plant weight is determined mainly by the stock.

Table 4.2 Mean plant weights (g) of stock and scion of grafts between L and S genotrophs of Stormont Cirrus (Durrant, 1962a).

Stock weight		Scion weight	Total weight	Number of plants
L	48	L 23	71	19
L	62	S 10	72	21
S	37	L 22	59	22
S	43	S 9	52	18

Plants from seed taken from stock and scion of six plants of each of the four types of grafts were grown in a general environment the following year. Table 4.3 shows that in all cases plants have the same characteristic difference in weight between L and S as occurs between ungrafted plants. The difference between L and S is greater in the progeny of the grafts than in the grafts themselves which is due to the mutilation in preparation, and pot culture, of the grafts. There is no evidence that factors determining the difference between L and S are graft transmitted and therefore they cannot themselves be nutritional or hormonal.

Table 4.3 Plant weights (g) of the progeny of stock and scion of grafts between L and S. Means of 30 plants (Durrant, 1962a).

Progeny of stock weight		Progeny of scion weight		Total weight
L	84	L	79	163
L	76	S	14	90
S	13	L	76	89
S	14	S	15	29

4.3 Reciprocal crosses between L and S genotrophs

The large (L) and small (S) genotrophs of Stormont Cirrus were reciprocally crossed in 1956, 1957 and 1958, (crosses 1 to 3) being respectively the 2nd 3rd and 4th generations of the plants grown in *npk* and *nk* fertilisers in 1954. The female parent (or the plant used as the female parent) is written first so in the cross L x S, S pollen is put on L stigma. Plants used for crossing in 1956 were grown in their respective *npk* and *nk* environments, the others in a general environment. The F_1 plants (the first generation from the cross) were grown in composts with a compound fertiliser in the summer. Although the L and S plants can be selfed, being the usual way of maintaining them from generation to generation, in the crossing regime, crosses are made between L plants (ie, L x L), and between S plants, in the same manner as crossing between L and S plants, and in all respects are treated in the same manner.

The plant weights of L x L in Table 4.4 are much greater than those of S x S, as expected. The plant weights of the reciprocal crosses, L x S and S x L, are about the same, i.e. inheritance is equilinear. There is a small reciprocal difference from the first cross which is probably due to the effects of the *npk* and *nk* environments in which the respective maternal parents were grown. Equilinear transmission, i.e. where male and female parents contribute equally to the next generation, is normally taken in genetic studies to show that the observed differences between the parents are due to nuclear differences, not to maternal or cytoplasmic differences. Therefore it appears that the *npk* and *nk* environments in 1954 induced changes in the nuclei, presumably in the chromosomes. This complements the grafting studies.

Table 4.4 also shows that the plant weights of the reciprocal crosses are about midway between those of the parents so that neither induced change is dominant over the other, and the effects of the changes induced by *npk* and *nk* average out in the plant weights of the F_1 plants. Grown in the greenhouse in winter L x L and S x S are about the same weight as previously recorded for L and S (Table 3.8), but the reciprocal crosses are substantially heavier than L x L and S x S (Table 4.4). So depending on the environment in which they are grown the crosses vary from no dominance to a value in excess of the parents, i.e. to heterosis. In other experiments in summer the crosses often show partial dominance in the direction of L depending on temperature and other conditions. Crosses between L and S of Liral Prince give similar results.

Table 4.4 Mean plant weights (g) of the first generation of crosses between L and S grown in three experiments in the summer, and another in winter (Durrant, 1962).

		L x L	L x S	S x L	S x S
Summer					
	Cross 1	62	40	30	13
	Cross 2	22	15	16	6
	Cross 3	64	49	51	17
Winter	Cross 4	10.0	15.0	16.9	11.9

When reciprocal crosses show complete dominance it is generally due to a single major gene difference between the two parents. When they vary from no dominance through to partial dominance, dominance and heterosis it is probably because the parents differ in a quantitative character determined by several or many genes, though it does not necessarily follow that the *npk* and *nk* environments induced changes at several places (loci) on the chromosomes. There could be induced changes at only one locus, or at least within one region, and the deviations from the mid parent value due not to dominance in the usual sense but to a balance struck within the crosses between such changes as have arisen in the induction of the L and S plants depending on the environment in which the crosses are grown.

There are similarities between Table 4.4, comparing the plant weights of L and S with their crosses, and Table 3.8 comparing the weights of L and S with Stormont Cirrus. Like the reciprocal crosses, Stormont Cirrus is midway between L and S in the summer but exceeds L and S in the winter, so with regard to this character it is as though the original variety is reconstituted in the reciprocal crosses between the L and S genotrophs derived from it, and physiological processes common to both respond to the different environments in the same way. On the other hand plant height in the crosses remained firmly intermediate between L and S in summer and winter whereas Stormont Cirrus plant height exceeds those of L and S at both times.

Patwary (1978, AU) grew 128 F_3 families from their respective F_2 parents in an attempt to pick up 3:1 or 1:2:1 ratios in plant weight among the F_3 family means, which if found would indicate the induced changes were largely confined to a small region of a chromosome. There was no clear evidence for ratios. Evans (1967, AU) analysed F_1 and F_2 generations of a diallel cross between L, S, L_w (L with an introduced white flower mutation) and four selections from an earlier

cross between L and S. Apart for the *w* mutation all differences between parents could be assumed to be genotrophic yet from their appearance none would normally be ascribed to anything other than normal quantitative genetic differences. The customary methods of genetic analysis and graphical presentation showed that the differences in plants weight between parents crossed could be explained in terms of many genes with normal Mendelian properties, though there were anomalies in plant variation between F_1, F_2 and parent plants, described below.

4.4 F_1 and F_2 variation in reciprocal crosses between L and S genotrophs

First generation (F_1) and second generation (F_2) plants from reciprocal crosses between genotrophs L and S were grown in several experiments from 1959 to 1962 (Table 4.5). The reciprocals L x S and S x L are about the same in mean plant weight and height as before (Table 4.4) though they show more dominance probably due to different environmental conditions. Overall there is nothing special about these results; they could easily apply to data of crosses between any pair of genetically different varieties.

Table 4.5 Means and variances of plant weights (g) and heights (cm) of F_1 and F_2 generations of crosses between L and S genotrophs. *CV*; coefficient of variation. (Durrant,1962b).

	L x L		L x S		S x L		S x S	
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
Weight F_1								
Exp.4	64	293	50	203	53	240	17	11
Exp.5	65	233	49	153	47	228	21	31
Exp.6	103	1130	81	1166	75	2503	26	382
Mean	77	552	60	507	58	987	21	141
CV	0.28		0.32		0.43		0.20	
Weight F_2								
Exp.1	33	167	27	172	28	353	7	11
Exp. 2	35	135	20	81	22	103	10	7
Exp.4	36	84	22	53	28	209	11	18
Mean	35	129	23	102	26	235	9	36
CV	0.35		0.42		0.55		0.37	
Height F_1								
Exp.1	85	15	81	19	77	30	71	8
Exp.2	61	15	61	19	60	41	52	18
Mean	73	15	71	19	68	35	61	14
CV	0.05		0.06		0.09		0.06	

But the variances are unusual, i.e. the amount of variation among the parent plants and among the crossed plants. Normally in crosses between two inbred varieties differing genetically, the variation among the F_1 plants is expected to be about the same as the variation among the parent plants, because the variation between plants of each parent, and between plants of each reciprocal cross is generally solely environmental. In the F_2, the variation among the plants of the reciprocal crosses is expected to be greater than in the parents or F_1 because of the segregation and recombination of the genes by which the two parents differ. Differences may occur because large plants tend to vary more than small ones so as a correction the square root of the variance is divided by the mean plant weight to give a coefficient of variation (*CV*). When these are compared the reciprocal crosses are more variable in the F_2 than in the parents, but so also are they in the F_1, in plant weight and height, and the variation is always greater in the S x L cross than in the L x S. It appears that environmentally induced changes in the chromosomes of L and S behave differently from normal gene differences when the two genotrophs are crossed, and that at least part of the F_2 variation may be due to the additional variation in the F_1.

To test this, F_2 family mean plants weights were compared with the plants weights of their parental F_1 plants for each of the four types of crosses. In the S x L cross the F_1/F_2 gave a significantly high correlation coefficient of $r = 0.94$, the L x S cross a high but non- significant $r = 0.62$, and the L x L and S x S crosses non-signifiant negative correlations (Durrant, 1962a). Therefore there appears to be a breakdown or instability when the L and S induced changes are brought together in the heterozygote giving heritable, apparently random, variation. Twice as much variation in S x L crosses as in L x S appeared in other experiments (Durrant and Tyson, 1964) but it seems in some environments and in later years this instability is arrested or reduced. Nicholas (1967, AU) had variable results and little evidence of correlation when the parents were grown in different environments. He had in many cases complete dominance, or heterosis, and it is possible, though there is no evidence, this could be due to a switch in the balance between L and S in the crosses towards L, leaving less scope for variation.

4.5 Crosses between genotrophs of Stormont Cirrus and Liral Prince

Although the L and S genotrophs of Stormont Cirrus are similar in many respects to L and S of Liral Prince, similar genetic changes may not have occurred in their induction in the two varieties, nor need they be at the same locus or loci on the chromosomes. A diallel cross was made between them

in 1959 to establish whether they were additive in their effects or showed interaction. Table 4.6 gives the plant weights of the F_1 generation of the 16 crosses grown in a randomised block field experiment in 1960 with 25 plants per cross.

Table 4.6 Mean plant weights (g) of F_1 families of a diallel cross between L and S genotrophs of Stormont Cirrus and Liral Prince. (Durrant, 1971).

♀ \ ♂	L Stormont Cirrus	S Stormont Cirrus	L Liral Prince	S Liral Prince
L Stormont Cirrus	48	37	50	42
S Stormont Cirrus	43	18	36	18
L Liral Prince	45	41	42	38
S Liral Prince	39	21	39	20

An easy visual assessment is to divide the 4 x 4 table into quarters, as shown, wherein four 2 x 2 tables appear. Each is a reciprocal cross with parents L x L, L x S, S x L and S x S but one concerns Stormont Cirrus, another Liral Prince and the other two are crosses between them. Allowing for moderate error variation, and a somewhat larger difference between L and S of Stormont Cirrus than of Liral Prince, the four 2 x 2 tables are virtually the same so that as assessed from these data alone, the two varieties appear identical in crosses within and between. The only observed inherited difference in the data is that between L and S, and not at all to the original variety difference. L and S are the new varieties apparently induced by similar genetic changes in the original varieties. This appears in a *beta* diallel analysis (Durrant, 1969, 1971) as a single major gene difference determining the whole of the inherited variation in the table.

4.6 Crosses between L and Pl and between S and Pl of Stormont Cirrus

The two stable genotrophs L and S of Stormont Cirrus behave as two genetically different varieties when they are crossed, apart from some instability in the first generation of the cross. A different result might obtain if L and S were each crossed with the plastic genotroph, Pl the original variety

Stormont Cirrus, in which the inherited changes to L and S were induced by the environments in the first place. Specifically, would Pl nuclear contributions to the cross be converted to L in crosses to L, and converted to S in crosses to S; or would L and S contributions revert to Pl? In the first case the plant weights of reciprocal crosses of L with Pl would be the same as L, and those of S with Pl the same as S; in the second case they would both be the same as Pl.. Generalising, the mean difference between the L and S crosses to Pl divided by half the parent difference is 2 for complete conversion of Pl to L and S respectively, 1 if their effects were additive (i.e. neither contribution influences the other) and 0 for complete reversion of L and S to Pl.

F_1 plants from two sets of crosses made in 1957 were grown in a general environment the following year. The L and S parents used in the crosses were grown in a general environment but for reasons given later the Pl parents were grown out of doors from sowing. The plant weights of the Pl x Pl cross in the two sets of crosses in Table 4.7 were calculated from the same plants, and are rather lower than previously described relative to L and S, possibly a maternal effect due to their different environment. The mean difference between the L and S crosses to Pl is

$$(44 + 54 – 31\text{-}25)/2 = 21,$$

and half the mean difference between L and S parents is

$$(63\text{-} 15)/2 =24,$$

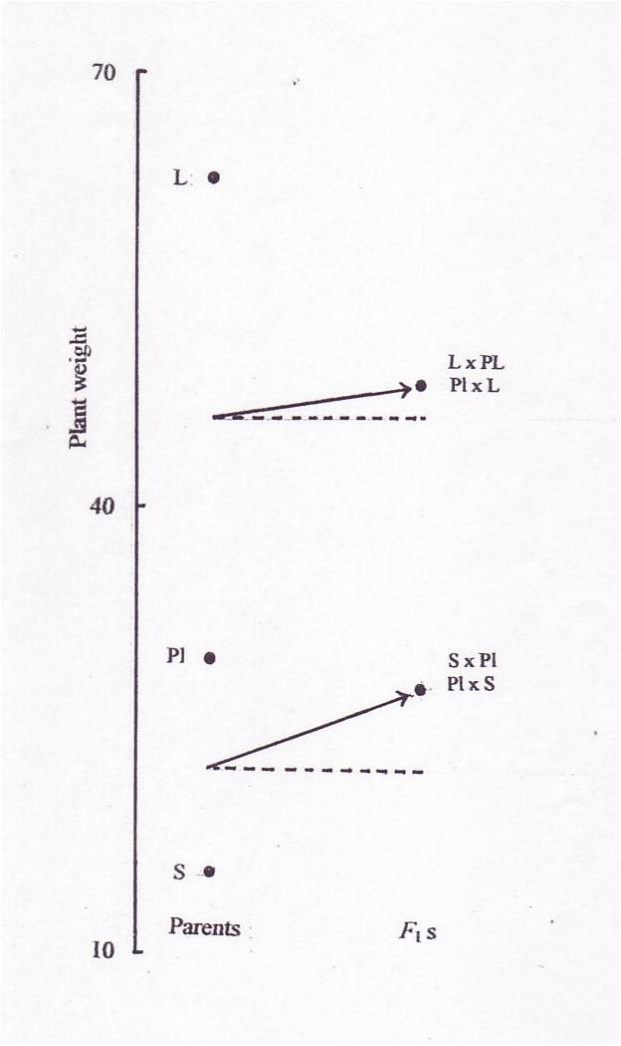
giving a ratio of 0.87, which is not significantly different from 1. In Fig.4.1, lines are drawn from the mid-parent values (46.5 for L and Pl, 22.5 for S and Pl) to the mean weights of their respective crosses. They would be parallel if the ratio were exactly 1. Factors determining L and S do not induce changes in Pl, nor do they themselves revert to Pl. Fig. 4.1 shows a moderate amount of dominance overall in a plus direction which, as it happens, would disappear if the Pl weight were raised to its usual mid-value between L and S, the arrows then virtually coinciding with the dotted lines.

To check these results from more crosses, Al-Saheal (1974, AU) studied the F_2 and F_3 generations and John A Bolton (1976, AU) the F_4 to see if changes eventually occurred. In the F_2 the ratio was 1.13, in the F_3 it was 1.36 and in the F_4, 0.94. Hence there is no good evidence for conversion of Pl nor reversion of L or S. At least, unlike the environmentally induced changes which convert the Pl plant weight to those of L and S in one generation, there is no such rapid change in Pl when heterozygous with L or S, nor in L or S when heterozygous with Pl.

Table 4.7 Mean plant weights (g) of F_1 families of reciprocal crosses between L and Pl, and between S and Pl, of Stormont Cirrus.

L x L	L x Pl	Pl x L	Pl x Pl
63	44	54	30
S x S	S x Pl	Pl x S	Pl x Pl
15	31	25	30

Fig. 4.1 Mean plant weights (g) of parents and F_1 plants of crosses between L and Pl, and S and Pl. Broken lines show mid-parent values. The arrows are nearly parallel indicating neither reversion of L or S nor conversion of Pl.



SUMMARY: CHAPTER 4

The induced changes are interpreted as being due to changes in gene regulation or gene activity in certain regions of the chromosomes.

Nuclear changes are confirmed by grafting and crossing experiments between L and S genotrophs. There is no transmission through reciprocal grafts nor to the progeny of the reciprocal grafts. In a general environment the L and S make equal contributions to the F_1 of the L x S and S x L crosses but show extreme heterosis in winter time.

The L and S induced changes appear to be unstable when they are brought together in crosses between the two genotrophs, shown by the greater variation among the F_1 plants which is correlated with the F_2 family means. Variation is also greater in the S x L than in the L x S cross in both generations.

The F_1 generations of reciprocal crosses between L and S genotrophs are essentially the same whether the crosses are made with the Stormont Cirrus variety, the Liral Prince variety, or between the two varieties. The absence of interactions suggests similar induced changes had occurred in the two varieties.

Crosses between L and Pl (the original Stormont Cirrus variety) and between S and Pl, show no reversion or conversion such that there is no change in the Pl contributions when heterozygous with L or S, nor in L or S contributions when heterozygous with Pl.

CHAPTER 5

ENVIRONMENTALLY INDUCED NUCLEAR CHANGES

5.1 DNA in flax genotrophs

The chromosome complement of a species can be studied cytologically under a microscope in suitably prepared cells, the chromosomes counted and their shapes and sizes recorded. But in comparing varieties or closely related species there may be differences in amount of genetic material, i.e. nuclear DNA, that are too small to be seen without additional aid. Feulgen photometry was developed (Leuchtenberger, 1954; McLeish and Sunderland, 1961) to measure the amounts of nuclear material in single cells and was used by H Rees and others in the department to trace the evolution of chromosomes and species (e.g, Rees and Jones, 1967, 1972; Narayan and Rees, 1976). Since it was believed that the environmentally induced changes in flax were located in the nucleus, yet no differences between the chromosomes could be observed under the microscope by the usual methods, there was a possibility that a difference might be detected using Feulgen photometry, although differences in amount of nuclear DNA would not normally be expected between plants of the same species with no chromosome aberrations.

Feulgen applied to specially prepared cells stain the DNA in the nuclei, and the more DNA the more stain taken up, and the more light absorbed which can be measured, so that if done under strictly controlled conditions the relative amounts of DNA in the cells of different plants can be measured. Evans (1967, AU) made some preliminary studies on the L and S genotrophs of Stormont Cirrus and found differences in nuclear DNA content by this method and decided that the best material for further studies was dividing cells in shoot apices of seedlings about 5 to 8 cm high. In a large experiment he measured the DNA in four plants of each of L, S and Pl of Stormont Cirrus and Liral Prince and a number of other flax and linseed varieties which had been grown under general conditions out of doors. As usual the slides were coded so that the genotype or genotroph being measured at any one time was unknown.

Table 5.1 summarises the results which are in arbitrary units because the actual values are dependent on the processing and stain used, but the differences between them are valid (Evans, Durrant and Rees, 1966; Evans, 1968a). Taking the genotrophs first, in both varieties L has more DNA than S, and Pl is intermediate. In Stormont Cirrus, L has about 16 per cent more DNA than S; in Liral Prince, L has about 10 per cent more than S. The differences between L, S and Pl of each

variety are highly significant and correlated with their plant weights; the more DNA the larger the plant.

Table 5.1 Amounts of DNA in arbitrary units in the genotrophs of Stormont Cirrus and Liral Prince, and in other varieties of flax and linseed (Evans, Durrant & Rees, 1966; Evans, 1968a)

Genotrophs

Stormont Cirrus		Liral Prince	
L	93.65	L	95.30
Pl	85.80	Pl	90.45
S	80.45	S	86.30

Flax varieties		Linseed varieties	
Percello	91.25	Dakota	94.30
Liral Monarch	88.50	Royal	87.60
Gossomer	88.25		
Hollandia	87.90		
Stormont Motley	87.70		
Rembrandt	87.00		
Norfolk Princess	86.50		
Mandarin	85.20		

These results were unexpected and a search was made for other factors which might give spurious differences in DNA measurements. To determine whether a difference between the L and S tissues, cell walls or cytoplasm could have affected the penetration of fixatives and stain, Evans measured 50 Feulgen-stained isolated nuclei on each of four slides for each of L and S and obtained a difference between their bimodal distributions (given by nuclei with divided and undivided chromosomes) comparable to that given by the cellular determinations. To test whether the higher growth rate of L, with presumably a higher metabolic turnover, had an effect, L and S were grown in fertile and poor soils, and at high and low temperatures. Table 5.2 shows these environments have no effect on either genotroph and overall L has about 18 per cent more DNA than S. Evans also checked the nuclear dry mass and nuclear size (Table 5.3) and found that these too paralleled the difference in DNA amount. The nuclear dry mass was separated into nucleolar dry mass and residual mass by Timmis (1971, AU) and found both were significantly greater in L than in S.

Table 5.2 Amounts of nuclear DNA in L and S genotrophs of Stormont Cirrus grown in soils of high and low fertility, and at high and low temperatures (Evans,1968a).

Fertility	Temperature	L	S
High	High	26.83	23.18
High	Low	26.75	21.68
Low	High	27.45	22.75
Low	Low	26.85	23.48

Table 5.3 Nuclear dry mass and nuclear volume of L and S genotrophs of Stormont Cirrus (Evans, 1968a).

	L	S
Nuclear mass (grams x 10^{-11})	3.52	2.83
Nuclear volume (μ^3)	55.793	47.794

The induced inherited changes in plant weight are therefore accompanied by induced changes in DNA amount, some treatments (*n* or *npk*) increasing plant weight and DNA, others (*nk* or *p*) decreasing them. The nature of these changes at first gave rise to speculation at a time when multiple gene sequences were unknown. Normally differences in DNA amount between plants are associated with visible aberrations such as the loss or gain of part of a chromosome, or a doubling of all or part of the chromosome complement. One suggestion was that the treatments had increased or decreased the number of strands of a chromosome or part of a chromosome. Flax has 15 pairs of chromosomes of approximately the same size and if the full 15 per cent difference was in one pair of chromosomes then in a hybrid of the L x S cross this would be seen cytologically as a pairing of a large with a small chromosome. This has not been seen so it appears that the DNA changes affect at least more than one chromosome pair (Evans, 1968a).

During cell division the nucleus goes through a mitotic cycle alternating between a resting stage and chromosome division and separation. Timmis (1971) compared the mitotic cycle time in L and S and found it was two hours shorter in L than in S, the difference being due mainly to S spending a longer time in the resting stage where it is suggested there is a slower build up of energy requirements.

The DNA measurements on the eight flax varieties are listed in descending order in Table 5.1. The differences between them are small, and non significant, compared with the differences induced in the genotrophs. The DNA of the plastic genotrophs of the original varieties, Stormont Cirrus and

Liral Prince, are just within their range. It may or may not be relevant but in a field experiment Percello plants were twice as large as Mandarin plants which have respectively the highest and lowest DNA values among these measurements. The large plants of the two linseed varieties have high DNA values, Dakota having about the same amount as the two L genotrophs.

The validity of Feulgen photometry has been questioned because DNA itself is not isolated and measured; only the amount of Feulgen staining is measured on the assumption it faithfully measures DNA amount, and only DNA amount (Ghogain, Byrne and Timmis, 1982). But it is clear there are measurable differences of some kind between the nuclei of the different genotrophs and, until disproved, results obtained by this method will continue to be referred to as differences in amount of DNA, for which there is other justification, described later.

5.2 Chromosome changes

Flax plants have 15 pairs of chromosomes in the nuclei of all cells except when the members of each pair separate in the formation of gametes which fuse during fertilisation to give the 15 pairs of chromosomes again in the next generation. This reduction division is easily seen in what are called *pollen mother cells*. The two members of each pair come together and are held together by cross-overs, or chiasmata, and as previously mentioned it is completely regular in L, S and Pl for all pairs of chromosomes. In crosses between L and S, pairing of L and S is also regular so the pairing ability of the chromosomes apparently had not been affected by the induction of the L and S genotrophs. There were no aberrant chromosomes and fertility was good.

Evans (1967, AU) argued that if the chromosome number was doubled artificially by treating the seeds with aqueous colchicine so that there were 15 sets of four chromosomes instead of 15 pairs of chromosomes (giving tetraploid instead of diploid plants) they might reveal previously unseen differences. This is because in tetraploids there is competition for pairing between the four chromosomes of each set so that instead of the four chromosomes associating in a group of four (quadrivalent) one chromosome may be left out to give a group of three (trivalent), or they may associate two and two, to give two bivalents. If there be differences between the L and S chromosomes then in the crosses, where there are two L and two S chromosomes in each set, the L and S chromosomes may be less likely to pair, leaving the two L chromosomes to pair, and the two S chromosomes to pair, with the result there would be more bivalents and fewer quadrivalents in the crosses than in the parents where all four chromosomes are either all L or all S. It would be necessary to check that the difference was not due to a difference in chiasma frequency since,

assuming random association, the frequency of quadrivalents increases with increase in chiasma frequency (Durrant,1960).

The mean bivalent frequencies per cell obtained by Evans, taken over all 15 chromosome sets, and over several plants, are in Table 5.4, where the few univalents and trivalents are omitted. The L x S and S x L crosses have 40 percent more bivalents than L x L and S x S, which is highly significant, showing that in the crosses, L chromosomes preferentially pair with L, and S with S, rather than L with S. This and the corresponding increase of quadrilavents in L x L and S x S cannot be ascribed to the slight increase in chiasma frequency in L x L and S x S plants. It appears there are structural differences between the L and S chromosomes, as well as differences in DNA amount, induced by the original environments, so that they pair less readily when in competition with the like pairing of L with L and S with S.

Table 5.4 Mean frequencies of bivalents and quadrivalents, and mean chiasma frequency per pollen mother cell in L, S and their reciprocal crosses (Evans, 1968b).

	L x L	L x S	S x L	S x S
Bivalents	10.30	14.29	14.70	10.38
Quadrivalents	9.52	7.33	7.22	9.42
Chiasmata	57.7	55.7	55.8	56.8

Small regions which stain with Feulgen solution may be seen in non-dividing nuclei of cells. They are due to condensed parts of chromosomes or heterochromatin and are called chromocentres. Although heterochromatin is generally regarded in itself to be inert its increase or decrease, i.e. in chromosome coiling, can have an influence on genetic activity in its region. Timmis (1971, AU) counted the number of chromocentres and measured their total area per cell to determine whether there was a difference between L and S. He projected the Feulgen stained slides and weighed cut-outs to determine area.

He found a highly significant difference in number of chromocentres, approximately 16 in L and 14 in S, and he reasoned that Pl would probably have 15, one for each pair of chromosomes. He notes that there were two fewer quadrivalent sets in the L x S and S x L crosses of Evans than in the L x L and S x S crosses and that therefore from these more sensitive tests the difference in DNA amount could perhaps be allocated to just one, or most two, chromosomes, though there are other data which do not necessarily support this. The total area per cell of the chromocentres in arbitrary

units was 1.55 for L and 1.34 for S, a difference of about 16 per cent which fortuitously or not ties in with the 16 per cent difference in DNA amount from Feulgen photometry.

5.3 Nuclear DNA in crosses between L and S of Stormont Cirrus

The F_1 plant weights of reciprocal crosses between L and S grown in a general environment in summer are approximately half way between the weights of the L and S parent plants. The nuclear DNA values of the genotrophs are correlated with the plant weights so the DNA values might be expected to follow the same pattern. This was confirmed by Joarder (1973, AU) who obtained the following values, each estimated from six plants using Feulgen photometry.

L x L	L x S	S x L	S x S
113.8	103.3	103.5	94.2

The reciprocals are the same and midway between the two parents, the difference between each parent and the crosses being highly significant, so there is here complete correlation between plant weight and nuclear DNA amount.

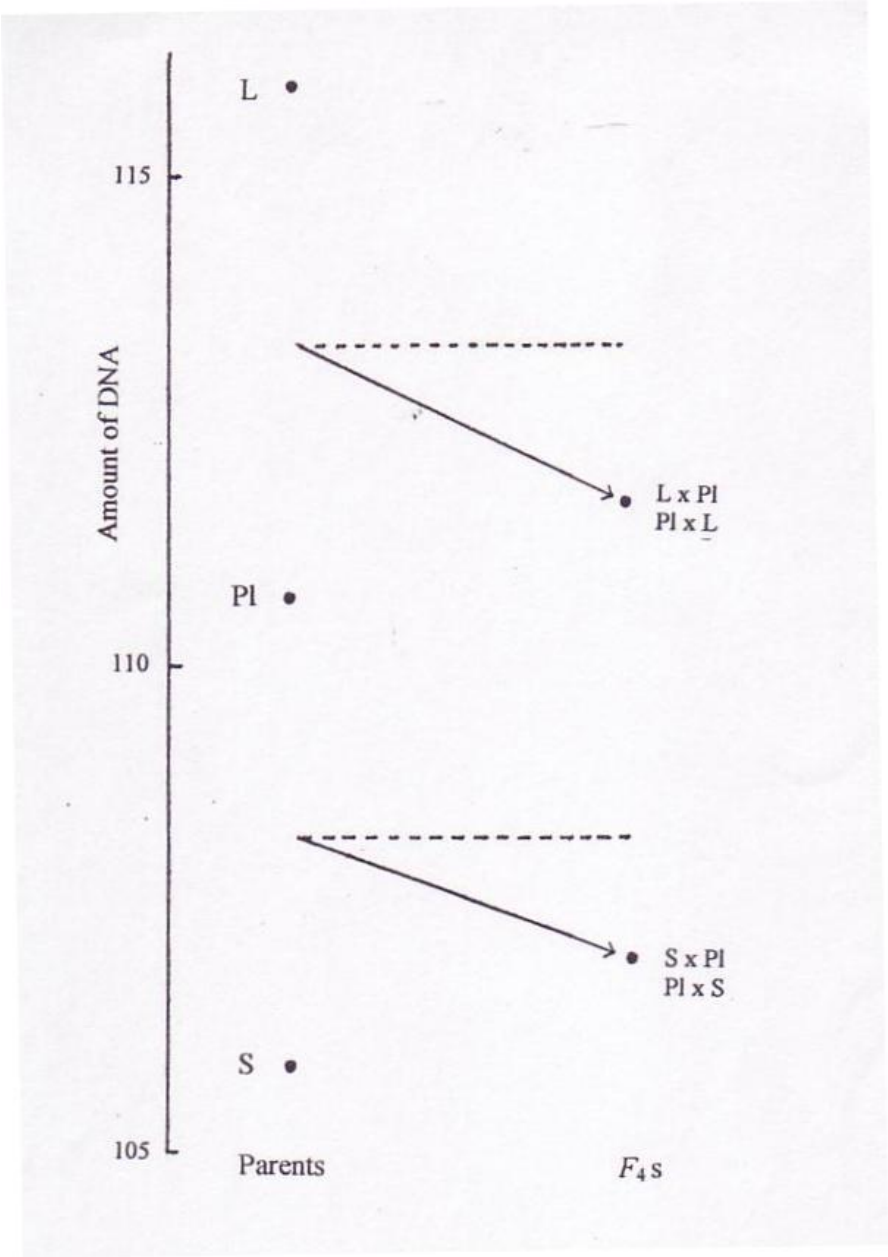
Earlier crosses had been made between L and S of Stormont Cirrus to determine whether a response could be obtained to the selection of large and small plants over several generations. At the 4th generation there was a substantial divergence in plant weight in the expected directions. By this time nuclear DNA differences among the genotrophs had been identified and Evans (1968a) took samples of the 5th generation plants and found a close positive correlation with amount of nuclear DNA. In other words, selection for high and low plant weight had also selected for larger and smaller amounts of nuclear DNA. It cannot be concluded from these close correlations that one is the cause of the other, as data given later show.

5.4 Nuclear DNA in crosses between L and Pl, and between S and Pl

Al-Sahheal (1974, AU) measured the amounts of nuclear DNA in the F_2 and F_4 generations of crosses between L and Pl, and between S and Pl. In the F_2 there was a movement in both sets of crosses towards Pl. In the F_4 this was reduced though there remained a moderate drop of DNA in both sets of crosses from the mid-parent values (Fig.5.1). This could be due to drift, interaction with the environment or changes induced by lower temperatures over the four generations. But the difference between the two sets of crosses is equal to half the parental difference, a ratio of 0.93, almost exactly 1. On this criterion (section 4.6) the parental contributions are unchanged in the crosses, there is neither conversion of the Pl contributions to L or S, nor reversion of L or S

contributions to Pl. Although the crosses had been selfed and maintained for several generations the F_4 samples still showed recognisable and distinct contributions of the original parents. A ratio of 0.94 was given by the plant weights in F_4 (section 4.6) so there is here again correlation between plant weight and amount of nuclear DNA in the plants.

Fig.5.1 Nuclear DNA amounts in parents and F_4 generation plants of crosses between L and Pl, and between S and Pl. Broken lines show mid-parent values. (Al-Saheal, 1974).



5.5 Induced changes in amount of nuclear DNA occur in the first five weeks of growth

It was thought that the induced changes in plant weight probably occur in the first five weeks of growth since plants grown from seed taken from all parts of the plant are changed. During this period the plants are reared in a moderately heated greenhouse in compost of average fertility and supplied with the inducing nutrients, after which they are placed outside and subsequently planted in the field. Whether factors actually determining the differences in plant weight are changed in this period was not clear, but at least it should be possible to check whether changes in amount of nuclear DNA occur during this period.

Seeds of Stormont Cirrus, the plastic genotroph, Pl, were sown in several 12cm pots. Some received *n*, or *p*, and were kept in the greenhouse, others received nothing and grown outside as controls. For the *n* treatment a 1 per cent solution of ammonium sulphate fertiliser was applied to saturation at sowing with subsequent free drainage and watering. The *p* treatment was similar except triple superphosphate was used and the compost had no lime added. Evans (1967) took samples each week and measured the amounts of nuclear DNA by Feulgen photometry. The slides were double-blind coded. The amounts in the *n* and *p* treated plants are shown in Fig.5.2 as percentages of the controls. In the first week the controls were too small to measure and there was no difference between the *n* and *p* treated plants. Thereafter there was a steady divergence in the expected directions and at the 5th week the amounts were virtually identical with those in L and S plants induced many generations earlier (section 5.1).

Evans grew the progeny of the *n* and *p* treated plants with those of earlier induced L and S plants and found their DNA amounts were almost identical;

L	S	*n*	*p*
143	127	143	131

Some of the first generation plants were also grown to maturity in a greenhouse where the progeny of the *n* treated plants were significantly larger than those of the *p* treated..

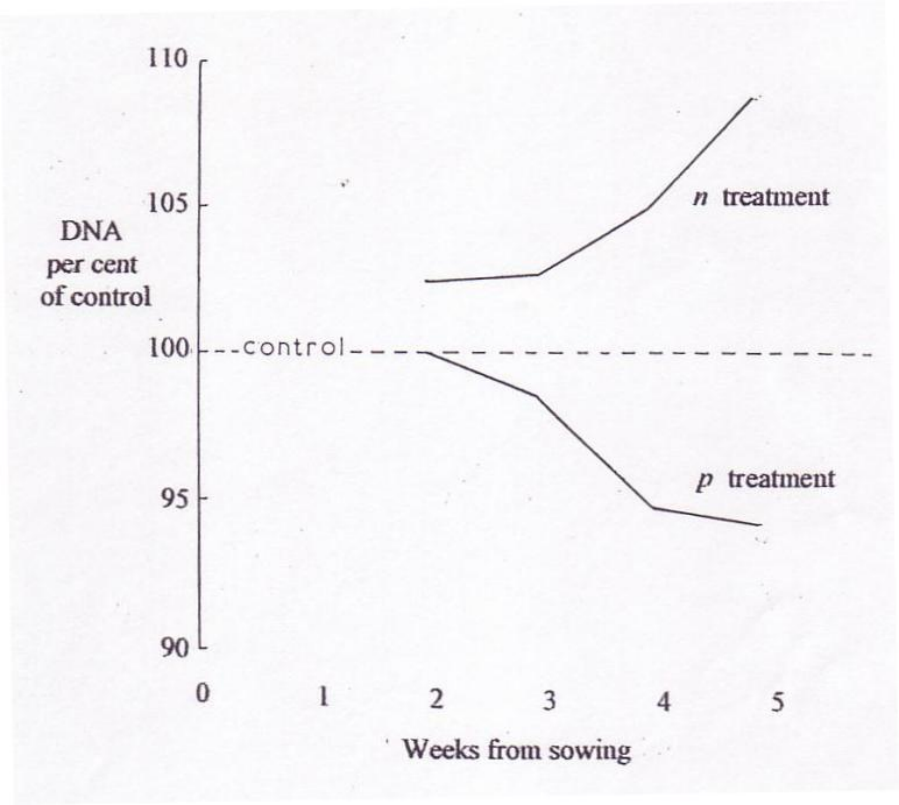
These results show that the inherited differences in DNA amount are induced by the environments since there is no possibility for selection by the *n* and *p* environments. It would not be credible that the DNA differences in the next generation were not those induced in the previous one.

The Pl genotroph has an intermediate amount of nuclear DNA between L and S (Table 5.1) which is increased by growing them in *n*, and decreased by growing them in *p*. There is no sudden change and it appears that the DNA can be of any amount from the lowest in S to the highest in L. This means that if conditions are not right, perhaps low temperature, persistent cloudy conditions,

low basic fertility and of course plants that are not plastic, the full 15 per cent difference may not be achieved. Another aspect is that measuring DNA amounts at about the end of April, i.e. at the 5th week from the beginning of the treatments at sowing, may give an early indication of an induced change and the possibility of eventual induced change in plant weight which otherwise could only be assessed by growing the plants to maturity normally in the field, collecting seed, growing their progeny and harvesting the plants in September the following year.

These data suggest that measuring DNA amount at about the 5th week from sowing is about right, but this applies under the conditions of these experiments. Using seed several years old or of other varieties, or a lower temperature, for example, may require a later sampling time. Begum (1974, AU) tested seven other flax varieties and a linseed variety, Dakota, for plasticity in DNA amount. It was already known that Liral Prince and Stormont Cirrus were plastic and the linseed variety Royal was not. Three varieties, Hollandia, Mandarin and Liral Monarch, gave a significant difference of about 8 or 9 per cent more DNA in *n* treated compared with the *p* treated plants. Rembrandt was marginal with 7 per cent. The others, Stormont Motley, Dakota, Percello and Stormont Gossomer, gave small non-significant responses of 2 per cent or less. But as Begum also points out, these values are not necessarily unique because in comparing them in batches with Stormont Cirrus they were sampled at the same time which is unlikely to be ideal for all. The values are likely to be partly measuring the rate at which the varieties develop, the rate at which changes can be induced in them and the rate at which they respond to the particular set of inducing environments. Even Stormont Cirrus in some of these experiments registered only 2 per cent DNA difference, although their progeny gave practically the full 16 per cent, showing that changes continued after the sampling time. Consequently further tests on the other varieties could yield different results. Begum also noted that changes induced in older seed were less than those in recently harvested seed probably because of their slower rate of development. She also found that DNA induction experiments can be satisfactorily done in October and November as well as in spring and summer and suggested that nuclear DNA may be better measured in side shoots than in the centre shoot since they have a further two weeks of growth before flower primordial are initiated.

Fig. 5.2 Changes in amount of DNA in the nuclei of plants of the plastic flax variety Stormont Cirrus, grown in inducing environments, occur during the first five weeks of growth (Evans, Durrant & Rees, 1966; Evans, 1968a).



5.6 Tests on nuclear DNA stability of L and S genotrophs

Inherited changes in plant weight and in amount of nuclear DNA are induced in the variety Stormont Cirrus, a plastic genotroph, Pl. No changes could be induced in the plant weights of the L and S stable genotrophs induced from Pl, but maybe the environment can induce nuclear DNA changes in L and S even though apparently it cannot induce changes in their plant weights. To find out Evans grew them in inducing environments described above together with seedlings of the plastic genotroph and made nuclear DNA measurements on their shoot apices after five weeks growth. All three genotrophs were also grown out of doors in a non-inducing environment as control plants (Evans, 1968a). Four plants of each genotroph were grown separately in pots in each environment (Table 5.5).

Table 5.5 Nuclear DNA in shoot apices of L, S and Pl plants grown in *n*, *p* and control. Totals of four replicates. (Evans, 1968a).

	n	*p*	control
L	92.59	93.07	96.65
S	83.65	82.60	83.00
Pl	92.10	81.95	89.55

The difference in nuclear DNA between L and S over all treatments is highly significant and again of the order of 16 per cent but there is no difference in L or S due to the *n* and *p* treatments. On the other hand as expected large changes are induced in Pl of about 13 percent so the initial conclusion from these data is that the environments cannot induce changes in nuclear DNA in L or S any more than they can in plant weight.

The induction in Pl is not quite symmetrical, the DNA amount being decreased more by *p* than is increased by *n* compared with the control, but environments cannot always be ideally applied, and a small change could have been induced in Pl by the control environment. S in control is virtually the same as S in *n* or *p*, whereas L in control has significantly more DNA compared with L in *n* or *p*. It appears therefore that L is not completely stable, at least as regards nuclear DNA. Evidence later shows that stable and plastic are relative rather than unvarying terms, but it is helpful to identify these basic types first.

A restricted environment can also induce change. Begum (1974) measured the DNA in L and S plants which had been grown previously for two and three generations crowded together in 13cm pots in the greenhouse (Table 5.6). Grown with controls in a general environment, Begum found the DNA in L and S had increased from 101 and 87 respectively to 108 and 99 units, a highly significant average increase of 10 per cent. Overall, after growth for three generations in the restricted environment L had about 24 per cent more DNA than S kept in a general control environment, which is a large amount (Joarder, Al-Saheal, Begum and Durrant, 1975).

Table 5.6 Data of Begum (1974) showing DNA amounts in L and S after growing them for two (G_2) and three (G_3) previous generations in a restricted environment, compared with control, all grown in a general environment (Joarder, *et al.*, 1975).

	Control	G_2	G_3
L	101	105	108
S	87	94	99

5.7 Repetitive DNA

The chromosomes of most species contain large amounts of DNA which apparently do not code for any genes and have no function. Towards the end of the 1960s it came to be generally realised that this non-functional DNA was made up of repeated DNA sequences lying end to end dispersed and interspersed along the chromosomes (Britten and Kohn, 1968). Some sequences are repeated thousands, or millions, of times, and different sequences of families of sequences may be intermixed. Since these regions of the chromosomes seem to have no detrimental effect on the growth and development of the organism they could be good candidates for the induced changes in the amount of DNA in flax. Cullis (1973, 1977) at the John Innes Institute, Norwich, categorised the DNA of L and S into highly repeated sequences, moderately repeated sequences and unique sequences and concluded there were gains and losses in both genotrophs but that L contained more of the moderately repeated sequences than S. In their studies Timmis and Ingle (1974) in the Department of Botany, Edinburgh University, concluded that DNA differences between L and S represented a wide spectrum of the flax genome in terms of composition, repetition and complexity of nucleotide sequences. Either way they compliment the results of Feulgen photometry in showing that the different environments have increased and decreased the amounts of DNA distributed over the chromosomes of L and S and that the initial changes, or some at least, might be implicated in the observed changes in plant weight.

5.8 Ribosomal DNA

Proteins in the cells of plants and animals are coded by genes on the chromosomes but manufactured in the cytoplasm on small roughly spherical bodies called ribosomes. A key component of ribosomes is ribosomal RNA (rRNA). This is produced by rRNA genes, or rDNA, and assembled in the attached nucleolus with other constituents before passing out into the cytoplasm. The rRNA genes are replicated thousands of times to supply enough rRNA for the large number of ribosomes required for the working of the cell. They vary in number from species to species, and during development. During amphibian oogenesis the number of rRNA genes increases hundreds of time to provide sufficient ribososmes for the rapid increase in proteins for the development of the egg from the primary oozyte. In some cases the number of rRNA genes is under the control of other chromosomes and their numbers are correlated with the vigour of individuals. They are a special class of repetitive DNA sequences because of their function in the working of the cell, unlike the reiterated sequences mentioned above.

The repetitive rDNA sequences are only a tiny fraction of the total DNA in the nucleus and they cannot be recognised by Feulgen photometry, but the nucleoli in which the large quantities of pre- and mature ribosomes are assembled are easily seen under the microscope. Timmis and Ingle (1973) noticed that the nucleolar dry mass was about 100 per cent greater in L than in S and suspected that this large increase of rRNA could be due to L having more ribosomal genes than S. Using standard hybridisation techniques they estimated that S had 2100 rRNA genes and L had 3500, 66 per cent more. Apparently environments inducing large plants also increase the number of rRNA cistrons in L relative to S, and by analogy with other organisms, the larger plants could be directly due to the greater vigour associated with more rRNA genes. But Jeremy Timmis later in the Botany Department, University College, Dublin, with John Ingle at Edinburgh (1975) found that Pl had about the same number as L although Pl has an intermediate weight between L and S. Cullis (1976) obtained similar results. L had 64 percent more rRNA genes than S, but he estimated Pl to have slightly more, about 12 per cent more, than L (Table 5.7).

Table 5.7 Estimates of numbers of ribosomal genes in L, S and Pl of Stormont Cirrus

	Timmis & Ingle (1973)	Timmis & Ingle (1975)	Cullis (1976)
L	3500	3287	2370
S	2100	2073	1430
Pl	-	3330	2660

Although the correlation is not complete the number of rRNA genes could be at least partly responsible for the differences in plant weight. There may be interactions with other unknown induced changes, and Pl has a higher plant weight than either L or S when all are grown in winter greenhouse conditions. Pl is also taller than L which is taller than S in winter and summer. More data on rDNA and plant weight are given later.

Like total nuclear DNA (section 5.5), changes in amount of rDNA in Pl plants can be traced from sowing. Cullis and Goldsbrough, John Innes Institute, Norwich (1981), grew Pl plants in compost without fertiliser in a controlled environment chamber and found the amount of rDNA declined in the upper one third of the plants from about the 5th week from sowing whereas in the lower third of the plants it remained relatively constant. The reduced amount appeared in the next generation. Later Cullis and Charlton, also at Norwich, (1981) grew one group of plants in Hoaglands nutrient solution and another group in Hoaglands plus a compound nitrogen, phosphorus

and potassium fertiliser With the compound fertiliser there is a rapid increase in rDNA at the 6th week; without it there is a steady decline. Buds taken from the bottom of the plants had unchanged amounts over this period. The results contrast with the Feulgen photometry measurements on total DNA by Evans (Fig. 5.2) where the changes are gradual and completed by the 5th week, suggesting perhaps that although the environment induces changes in total nuclear DNA and in rDNA they are otherwise independent events. But a direct comparison is not possible because of the different conditions and nutrients and perhaps of unknown changes in Pl in the ensuing 13 years. Even the induced changes in nuclear DNA may be retarded if the conditions are not right.

Cullis (1979a) also found substantial variation in the proportion of rDNA among L, and among S, plants which is inherited by their progeny. There was even greater variation among F_1 plants of reciprocal crosses between L and S as was the case with plant weight (Table 4.5) but there was little if any response to selection thereon, the F_2 family means regressing towards a mid value as though there were some favourable rDNA amount to which the plants adjusted. Ghogain, Byrne and Timmis (1982) in the Botany Department, University College, Dublin, also found differences in number of rRNA genes among plants of each of the genotrophs L and S, up to 45 per cent differences, but they were unable to detect any additional variation in the F_1 in the reciprocal crosses, again perhaps because of the different experimental conditions. The numbers of ribosomal genes in the reciprocal crosses averaged out to

L x L	L x S	S x L	S x S
2557	1926	2032	1477

The F_1 means are almost exactly midway between the two parental values with no significant reciprocal difference. Similar results were obtained with plant weight (Table 4.4) and nuclear DNA amount (section 5.3).

Although results described so far show there are good correlations between induced changes in plant weight, total nuclear DNA and number of ribosomal genes it will be shown later these are not absolute; that differences induced in one are not necessary the cause of differences in another, nor even that environments inducing changes in one inevitable induce changes in another. More information is in Chapter 8.

SUMMARY: CHAPTER 5

The large genotroph L induced from the flax variety Stormont Cirrus has 15 per cent more nuclear DNA than the small genotroph S as estimated by Feulgen photometry, and the untreated Stormont Cirrus variety itself has an intermediate amount. Similarly the large genotroph L from the flax variety Liral Prince has 12 per cent more than S and Liral Prince has an intermediate amount.

There were no significant differences in DNA amount between eight other, untreated, flax varieties with values scattered around those of the untreated Stormont Cirrus and Liral Prince varieties.

Reciprocal crosses between L and S had intermediate amounts of DNA. Crosses with Pl (the original Stormont Cirrus variety) gave no evidence of either reversion or conversion .

Cytological studies showed normal pairing and no aberrant chromosomes, but in crosses between artificially obtained autotetraploids there were fewer quadrivalents and more bivalents in L x S and S x L than in L x L and S x S crosses, suggesting there were structural differences between the chromosomes of L and S. There were more chromocentres in L than in S.

Changes are induced in amount of DNA in the first five weeks from sowing in plants grown in inducing environments. L and S genotrophs are not entirely stable in amount of DNA when grown in some environments.

Different distributions of DNA repetitive sequences are found in L and S, and L has about 60 per cent more ribosomal genes than S.

6 OTHER CHARACTERS

6.1 Hairy/hairless septa in the capsules

More differences found among the genotrophs, together with those already described, reveal the many changes induced by the environment. A search was made in 1964 to determine whether there were other morphological or anatomical differences between the three genotrophs of Stormont Cirrus; Pl, the original variety and large L and small S types induced from it. Nicholas (1967, AU) found Pl and S had numerous hairs on the false septa of the capsules whereas L had none (Plate 5). This character had been reported before in *Linum* (Tammes, 1928; Dillman, 1936) and has been variously referred to as pilous *v* glabrous, and ciliate *v* smooth. 3:1 ratios were recorded with hairy septa dominant to hairless. Most non-dehiscent forms of flax have hairy septa but the degree of hairiness varies. Stormont Cirrus variety, i.e. Pl, has hairy septa and conforms to the general pattern, as also does the small genotroph S. The large genotroph L does not and it was suspected that its induction by the environment was associated with a change from hairy to hairless septa. Capsules of hundreds of plants growing in the field and in store, and later experiments, agreed with this. Many experiments were done subsequently demonstrating the genetic instability of this character, here designated *H*, *h* for hairy *v* hairless septa. Thus the genotypes (genetic constitutions) and phenotypes (appearances) of the three genotrophs with respect to this character are

Pl	*HH*	*H*	(hairy)
S	*HH*	*H*	(hairy)
L	*hh*	*h*	(hairless)

A large number of crosses had been made in 1960 between the three genotrophs in all combinations with L and S parents grown in *npk* and *p*, and Pl grown in *npk*, *p* and *nil.* Examination of the capsules of about 500 F_1 plants left in store showed that L x L, S x S and Pl x Pl plants from all environments bred true. Most crosses between different genotrophs had hairy septa as expected, but some anomalous results appeared when L and S parents were grown in *npk* and Pl parents in *nil* (Table 6.1).

Table 6.1 Some anomalous results appeared in the hairy/hairless character in crosses between genotrophs grown in some environments shown in brackets (Durrant and Nicholas, 1970).

Cross	F_1 expected		F_1 obtained	
	H	*h*	*H*	*h*
L (*npk*) x S (*npk*) *hh* x *HH*	24	0	21	3
L (*npk*) x Pl (*nil*) *hh* x *HH*	38	0	9	29
Pl (*nil*) x L (*npk*) *HH* x *hh*	10	0	1	9

All the plants in this first generation (F_1) should be genetically heterozygous *Hh*, or homozygous *HH*, and have hairy capsules whereas three hairless *h* plants appeared in the L x S cross. In the crosses between L and Pl most F_1 plants are hairless. It appears that *H* changes to *h* in some of the F_1 *Hh* heterozygous plants so that instead of being *Hh* they are *hh.* Since they occur with high frequency they cannot be categorised as mutations which are rare events as originally defined, but to instability, or repression of *H* when heterozygous with *h*. The classified *H* plants in Table 6.1 should be *Hh* and give 3:1 ratios of *H*:*h* in the next (F_2) generation but there was again an excess of *h* plants.

This supports the notion that the environmental induction of the large plant L from Pl was associated with *HH* changes to *hh.* There was also instability in plant weight and height in the F_1 of L x S and S x L crosses (Tables 4.4, 4.5), and in the F_2. Among thousands of plants only very rarely was an L plant found with hairy septa, or an S or Pl plant with hairless septa.

Many cases are known of instability in plants. Harrison and Fincham (1964) demonstrated the influence of temperature on the amount of instability at the *pal* locus in *Antirrhinum major.* Mikula (1967) showed that the changes at the *r* locus in maize are influenced by day length. In flax this character, *H,h*, could be useful as a marker gene for induced changes in plant weight. The capsules of plants receiving the treatments can be checked five months from sowing, whereas to confirm changes in plant weight the plants are normally recorded at harvest of the next generation the following year, about seventeen months later.

Plate 5 Hairless capsule of *hh* plant and hairy capsule of *HH* plant.

6.2 Hair number of F_2 heterozygotes

Nicholas (1967, AU) dissected out the septa of a number of capsules from F_1 and F_2 generation plants of crosses between L and S, and between L and Pl, to determines whether the *Hh* heterozygous plants had the same number of hairs as the *HH* homozygotes. If they had the same number then *H* would be completely dominant over *h*. It became apparent this was not the case because the number of hairs per septum varied from about 30 to over 70 whereas between septa within a capsule, or between capsules on the same plant, the difference was only about 3 or 4 hairs per septum. The hair numbers of different plants also appeared to fall into distinct groups and a comparison of the F_1 and F_2 distributions suggested that the highest group with 60 or more hairs per

septum contained the homozygous *HH* plants, the remaining groups with lower hair numbers being composed of the heterozygous *Hh* plants.

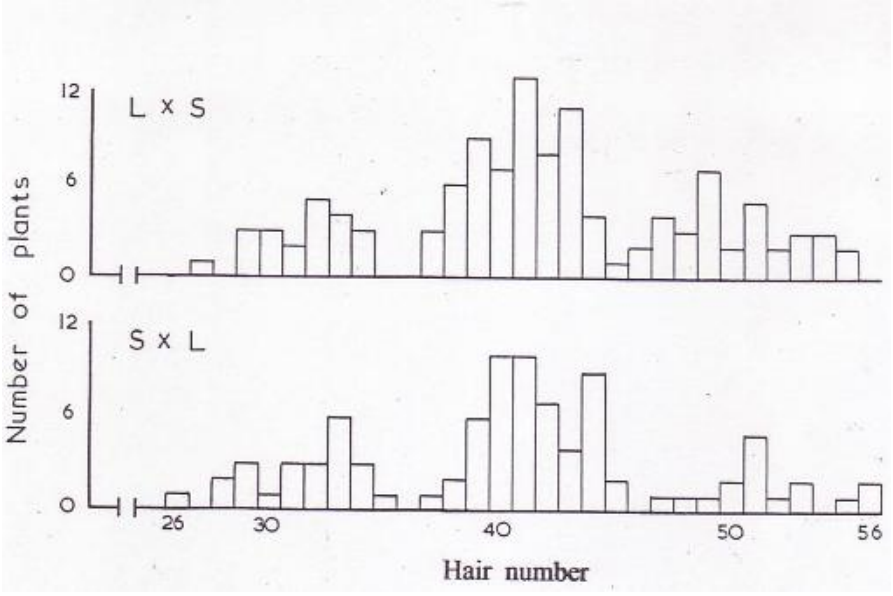
To examine the distribution in detail Nicholas scored the hair number of all F_2 plants with hairs, grown in 1965. One capsule was collected from each plant and if the hair count of one of its septa fell within the presumed *Hh* range two more septa were counted and the mean hair of the three septa taken. If the septum had more than 60 hairs it was presumed to be *HH* and no more septa were counted. The hairless plants were re-examined for absence of hairs.

The distribution of the *Hh* heterozygotes in the F_2 generation of the L x S and S x L crosses shown in Fig.6.1 is trimodal with modes at 33, 41 and 51 hairs per septum, designated $(Hh)_3$, $(Hh)_4$ and $(Hh)_5$ (originally I, II and III, Durrant and Nicholas, 1970; McLellan and Durrant, 1973). The numbers of plants in each of the three classes and in the two homozygous classes in the F_2 generation studied were

(hh)	$(Hh)_3$	$(Hh)_4$	$(Hh)_5$	(HH)
113	43	112	48	73

There were no significant reciprocal differences and there was again an excess of *hh* plants compared with *HH*.

Fig. 6.1 Distribution of hair numbers among *Hh* heterozygotes in the F_2 generation of L x S and S x L crosses grown in 1965 (Nicholas, 1967).



Nicholas grew the next generation (F_3) in 1966 to determine whether the three clearly recognisable heterozygous *Hh* F_2 classes all gave *H*:*h* ratios of 3 : 1. F_2 plants with capsules covering the range of hair numbers of each of the *Hh* classes were selected, and up to ten seeds from each capsule grown in a pot in a cool greenhouse and placed out of doors after three weeks. He repeated the experiment in 1967, growing the plants from seed to maturity out of doors using another selection of capsules. McLellan (1970, AU) repeated it again over winter 1967-1968 in a greenhouse with supplementary heating and lighting. The F_3 *H*: *h* ratios calculated from all plants in all experiments

F_2 plants	*hh*	$(Hh)_3$	$(Hh)_4$	$(Hh)_5$	*HH*
F_3 ratios	All *h*	2.2:1	3.5:1	4.3:1	All *H*

are related to the hair numbers of the three *Hh* classes in F_2. Plants from the intermediate $(Hh)_4$ F_2 class give a ratio nearest to a 3 : 1 ratio normally expected from heterozygotes such as *Hh.* Plants from class $(Hh)_3$ in F_2 with fewest hairs give F_3 families with excess hairless plants, and $(Hh)_5$ plants with most hairs give F_3 families with excess hairy plants. It is possible some misclassification could have occurred in class $(Hh)_5$ in the F_2 since this gave several F_3 families that contained all *H* plants (i.e. the F_2 plants could be *HH* with a lower hair number). M^{c}Lellan therefore grew F_4 families from *H* plants in the F_3 segregating families to check the ratio of *HH* : *Hh* in the F_3, which should be 1 : 2 but it was found to be near to 2 : 1, showing that F_2 $(Hh)_5$ heterozygotes were actually changing *Hh* → *HH*, with an estimated frequency of about 35 per cent (McLellan and Durrant, 1973). Summarising, F_2 *Hh* plants with high hair number have a higher *H* potential giving *h* → *H* changes; *Hh* with low hair number have a low *H* potential giving *H* → *h* changes.

6.3 H,h in later generations

Joarder (1973, AU) made further studies on the hairy septa character in several crosses and generations and confirmed that *HH* and *hh* homozygotes are very stable grown in a general environment, only rarely is an aberrant plant found, but once *H* and *h* are brought together in the *Hh* heterozygote, instability results. There now appeared four *Hh* classes with an extra class of about 20 hairs per septum, $(Hh)_2$. And the *HH* homozygotes showed two distinct classes with 60 and 70 or more hairs per septum, $(HH)_6$ and $(HH)_7$. Sometimes there is a suspicion of an 8th *HH* class with about 80 hairs. Backcrosses confirmed *h* → *H* and *H* → *h* changes occurred in heterozygotes with high and low hair numbers respectively. The environment in which the F_2 generation is grown influences its distribution which in turn largely determines the *H* and *h* frequencies and ratios in the

next generation (Table 6.2). Other distributions occur. In one year unknown environmental factors increased the hair numbers of the three original classes by about six hairs per septum, to 37.2, 46.5, 55.7. Sometimes all *Hh* and *HH* classes merge into a single broad distribution.

Table 6.2 Significantly different *Hh* frequencies occur in two F_2 families grown in different years though descended from the same F_1 family. (Durrant & Joarder, 1978).

	1971		1972	
	Mean hair number	Number of plants	Mean hair number	Number of plants
hh	0	86	0	99
$(Hh)_2$		0	21.5	32
$(Hh)_3$	30.5	46	30.7	34
$(Hh)_4$	41.2	102	40.7	42
$(Hh)_5$	50.5	82	49.5	40
HH	65.0	94	66.5	123

Three or four *Hh* classes have been found in all generations up to as far as have been studied in F_5 but they have progressively less effect on the ratios in the next generation. The F_2 *Hh* classes have little effect on F_4 ratios. The F_3 *Hh* classes have more effect on F_4 ratios but they are small compared with the effect of F_2 *Hh* classes on F_3 ratios. The four *Hh* classes in F_4 and F_5 have virtually the same frequencies. The numbers of plants in F_5 families from F_4 *Hh* plants (Joarder, 1973), for example

hh	*Hh*	*Hh*	*Hh*	*Hh*	*HH*
434	205	221	220	221	396

agree with a normal 1:1 ratio for *HH*:*hh*, 1:1 ratio for *Hh*:(*HH*+*hh* and a 3:1 ratio for *H*: *h.* Thus crossing *HH* and *hh* parents gives rise to unstable *Hh* heterozygotes which become stable *Hh* heterozygotes in later generations so that although the different *Hh* classes appear each generation, as far as they have been studied, they are no longer able to influence the *H*:*h* ratios of succeeding generations. Therefore *Hh* heterozygotes in earlier generations are different from those in later generations (McLellan and Durrant, 1973; Durrant and Joarder, 1978).

This can be interpreted in two ways. When *H* and *h* are first brought together in *Hh* heterozygotes their instability results in plants with different hair numbers, but also in their capacity, or potential, to follow through and initiate changes of $H \rightarrow h$ or $h \rightarrow H$. Those that have, give *HH* and *hh* plants in the next generation leaving in the pool of heterozygotes those *Hh* plants with a

lower or no capacity for initiating change. The other interpretation is that all *Hh* heterozygotes have this capacity whose influence wanes over generations.

A consequence of this fade out is that while the effect of the environments on the *Hh* class frequencies in the F_2 generation for example influence the ratios of later generations, environments of later generations do not influence generations that follow them. Crosses between L and Pl grown at the same time and in parallel with the L x S crosses gave similar results except the *Hh* hair number classes cease one generation earlier to affect the ratios of the next generation.

The mean hair number of *HH* plants in F_2 and F_3 after extraction from $(Hh)_5$ heterozygotes is enhanced by about 3 or 4 hairs per septum over the hair number of *HH* parents, and appears to be due to an increase in number of $(HH)_7$ plants. After extraction from $(Hh)_2$ plants there is no enhancement. Enhanced *HH* plants gradually revert on selfing for three generations to the original mean hair number of *HH* parents. It may be suggested that results given by studies extending over many years may be misinterpreted in that they could be due to changes in technique, facilities, seasonal variation, workers, etc. The interpretations are valid because of the use of controls, repetition over years and overlapping of experiments.

6.4 Linkage studies on *H,h* with L and S plant weights

From a practical view point inherited differences in plant size induced by the environment are more interesting than seemingly inconsequential differences in the numbers of septum hairs in the capsules. On the other hand, although L and S genotrophs are always easily distinguished because of their large difference in plant size they are still subject to environmental variation which can reduce the sensitivity of analyses specially in the derivatives of crosses and, taking a broader view, in assessing the possible occurrence of further induced changes in them. The capsule character *H,h* is hardly affected by the environment, apart from its role in induction and influence on ratios, in the sense that *HH* and *hh* plants are always clearly distinguished, and often heterozygotes as well. There is obviously some relationship between *H,h* and plant weight if only because induced changes occur in them at the same time, so their study may be mutually helpful and a priority was to determine whether they were linked, i.e. whether they were close together on the same pair of chromosomes .

The first studies on the association of *H,h* with the plant weights of L and S was made by Nicholas who scored the hairy/hairless character in about a thousand F_2 seed samples from reciprocal crosses between L and S, and between L and Pl, both of which involve crosses between *HH* and *hh.* They had been held in store from plants whose weights had already been measured in

1964 and 1965. If L and S (i.e. chromosome regions involved in the induced changes in plant weight) and *H,h* are not linked, that is are well separated on the same pair of chromosomes, or on different chromosomes, they would segregate independently in the F_1 heterozygotes whence there should be no significant difference between the weights of segregating *H* and *h* plants in the F_2 generation. If they are linked, *h* would tend to stay with L, and *H* with S (or with Pl in the other cross), so in the F_2 generation *h* plants would be expected to be larger than *H* plants, which is what Nicholas found. The difference was not large but consistent over all crosses and highly significant (Table 6.3).

Table 6.3 Weights (g) of plants with hairy (*H*) and hairless (*h*) septa in F_2 generations grown in 1964 and 1965 (Durrant and Nicholas, 1970).

	1964		1965		Mean difference
	H	*h*	*H*	*h*	*h* - *H*
L x Pl	29.7	33.7	77.4	81.1	3.1
Pl x L	33.0	37.4	72.1	72.4	
L x S	27.2	29.5	48.0	51.6	3.6
S x L	24.0	27.8	60.2	63.8	
Mean	28.5	32.1	64.4	67.5	

If *H,h* and L and S are so close on the chromosomes that they do not recombine at all (complete linkage), assuming no dominance of plant weight, the *h* plants in F_2 should be larger than the *H* plants by about two thirds of the weight difference between L and S parents. If they are close but sufficiently far apart to allow some recombination, the difference in weight between *H* and *h* plants in F_2 would be between 0 and two-thirds of the parental difference. Hence it is useful to take an association ratio of:

$$R = \frac{\text{3 x difference in weight between } H \text{ and } h \text{ plants in } F_2}{\text{2 x difference in weight between L and S parents}}$$

as a measure of linkage, which is 0 for no linkage and 1 for complete linkage. A problem here is that this would give a wrong estimate if parents and F_2 plants respond differently to the environment (e.g, Tables 3.8, 4.4).

Al-Saheal (1974, AU) grew a large number of F_2 and parent plants of reciprocal crosses between L and Pl and again found the *h* plants larger than *H* plants. But the difference in weight was 70 per cent of the parental difference, an association ratio of about $R = 1$, implying factors for plant weight and *H,h* are held closely together on the same pair of chromosomes without recombination; complete linkage.

6.5 Coupling and repulsion

Several inherited changes have been induced in flax over the years but to maintain some consistency, and to avoid introducing initially more detail unnecessarily, most of the observations so far described have been on the large L and small S genotrophs induced by *npk* and *nk* in the Stormont Cirrus variety in 1954. These are phenotypically L*h* and S*H.* Later, apparently typical L and S plants were induced with *n* and *p* fertilisers which were *HH* and *hh* respectively. So a change from *HH* to *hh* can accompany the induction of a large plant or a small plant. These new combinations, L*H* and S*h*, are described as coupling and the original ones, L*h* and S*H,* as repulsion.

Joarder (1973, AU), using Feulgen photometry, estimated L*H* to have about 9 percent more DNA than S*h*, compared with the 15 per cent increase of L*h* over S*H*. Cullis (1976, John Innes, Norwich) obtained a similar estimate, about 8 per cent and also estimated L*H* to have 65 per cent more ribosomal genes than S*h*, the same as the increase of L*h* over S*H*.

Crosses were made in 1971 between combinations of genotrophs yielding segregation of *H* and *h* in F_2 of which about a thousand plants were grown in 1973 by Al-Saheal. Crosses between L and S (Table 6.4, sets 1 and 2) gave mean plant weights similar to those described in earlier chapters (e.g, Table 4.4). L is two to three times the weight of S, and the reciprocal crosses have about mid-parent values, but coupling parents L*H* and S*h* are significantly larger than repulsion parents, L*h* and S*H* respectively. In sets 3 and 4, the results are rather different. There is heterosis in crosses between the two L genotrophs, L*h* x L*H* and L*H* x L*h*, i.e. the F_2 plants are bigger than either parent, and dominance in the S genotroph crosses, S*H* x S*h* and S*h* x S*H*, ie, the F_2 is as large as the larger parent. The induced changes in plant weight in the two phases are therefore not exactly the same. This is in contrast to L and S crosses between the two varieties Stormont Cirrus and Liral Prince

(Table 4.6) where all had mid-parent values and there were no significant differences between the varieties themselves.

The segregation of *H* and *h* gave a normal 3:1 ratio in each of the reciprocal crosses shown in Table 6.4, giving an acceptable basis for linkage estimates in crosses between L and S, which could now be made in both phases. Al-Saheal grew about 100 plants of each of the parents and F_2 generations of the relevant reciprocal crosses in 1973 and found highly significant linkage in the repulsion phase with $R = 0.61$, but less in coupling with $R = 0.37$ (Table 6.5).

Table 6.4 Plant weights (g) of four genotrophs, *Lh* and S*H* in repulsion, *LH* and *Sh* in coupling, and of F_2 plants of crosses between them (Al-Saheal, 1974).

Set 1	L*h*	S*H*	L*h* x S*H*	S*H* x L*h*
	17.8	4.7	12.5	11.4
Set 2	L*H*	S*h*	L*H* x S*h*	S*h* x L*H*
	22.2	8.8	17.7	15.7
Set 3	L*h*	L*H*	L*h* x L*H*	L*H* x L*h*
	17.8	22.2	26.1	25.5
Set 4	S*H*	S*h*	S*H* x S*h*	S*h* x S*H*
	4.7	8.8	8.4	9.0

Table 6.5 Association between *H,h* and L and S plant weights in coupling and repulsion phases. Mean plant weights (g). (Al-Saheal, 1974).

	Parents			F_2			
Repulsion	L*h*	S*H*	L*h* - S*H*	*H*	*h*	*h* - *H*	*R*
	17.8	4.7	13.1	10.3	15.7	5.4	0.61
Coupling	L*H*	S*h*	L*H* - S*h*	*H*	*h*	*H* - *h*	*R*
	22.2	8.8	13.4	17.6	14.3	3.3	0.37

A positive association in coupling and repulsion is normally regarded as good evidence for genetic linkage but its estimate here may not be valid because of instability occurring in both plant weight and *H,h* when the genotrophs are crossed, nor appropriate if as previous data suggest they are in any case closely linked. Furthermore there is good evidence of negative associations which

obviously cannot be interpreted in terms of usual genetic linkage estimates. Instead it is supposed an interaction between L and S, or *H* and *h*, or both, leads to the transfer of elements, or influence, from one of the pair of chromosomes to the other, giving an apparently looser linkage than would ordinarily be the case. This means that in Al-Saheal's data, repulsion with the highly significant value $R = 0.61$ has fewer transfers than coupling with a linkage value of $R = 0.37$. This might be achieved for example by heterochromatinisation (coiling of the chromosomes switching off included genes) disappearing from one chromosome and appearing on its homologue thereby switching $H \rightarrow h$ on one chromosome and $h \rightarrow H$ on the other. This would preserve the frequencies of *H* and *h*, and 3 : 1 ratios in the F_2 generation and at the same time slacken the linkage between plant weight and *H,h*. Or factors determining L and S could interchange. The ratio *R* can be scaled to give a measure of the frequency of transfer from:

$$f = (1 - R)/2.$$

$f = 0$ (or 0 per cent); no transfer in any of the heterozygous F_1 meiotic cells; the difference between *H* and *h* plants in F_2 is in the same direction as in the parents and linkage is apparently complete between *H, h* and plant weight; they segregate as though they were a single unit. $f = 0.5$ (or 50 per cent); transfer occurs in 50 percent of the meiotic cells; there is no significant difference between *H* and *h* plants in F_2 and there is no apparent linkage. $f = 1$ (or 100 per cent); transfer occurs in every meiotic cell; the difference between *H* and *h* in F_2 is in the opposite direction to that in the parents and linkage is apparently complete in a negative direction, which cannot be interpreted in terms of normal linkage data. Al-Saheal's transfer frequencies were 31 per cent in coupling and 19 per cent in repulsion (Table 6.6).

Cundall (1978, AU) grew and crossed parents of both phases in combinations of fertilisers in 1974. The F_1 was grown in the greenhouse in the winter and the F_2 in the field in the 1975 summer. The repulsion crosses gave positive associations with 38 per cent transfers, the coupling crosses negative associations with 60 per cent transfers, the difference being significant (Table 6.6). Transfers are nearly twice as frequent in coupling as in repulsion and therefore related to the initial arrangements of *H* and *h* with L and S on the chromosomes. Apparently L*h* and S*H* associations are preferred to L*H* and S*h,* since the transfers to give L*h* and S*H* are more frequent than the transfers giving L*H* and S*h.* The mean plant weights of heterozygous *Hh* plants in F_2 were half way between those of *HH* and *hh* as predicted on the model. Al-Saheal had similar results (Table 6.6) but with lower frequencies. The parental environments in Cundall's experiment had no significant effect but growing the F_1 generation in the winter may have been responsible for the greater frequencies in his

F_2. Cundall found no significant association between *H,h* and plant height. This series of experiments supports the belief that at least part of the difference in plant weight induced at the same time as *H,h* occurs on a pair of chromosomes close to *H,h*. Evans (1968a) found a positive correlation between amounts of nuclear DNA and plant weight in the 5th generation of crosses between L and S (section 5.3), also suggesting linkage, but there are other data in chapter 8 to be considered.

Table 6.6 Estimated frequencies of transfers between homologous chromosomes in F_1 heterozygotes at meiosis in coupling and repulsion. (a) Al-Saheal (1974); (b) Cundall (1978).

	Repulsion		Coupling	
	L *h* / S *H*		L *H* / S *h*	
	↓		↓	
	no transfer	transfer	no transfer	transfer
	L *h* / S *H*	L *H* / S *h*	L *H* / S *h*	L *h* / S *H*
(a)	81%	19%	69%	31%
(b)	62%	38%	40%	60%

6.6 Floral character, *an*

Plants of the plastic genotroph (Pl) of Stormont Cirrus are maintained each generation by growing them in pots out of doors with small amounts of nutrients assuming this maintains their plasticity, i.e. they remain capable of environmentally induced changes. These conditions are not always suitable for crossing or comparing Pl with other plants so Pl plants are sometimes grown in the field with additional nutrients, or in a general environment over generations for comparison with derivatives of crosses. In 1971 some Pl plants that had been maintained in pots were grown alongside Pl plants that had been maintained in the field. During the crossing process a difference was noted in that the stamens of the field plants were longer relative to the length of the sepals than in the pot plants. A change in floral characters could affect breeding behaviour in this normally

hundred per cent selfing variety, or species, and studies were made to determine whether the difference was due to the environment, maternal effect or induced change.

To check and quantify this observation Begum (1974, AU) grew five plants of each of two lines of the Pl plants and of two lines of the field plants, denoted Plf, in 1971. On the morning of a sunny day when the flowers were fully open she took five flowers from each plant and measured the lengths of the five stamens (filaments plus anthers) and five sepals of each flower under a dissecting microscope. The difference in length stamens – sepals was called andryx, *an*, (androecium – calyx). Table 6.7 shows that the Plf sepals are shorter, and the filaments longer, than those of Pl, differences that are highly significant. The Plf stamens protrude from the sepals whereas the Pl stamens are well inside (Plate 6).

Table 6.7 Lengths (mm) of anthers and sepals on Pl and Plf plants grown in the field in 1971 (Begum, 1974).

		Stamens	Sepals	Stamens – sepals (*an*)
Pl	Line 1	6.99	8.25	- 1.26
	Line 2	6.91	8.08	- 1.17
Plf	Line 3	7.85	7.64	0.21
	Line 4	7.63	7.52	0.11

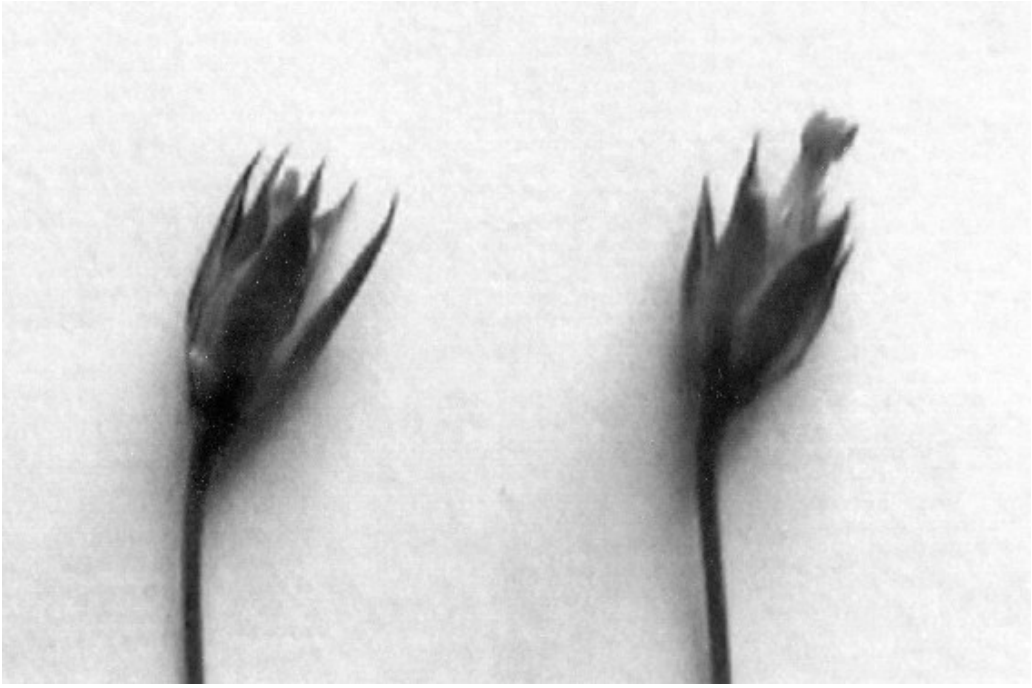
The field plants, Plf, are descended from the pot plants, Pl, and by growing plants from small samples of seed in store Begum assessed the change to Plf occurred in 1966 or 1967 when *an* dropped from about 0.8 to 0.2. Reciprocal crosses between Pl and Plf had intermediate values. The plant height of Plf was about nine centimetres shorter, and its weight 15 per cent greater, than Pl, but there was no difference in nuclear DNA amount that could be detected. Bolton (1976, AU) also found Plf was heavier and shorter and had about 9 fewer hairs per septum. Fertilisers reduced the magnitude of *an* on the plants receiving them but otherwise apparently had no inherited effect. Plants of the S genotroph were consistently of the Plf type with protruding stamens but L plants were variable, often intermediate.

There are two observations. The first is that one would hardly expect Pl plants in which large inherited changes in plant size can be induced by the environment to remain totally unchanged when maintained over generations, especially if grown in a different environment.. Some inherited variation may occur either from some intrinsic genetic instability or from general environmental

exigencies that must occur from year to year. The reduction in plant height and increased weight may be related to the induced changes in 1954 which gave rise to the L genotroph.

The second observation is that changes in the relative sizes of floral parts can have a important effect on the breeding behaviour of a species which in turn can affect the amount of genetic variation and evolutionary prospects of the species. Flax, *Linum usitatissimum*, is considered to be virtually hundred per cent inbreeding and although the change to Plf is in the direction that could promote outbreeding it is not believed in this case that this actually does occur to any marked degree, if at all, because the anthers surround the stigmata and burst just before, or when, the buds open. Even so it suggests the environment could in other situations induce changes in the breeding behaviour of a species when for example it migrates or is transferred to another country with different temperature and day length. It is generally thought that *L. usitatissimun* is derived from the outbreeding species *L. angustifolium* two or three thousand years ago. Breese (1959) at the Genetics Department, Birmingham University, demonstrated the effect of changes in floral characters by selecting for the amount the stigma projected above the anthers in an heterogeneous population of *Nicotiana rustica*, which significantly increased the percentage outcrossing from 10 to 27 per cent.

Plate 6 Anthers exposed beyond the calyx in the Plf plant, on the right, compared with the nearly enclosed anthers of the Stormont Cirrus plant, Pl.



6.7 Peroxidase enzyme

There are large differences in plant weight among the flax and linseed varieties held in the department but the most consistent and easily recognisable differences are the greater height of the flax varieties and the greater seed weight of linseed. The plastic Stormont Cirrus plant, Pl, is a tall flax plant but whereas L plant weight is greater, and the S plant weight is less, than Pl, both are shorter than Pl. From these observations neither L nor S can be categorised as flax or linseed type unless L, being taller of the two, is dubbed more flax like than S, but S itself can hardly be called a linseed type because of its smaller size and seed. Some physiological studies were made to see how the genotrophs compared one with another and among flax and linseed varieties in general.

Tyson (1959, AU) studied peroxidase acivity in the main stems of several varieties. This enzyme is implicated in the indole-acetic acid (IAA) oxidase system which is inversely correlated with growth. He found S plants had greater peroxidase activity than L. In the first generation (F_1) of the their reciprocal crosses both L x S and S x L were intermediate between L and S, showing the difference in peroxidase activity between L and S was an inherited nuclear difference induced by the environment at the same time as the induced plant weight difference, and is as likely to be an effect as a cause. An untreated linseed variety Royal assayed at the same time had greater peroxidase acitivity, like S, than an untreated flax variety Mandarin, like L.

Fieldes and Tyson (1973) in the Biology Department, McGill University, Montreal, recorded a rapid increase in peroxidase activity in the main stem of L up to about the 24th day and an even greater increase in S, both levelling off afterwards. Activity of the esterase enzyme measured at the same time was greater in L than in S without any significant change during development. Later Fieldes and Ross (1991), also at Montreal, made corrections for developmental age and measured peroxidase activity in the first and second generations of reciprocal crosses between L and S. Activity was regulated by a single gene difference, with L dominant, and correlated with plant height.

Since L had been induced by *npk* treatment and S by *nk*, Fieldes and Tyson (1972) applied both treatments to L and S, grew their progeny for two generations in a common environment and measured their peroxidase acivity. Table 6.8 shows that the new *npk* and *nk* treatments impose significant inherited differences on the earlier ones, and are cumulative, *npk* decreasing and *nk* increasing activity. These induced changes, along with those in DNA amount described in section

5.6, are additional evidence that L and S genotrophs are not completely stable. More inherited changes are described later.

Table 6.8 The S genotroph (induced by *nk* in 1954) has more peroxidase activity than the L genotroph (induced by *npk* in 1954). More *nk* and *npk* treatments were applied to L and S in a later generation (1970) which induced more changes in peroxidase activity in the same directions as before and which were inherited for at least two subsequent generations grown in a common environment (Fieldes and Tyson, 1972).

	npk in 1954	*nk* in 1954	Mean
	L	S	
npk in 1970	0.326	0.442	0.384
nk in 1970	0.416	0.495	0.455
Mean	0.372	0.468	

Wanigratne (1963, AU) made many comparative studies on the L and S genotrophs of Stormont Cirrus and Liral Prince, on two linseed and two other flax varieties, which included estimates of peroxidase, acid phosphatase and mineral contents. The overall picture showed a lot of variation between the flax varieties, between the linseed varieties and between flax and linseed, but the most consistent feature was the close similarity between the two L genotrophs, and between the two S genotrophs, of Stormont Cirrus and Liral Prince, compared with the differences between these two varieties and other flax and linseed varieties, apart from one instance given below.

L plants of both varieties have more side shoots at the base than S. In general, IAA produced at the apices of plants inhibits side shooting and it was thought might play a part in mediating the difference between L and S. Tyson (1959, AU) sprayed L and S seedlings of Stormont Cirrus with weak IAA solutions commencing at 18 days from sowing and continued for three weeks. At the same time he similarly treated the flax variety Mandarin which is tall with few side shoots and a linseed variety Royal which is short with much side branching. IAA had the opposite effect to that expected; it increased side shooting. Linseed and L had moderate increases whereas in S and the flax variety side shoots increased from about 30 per cent to 85 per cent of the total plant weight. Wanigratne (1963) applied a paste containing IAA to the apices of L, S and Pl plants of Stormont Cirrus and Liral Prince and compared the effect on side shooting with control. He obtained the same result with Stormont Cirrus as Tyson and also found that Pl was intermediate between L and S. The opposite result was obtained with Liral Prince; side shoots were reduced on all L, S and Pl plants.

Hence excess IAA gives conflicting results evidently due to the different genetic backgrounds of Stormont Cirus and Liral Prince.

6.8 Isozymes

Enzymes occur in several forms, called isoenzymes or isozymes, in different amounts, in different parts, and during development in the same individual. They are sufficiently distinct to be separated and recognised by electrophoresis whereby their movement, or mobility, R_m, in a gel is measured. They appear as distinct bands, each band corresponding to different isozyme. A number of studies have been made by various authors on isozymes of the flax genotrophs of Stormont Cirrus, with the aim of gaining more information on the induced differences. Some are mentioned below. Fieldes and Tyson (1972) distinguished four peroxidase isozymes from their relative mobilities in preparations from the main stems of L and S, which they numbered 1 to 4. The environments had induced greater inherited activities, but lower relative mobilities, in S than in L, the difference between L and S being greatest for isozyme 2. In the F_1 of L x S and S x L crosses, isozyme activities were intermediate between their parents but the relative mobilities were clearly dominant towards L, i.e. the F_1 plants had isozymes with the same mobilities as their L parent. More differences appeared during the development of the main stem; the R_m of isozyme 2 increased in L; R_m of isozyme 4 decreased in L and S. It was supposed the peroxidase locus was complex whose expression was influenced by other factors. Two esterase isozymes in L had greater relative mobility than those in S, but they showed no significant change with development and no dominance in the F_1 of crosses between L and S.

Cullis and Kolodynska at the John Innes Institute, Norwich (1975) extended the peroxidase isozyme analysis to roots and shoots of Stormont Cirrus and compared L and S with the plastic genotroph, Pl, from which they were derived. In the stem, L and S each lacked isozyme bands present in the other, whereas they were all present in Pl. In the roots, L and Pl had the same isozyme band pattern but S had additional bands not present in the other two, i.e they were *s*witched on by the *nk* (1954) treatment. There is a possibility that Pl itself could have changed over several generations of multiplication since the original induction period.

Tyson, Taylor and Fieldes (McGill, 1978) made a genetic analysis of peroxidase isozymes 1 and 2. They reciprocally crossed L and S and grew the first, second and backcross generations. The R_ms of both isozymes segregated to give clear Mendelian ratios, L possessing the dominant allele and S the recessive. Cullis (John Innes, 1979b) grew large numbers of second generation (F_2) plants

and recorded the segregation of six peroxidase isozymes in roots and shoots, each of which differed in the L and S parents. He found all gave Mendelian segregations, all had the dominant allele in L and all segregated as one, indicating that the environmentally induced change had altered the expression of a single peroxidase gene, compound gene or closely linked genes. The induced isozyme differences had been maintained through many generations and remained unchanged when brought together heterozygous in the F_1 plants, which were L type, to reappear among the F_2 plants. Cullis and Kolodynska (1975) recorded acid phosphatase isozyme differences between L and S and in later studies Cullis (1979) showed all F_1 plants were L type, but there were no convincing Mendelian segregations in the second generation, nor associations between the isozymes. The induced differences between L and S may in this case be unstable when brought together in the F_1.

SUMMARY: CHAPTER 6

The environments induced changes in a gene, *H,h,* which determines hairs on the septa in the capsules. It gives simple Mendelian ratios, has positive and negative associations with plant weight in the F_2 of crosses between L and S with which it has been obtained in coupling and repulsion. It is unstable when heterozygous, giving plants with different hair numbers and correlated ratios in the next generation. *H,h* is associated with induced plant weight changes in segregating generations and they are probably closely linked, transfers between homologous sites apparently favouring L*h* and S*H* combinations. *H,h* may be used as a marker gene indicating the probable occurrence of an induced change in plant weight.

Small changes in cultural conditions of Stormont Cirrus variety, Pl, induced a greater exposure of the stamens beyond the calyx. This characteristic was already present in S genotroph.

Many studies by various workers demonstrated inherited differences between L and S in peroxidase activity and isozymes, other enzymes and mineral content, with evidence of some Mendelian segregations, but neither L nor S could be categorised as either a flax type or linseed type.

7 OUTCROSSING

7.1 A diallel cross between L, S and linseed and flax varieties

The two genotrophs L and S induced from the flax variety Stormont Cirrus have the appearance and behaviour of two distinct genetic types, breeding true in plant weight over generations, and in crosses and grafts, apart from one feature. The variation among the plants in the first generation (F_1) of crosses between L and S is larger than the variation among the parent plants, and the variation is always greater in the F_1 of the S x L cross than in the L x S cross (Tables 4.5, 4.6), although there is no significant difference between the reciprocal F_1 mean plant weights. The variation is inherited by later generations and is regarded as a genetic instability arising on bringing together the two induced types, L and S, in the hybrid. Therefore if the origins of L and S were unknown they would be regarded as two ordinary genetic types unless note were made of the F_1 variation which might then be simply relegated to residual genetic variation, or developmental instability. Differences in a character such as the hairy septa character, *H, h*, may not be recognised and if it were it might be viewed as another example of an unstable gene. So there could be differences between varieties and cultivars which were at least partly due to environmentally induced inherited changes.

Many crosses have been made between L and S and other varieties using customary experimental designs and analyses to see, knowing the origin of L and S, whether any deviant behaviour occurs. The diallel cross is a commonly used design where a number of varieties are crossed in all ways and which boasts several forms of analysis. A summary below gives the main results of two analyses on a diallel containing L and S, two flax varieties, Percello (P) and Mandarin (M) and two linseed varieties Royal (R) and Dakota (D) grown in a general environment.

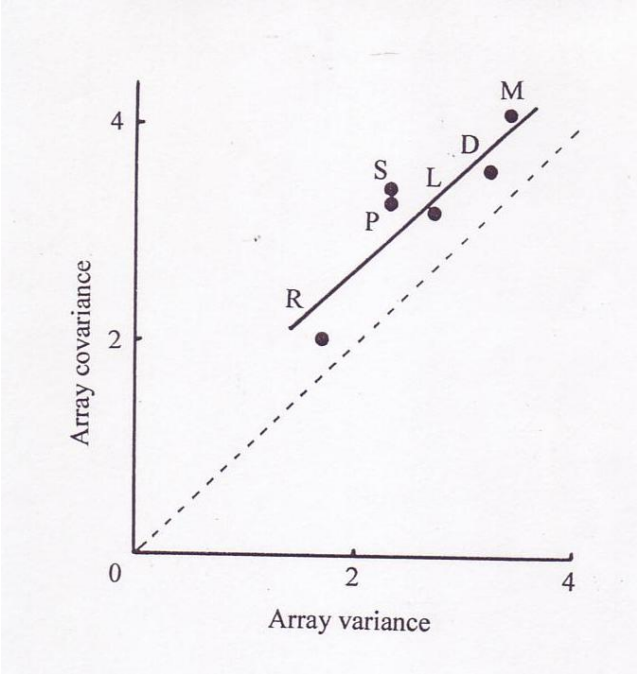
Table 7.1 F_1 mean plant weights and family variances (x 10^4) of F_2 log plant weights, in that order, of a diallel cross (Durrant and Tyson, 1964).

		Male parents						
		P	M	R	D	S	L	Total
Female parents	P	68 145	59 303	110 197	89 227	63 290	80 195	469 1357
	M	52 193	31 122	98 272	79 392	35 335	39 183	334 1497
	R	130 240	114 237	121 173	146 136	89 332	105 278	705 1396
	D	100 208	69 298	146 188	91 178	66 330	97 280	569 1482
	S	59 32	25 167	93 260	67 288	27 305	54 382	325 1725
	L	91 255	41 238	83 310	120 223	57 200	61 253	453 1479
	Total	500 1364	339 1365	651 1400	592 1444	337 1792	436 1571	2855 8936

A standard covariance/variance graph in Fig.7.1 calculated from the mean F_1 plant weights in Table 7.1 is interpreted as showing no gene interactions but the presence of dominant genes distributed among the six varieties as shown by their order along the straight line of unit slope. The large linseed variety R has most dominant genes, the small flax variety M at the other end has most recessive genes, so dominance is plainly in a plus direction. The two genotrophs, L and S, are in the middle with excess of neither dominant nor recessive genes but, noteworthy, they are together implying the environment has not induced differences in them with regard to their dominance relations with the other varieties. Basically in this respect L and S appear the same with no gene interaction and a simple mainly additive relationship between them. This was also the case in plant weight and nuclear DNA in crosses between L and S, and between these and Pl (sections 4.3, 4.4, 4.6, 5.3) when grown in a general environment, though dominance and heterotic effects occur in

other environments. From these results L and S appear to be essentially no different from the other varieties.

Fig. 7.1 Array covariance/variance (x 10^{-3}) regression of F_1 plant weights from a diallel cross of L, S and flax and linseed varieties. Regression coefficient $b = 0.94$ (Durrant and Tyson, 1964).



Where the data would be considered unusual are in the variances. Although there were no significant differences between the means of the reciprocal crosses between L and S in the F_1 or F_2, the variance of the log plant weights in the F_2, (i.e among the plants in the families of the second generation) of the S x L cross was nearly twice as large as recorded for the L x S cross, 382 compared with 200. This agrees with results obtained in earlier crosses between L and S (Table 4.5). It is reflected in crosses with the other varieties (Durrant and Tyson, 1964; Durrant, 1965)) where the amount of F_2 variation is determined mostly by whether L or S, male or female, was used in the cross. Reciprocal differences and cytoplasmic effects are commonly found in other species, plants and animals, and their occurrence in the genotrophs is no indication of their origin being different from recognised gene differences, but these studies at least identify one of their features.

7.2 Crosses of L and S with individual flax and linseed varieties.

In section 4.6, L and S were each crossed with Pl of Stormont Cirrus, from which they had been induced, to determine whether the induced changes in L and S would revert to Pl, in which case both sets of crosses would be the same as Pl in plant weight, or whether L and S instead would each induce changes in Pl to L and S respectively. In the first case a ratio of the difference between the L x Pl and S x Pl crosses, including their reciprocals, in F_1 and later generations divided by half the difference between their L and S parents would be 0 for complete reversion of L and S to Pl; 2 for complete conversion of Pl to L and S; and 1 if their contributions to the crosses were unchanged, i.e the contributions were additive. In those studies the ratio was approximately 1 for plant weight and DNA amount; there was neither reversion nor conversion.

If L and S were outcrossed to other varieties would the varieties revert the L and S induced changes so there was no difference between the L crosses and the S crosses to them in the F_1, F_2 and later generations? Or are L and S capable of inducing changes in other varieties similar to those which they themselves have undergone; in effect acting as agents in inducing changes in other varieties? Or would they simply add or subtract to the plant weights of the respective crosses? Crosses were made to several varieties but two of them have been studied in more detail.

The first of these was the linseed variety Royal (R). Reciprocal crosses L x R and R x L, denoted LR, and S x R and R x S, denoted SR, were made in 1962 and selection for high and low plant weight made over five generations. There were no significant reciprocal differences, the same R plants were used for crosses to L and S, and the five groups of plants, L, S, R, LR and SR, were treated the same with regard to culture and numbers of plants. Four high and four low selection lines were begun with the selection of the four largest and the four smallest plants from each of the 40 F_2 plants in each group. Ten F_3 plants were grown from each of the eight plants selected giving 80 plants in each group, 400 F_3 plants in all and the procedure repeated up to the 6th generation grown in 1969.

There was a gradual increase in the ratio over years suggesting a trend towards the incorporation of the induced changes into the crosses (Durrant, 1972) but the main interest here are the plants weights in the 6th generation, in Table 7.2. Selection for high plant weight in the crosses of L with Royal gives a much larger plant, more than twice the weight, than selection for high plant weight in crosses of S to Royal. Similarly, selection for low plant weight gives plants nearly twice the weight in LR than in SR crosses. Therefore L and S induced changes show no sign of reverting in the crosses but apparently continue to exist in the different genetic background, so the eventual

size of the plant is largely determined at the outset whether L or S is used for the crosses. Selection for high plant weight in SR gives about the same plant weight, 17.4, as selection for low plant weight in LR, 15.1, so the genetic difference due to the high and low selections apparently has approximately the same effect on plant weight as the genotrophic difference between L and S. There was no response to selection in L, S or R.

Table 7.2 Mean plant weights (g) in F_6 after selecting high and low plant weights for several generation in L, S, Royal (R), reciprocal crosses L x R, R x L (LR) and reciprocal crosses S x R, R x S (SR). Ratio calculated on means, (LR – SR)/½(L – S) = 2.64. Durrant, 1972).

	High	Low	Mean
L	15.4	18.0	16.7
S	4.5	4.6	4.5
R	43.4	46.6	45.0
LR	43.1	15.1	29.1
SR	17.4	8.7	13.0

A different result was given by crosses to the flax variety Liral Monarch (not Liral Prince in which inherited changes have been induced, section 3.8). Joarder (1973, AU) grew several generations from crosses with L and S in different years and obtained complete reversion in plant weight but he also found that differences in the general environment from year to year could delay reversion, though it was always progressive. Table 7.3 gives the plant weights of Liral Monarch (denoted K), L, S, the reciprocal crosses L x K, K x L (LK) and the reciprocal crosses S x K, K x S (SK) in the F_3 in 1971. LK and SK have almost the same weight giving a ratio virtually zero. This result contrasts with the apparently unchanged contributions of L and S in crosses to the original variety Stormont Cirrus (Pl). It seems Liral Monarch has genetic factors able to revert the induced changes, or some of them, which Stormont Cirrus does not have. Perhaps this is partly why induced changes are found in Stormont Cirrus. Joarder measured Stormont Cirrus, Pl, which here has an identical weight to Liral Monarch, and found its crosses (PlK) to Liral Monarch are the same as LK and SK which are the same as Pl and K.

Table 7.3 Mean plant weights (g), heights (cm) and nuclear DNA amounts (except Pl, PlK) of L, S, Pl, Liral Monarch (K), and reciprocal crosses to Liral Monarch; LK, SK and PlK. (Joarder, 1973). Ratios are (LK – SK)/½(L – S).

	Weight (F_3)	DNA (F_1)	Height (F_3)
L	70.1	110.5	82.0
S	37.4	96.0	57.8
Pl	47.4	-	87.1
K	47.4	102.3	75.0
LK	49.6	103.2	75.1
SK	48.5	102.6	68.3
PlK	49.5	-	78.2
Ratio	0.10	0.08	0.56

Joarder also selected for high and low plant weight over a couple of generations and obtained a good response, but there was no difference between LK and SK. The weights of the plants in the high selection lines were the same in LK as in SK and similarly the low selection lines were the same, the inference being that since the induced changes of L and S had reverted to Pl the response to selection occurred among the background genetic differences between the two varieties, Stormont Cirrus and Liral Monarch, although the chromosome region involved in the induced changes in plant weight cannot be ruled out. Other studies (sections 6.4, 6.5) showed that induced changes in plant weight and the hairy septa character, *H,h*, are associated on the chromosomes and when heterozygous reveal genetic instability in this region. Liral Monarch has hairless septa *hh* so Joarder could study the segregation of *H* and *h* in the crosses with S and Pl respectively which are both *HH*. He recorded the plant weights of *H* and *h* unselected plants in F_2 and F_3, as follows.

	H	*h*		*H*	*h*
SK	33.4	40.1	PlK	37.1	42.1

The differences, 20% and 13%, in the direction of linkage are highly significant, and implicates the region on the Liral Monarch chromosome homologous to the one on the Pl chromosome carrying the reverted induced changes. Bolton (1976, AU) examined Joarder's material and data for evidence of reversion in hair number. S has about 64 hairs per septum, Pl has 52, but in the crosses to Liral Monarch they have the same hair number of 48, i.e S contribution was the same as Pl, implying that S had reverted to Pl in this character as well. In addition hair number counts showed that *Hh* plants had changed to *HH* and since *h* was donated by Liral Monarch this variety must also carry unstable elements, or they become unstable, in this chromosome region, revealed when made heterozygous

with Stormont Cirrus. Bolton found hair number reverted in F_3 in several varieties depending on the time of sowing of F_2 from the end of March to the middle of May.

Joarder also measured the amount of nuclear DNA in the F_1 generation of LK, SK of Liral Monarch and in their parents (Table 7.3). Liral Monarch (K) has an amount half way between L and S and thus has the same amount as Pl as recorded in previous studies. The crosses revert to almost exactly the same amount as in K. Within one generation heterozygous with Liral Monarch the induced changes in nuclear DNA amount disappear, maintaining the correlation between DNA amount and plant weight noted in previous chapters. Plant height is only partly reverted, if at all (Table 7.3). The ratio is 0.56 for L and S, more for Pl and S, 0.68. In the F_4 generation the ratios for L and S were 0.02 for weight, 0.78 for height. This is in line with previous observations that different induced changes affect weight and height and they could be dispersed on the chromosomes.

Thus there is a big difference in plant weight according to whether L or S is used when crossing to varieties such as Royal. Using L instead of S gives a much larger plant in later generations. On the other hand it makes no difference in crosses to Liral Monarch whether L or S is used. They revert to Pl and the crosses might as well be made using the original Stormont Cirrus variety. Crosses to other varieties have been studied less and there is a question as to how much the results are affected by variation in the general environment. Averaging over F_2 data (Durrant, 1972) and F_2 and F_3 data of Bolton (1976), varieties like Royal were Hollandia 1.34, Percello 1.14, Dakota 1.31. Two varieties which may be causing reversion, like Liral Monarch, though incomplete were Mandarin 0.66 and Stormont Motley 0.48. Dakota is linseed, the others flax. Finally Patwary (1978, AU) in a large experiment with several sowings grew the F_4 of crosses to two of the varieties and confirmed Hollandia had a ratio of 1.47, Stormont Motley a ratio of 0.05. It would be useful to test the derivatives of crosses between the two types, those causing reversion and those not.

7.3 Association with a white flowered mutant.

Some mutant genes were introduced in the genotrophs by physical and chemical means to get marker genes, which segregate clearly to give Mendelian ratios allowing the chromosomes they are on to be tagged, and to check whether any plant weight mutations interact with the environmentally induced changes in plant weight. One marker gene has been described and used already; the hairy/hairless septa gene, *H,h*, (section 6.1). This segregates and is apparently associated on the

chromosomes with the environmentally induced changes in plant weight, although it itself is unstable and is changed by the environment.

A mutation giving white flowers instead of the blue flowers in Stormont Cirrus was obtained by subjecting L plants to irradiation form a radioactive Co^{60} source, and selfing the plants. Plants homozygous for this mutation, *w*, were otherwise similar in appearance to the original L plants. Thus the genotypes and phenotypes with regard to this character are

Pl	*WW*	Pl*W*	blue flower
L	*WW*	L*W*	blue flower
L	*ww*	L*w*	white flower
S	*WW*	S*W*	blue flower

L*w* does not appear to have any chromosomal abnormalities and it has the same amount of nuclear DNA as L*W*, i.e 16 per cent more than S*W*. More tests showed that L*w* and L*W* and their reciprocal crosses all had the same plant weight.

Crosses between S*W* female and L*w* male (blue flowered S and white flowered L) had normal intermediate plant weights between their two parents and, in the F_2, blue and white segregated in a normal 3:1 ratio. But there was a highly significant difference in the F_2 plant weights, blue being larger than white flowered plants, i.e in the opposite direction to linkage, and also among the segregating plants in F_3 (Durrant, 1974). Selection for large or small plants also selects for blue or white flowered plants respectively.

This suggests there could also be some relationship with the capsule character *H*,*h* which is associated in positive and negative directions with plant weight (section 6.5), apparently due to variation in the frequency of transfers of some kind between homologous chromosome regions determining both the induced changes in plant weight and *H*,*h*. The earliest data from the cross, S*WH* x L*wh*, showed the segregations of *H,h* and *W,w* were normal and no linkage between *h* and *w*, but in this experiment there was no association of *h* with plant weight although the negative association between *W*,*w* and plant weight was as strong as before.

More crosses between S*WH* and L*wh*, with both reciprocals, were made and there was again no evidence of linkage between *h* and *w* but there were significant positive associations of *h*, and significant negative associations of *w*, with plant weight. The apparent linkage, *R*, and transfer frequency, *f*, (section 6.5) of *h* and *w* in each of the three crosses, *a*, *b* and *c*, with respect to plant weight in Table 7.4 are shown in Fig. 7.2. There are big differences in the frequency of transfers

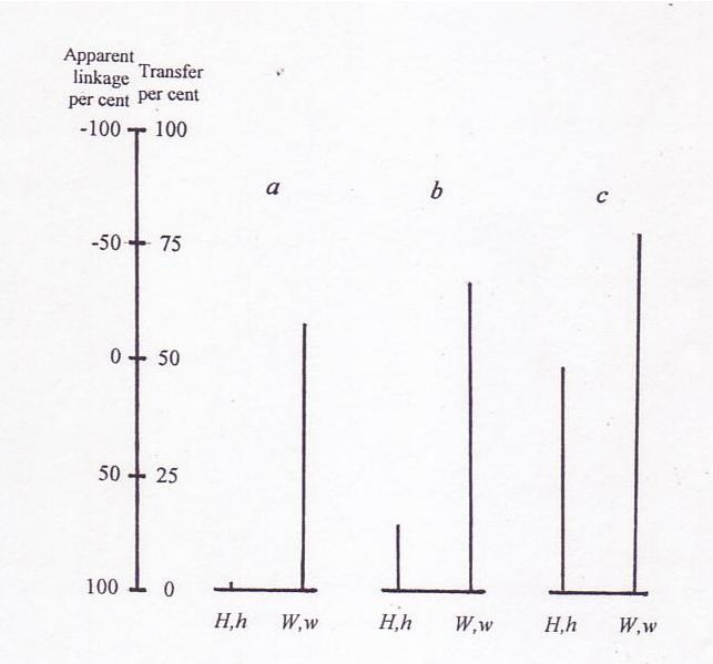
between the three crosses, and the *h* and *w* frequencies are correlated. *w* transfers are on average nearly three times more frequent than *h* transfers. The associations L*hW* and S*Hw* are favoured, as were L*h* and S*H* in a previous analysis (Table 6.6), since the lower transfer frequencies of *h* means L and *h* tend to stay together while, with higher transfer frequencies, *w* tends to associate with L instead of S. It appears that *h* and *w* could be located at opposite ends of a chromosome region bearing the induced plant weight changes, a region which, like *H*,*h*, has already shown instability on bringing the contrasting induced changes together in the heterozygote when L and S are crossed.

Table 7.4 Apparent linkages, *R*, (per cent) of *H,h* and *W,w* with induced L and S plant weights, converted to transfer frequencies, *f*, (per cent).

	R		*f*	
Cross	*H,h*	*W,w*	*H,h*	*W,w*
(a)	113	-18	0	59
(b)	69	-32	15	66
(c)	1	-58	50	79

The variety Stormont Motley also has white flowers and crosses with L*w* give all white flowered F_1 and F_2 plants, indicating the white flower mutations in Stormont Motley and L*w* are located in the same gene. Stormont Motley has hairless septa, *hh*, and is one of the varieties classified as reverting the induced changes in plant weight in L and S on crossing with them (section 7.2). Since in crosses of Stormont Motley to either L or S there would be no L/S heterozygotes and additionally the L and S induced changes may be partly or wholly reverted in their respective crosses, presumably there would be little likelihood of reciprocal transfers like those above. In effect there was no significant association, positive or negative, of either *W*,*w* or *H*,*h* with plant weight in the F_2 where reversion had already reduced the ratio (section 7.2) to 0.2. The segregations of *H*:*h* and *W*:*w* were normal and there was no association, ie, linkage, between them. Bolton (1976, AU) found small but significant associations between *H*,*h* and plant weight in crosses to several varieties and there was a significant positive association between *H*,*h* and plant weight in crosses to Liral Monarch (section 7.2) even though there was here reversion in plant weight, amount of nuclear DNA and hair number. Overall, the environmentally induced changes in plant weight appear to be at least partly located in a particular chromosome region where transfers, instabilities, reversions and associations occur.

Fig. 7.2 Transfer frequencies scaled against the apparent linkage frequencies of *H,h* and *W,w* with induced Land S plant weights.



7.4 EMS induced mutations

Begum (1974, AU) germinated L, S and Pl seed in ethyl methanesulphanate (EMS), a mutagen, to compare the kind of changes produced with those environmentally induced, and to generate more marker genes. She immersed the seeds in 0.5% and 0.75% EMS concentrations for 24 hours and in 1% for 12 hours, at 25^{o}C. These and their progeny were selfed over several generation where it was clear the treatments had a substantial disruptive effect on the genetic material. Begum found a high frequency of back mutations and instabilities suggesting the induced changes were in gene control processes or heterochromatic regions. Apart from mutants that were lethal at an early age, she scored 38 that could be characterised; 14 in L, 10 in S, 14 in Pl. Of these 22 were eventually lethal, six white flower mutants did not set seed or were otherwise lost and four more were unstable. Among the few remaining which might be useful were a procumbent form in Pl, purple and white flower types in L and pink flower, yellow anther, black seed and dwarf in S. Later studies showed pink flower, yellow anther and black seed to be either very closely linked or due to a single pleitropic gene, *d*, referred to as pink flower.

Following some initial work of Begum, Cundall (1978, AU) included the *D,d* gene in crosses between L and S which were therefore L*hD* x S*Hd* and its reciprocal S*Hd* x L*hD.* Applying the customary genetic analysis on the F_2 segregations he found *d* was linked to *h* with a recombination frequency of about 30 per cent, i.e. *h* and *d* are fairly close on the same chromosome. A backcross of the F_1 to *hhdd* plants obtained from an F_2 field experiment of the same cross gave a linkage value of the same order. Next he crossed S with the pink flower gene to L with white flower gene described in the previous section so the crosses were L*hwD* x S*HWd* and its reciprocal, giving three segregating genes. The *h/d* linkage was about 30 per cent as before. There was no evidence of linkage between *h* and *w* but there was a marginal non-significant *w/d* linkage value of 40 per cent. Additional support for the *w/d* linkage is that *h* and *w* have both been associated with induced plant weight differences, and *h* is linked to *d*. If this be accepted then the order of the three genes on the chromosome would be *h - d - w* with the environmentally induced plant weight changes also in this region. Cundall found some weak, mainly insignificant associations of *h*, *d* or *w* with plant weight and also with plant height which could be due to the introduction of the two genes or a reflection of plant weight differences in rather poor, drought conditions, at the time.

Cundall also noted that all three genes were unstable with $H \rightarrow h$ $d \rightarrow D$ and $W \rightarrow w$ changes, thus tending towards the configuration of the L parent used *hDw*. In some cases $d \rightarrow D$ changes were accompanied by $W \rightarrow w$ changes suggesting heterochromatic adjustment along the chromosome. Ene-Obong (1979, AU), 1986) also found instability of these genes, significant *h/d* linkage of about 30 per cent and similar associated changes in *D,d* and *W,w.* These results further implicate an unstable chromosome region bearing both environmentally and EMS induced changes.

Begum found many EMS induced mutations affecting plant weight primarily in the L and Pl treated plants although a dwarf type appeared in S. To separate the weight changes, Begum selected high and low plant weights and heights for a couple of generations and grew 25 families of five plants each from L, S and Pl EMS treated and untreated lines, all randomised in the field. Dwarf S was excluded. EMS had little effect on S genotroph but induced similar substantial increases and decreases, about 25 per cent or more, in the plant weights of Pl and L, although the mutations are unlikely to be the same in both (Table 7.5). More studies would be required to see if they have any association with factors involved in the environmentally induced weight changes. Some plant weight changes were unstable, even the dwarf type on crossing. Rahman, (1982, AU) grew a thousand plants of later generations of selfings from crosses between dwarf plants and L and S and found only 11 plants that could possibly be classified as dwarf, and these may not have been true dwarfs. In fact

instability appears to be a characteristic of EMS induced changes in these plants. On the other hand, unlike environmentally induced changes in DNA amount accompanying plant weight changes, there were no DNA changes accompanying the EMS induced plant weight changes. Begum measured the amounts in 54 plants using Feulgen photometry and found no significant differences between the largest and smallest plants taken from the selection lines.

Table 7.5 Mean weights (g) of L, S and Pl plants and of high and low plant weight selections following EMS treatments. (Begum, 1974).

	Untreated	High selection	Low selection
L	20.3	25.5	13.9
S	9.9	9.0	7.4
Pl	16.2	20.3	11.4

SUMMARY: CHAPTER 7

L and S genotrophs appeared to behave no differently from other flax and linseed varieties to which they were crossed, apart from reciprocal differences in F_2 variances which probably would be considered of little significance. Hence customary genetic studies on other varieties are unlikely to reveal the presence of environmentally induced change.

When L and S are crossed to some varieties like Liral Monarch there is eventually no difference between the two crosses, ie, the variety reverts the induced plant weight changes in L and S to an intermediate value. This variety also reverted the induced DNA changes. In crosses to other varieties there is no sign of reversion. In Royal for example after several generations, plant weight is largely determined by whether L or S was used in crossing to Royal in the first instance.

A white flower mutant, *w*, obtained in the L genotroph by radiation segregated normally but it was associated in a negative direction with induced plant weight. There was no evidence of direct linkage with *h*, the capsule character, but *h* and *w* were correlated in their transfer frequencies with respect to plant weight.

Many EMS induced mutations reverted later or were unstable. One mutant, *d*, giving pink flowers instead of blue in L was linked to *h*, and possibly loosely linked to *w*. All three were unstable suggesting they and factors for the induced plant weight differences were located together in a particular chromosome region. Some mutants increased or decreased plant weights in L and Pl, but had no effect on DNA amount.

8 GENETIC FACTORS

8.1 Crosses between varieties Stormont Cirrus and Royal

Large inherited changes in plant weight and amount of nuclear DNA occur in the plastic flax variety Stormont Cirrus when grown in different environments. As far as is known the linseed variety Royal is not plastic since no inherited changes have been induced in it, at least in the environments in which it has been grown. It may be non-plastic because it has lost its plasticity in environments of previous generations, as have some of the Stormont Cirrus progeny, or because of its genetic constitution, i.e. its genotype, in which case how does Stormont Cirrus differ genetically from it? The following experiments were aimed at finding evidence for genetic factors determining plasiticity.

Instead of using the character plant weight which is measured in the following generation at least 18 months after the inducing treatments are applied, the amount of nuclear DNA at the fifth week was assayed in the treated plants themselves. This also avoids unwanted genetic variation in the next generation due to segregation and recombination, at least in the first instance. Two sub-lines of Stormont Cirrus (Pl) and of Royal (R) were maintained and crossings made between sub-lines and between Pl and R in 1964 followed by backcrosses and more crosses in subsequent years. The environments for testing plasticity were the same as used earlier consisting of 1 per cent solutions of *n* or *p* applied to plants in 12 cm pots in the greenhouse in April, *n* to increase and *p* to decrease the amounts of nuclear DNA. Pl and R plants were monitored over the years to confirm they had not changed and seeds from the same capsules allocated to the *n* and *p* treatments. DNA measurements in the first sequence of experiments were made by Timmis (1971, AU).

Pl and R plants, and F_1 plants of Pl x R and R x Pl crosses were tested for plasticity in 1968 on three plants of each of the eight genotype and treatment combinations. (Pl x R indicates Pl used as female parent and R pollen placed on Pl stigma; R x Pl indicates R used as female parent and Pl pollen placed on R stigma). As expected the treatments induced a difference in DNA amounts in Pl but not in R (Table 8.1) but there is a marked difference between the reciprocals where there is a highly significant difference of 13.1 per cent between *n* and *p* treated plants in Pl x R but not in R x Pl. The data divide into two groups, Pl and Pl x R in one group and R x Pl and R in the other the difference being highly significant. There is either a cytoplasmic factor contributing to plasticity or there are strong maternal effects. The full 16 per cent difference is not obtained in Pl probably because the duration of the treatments was too short.

Table 8.1 Amounts of nuclear DNA in plants of Stormont Cirrus (Pl) and Royal (R) and in the F_1 of their reciprocal crosses, grown in *n* or *p* inducing environments. DNA values are given in arbitrary units as before. (Durrant and Timmis, 1973).

	n	*p*	*n* – p	Percentage difference
Pl	61.0	56.2	4.8	8.5
Pl x R	65. 5	57.9	7.6	13.1
R x Pl	56.3	58.8	-2.5	-4.4
R	57.9	58.7	-0.8	-1.4

The amounts of nuclear DNA were measured the following year in the progeny of the 24 parent and F_1 plants. The object was to check the results obtained from the treated plants by measuring the inherited effects and to determine whether they were inherited. All the progeny were grown alike in a general environment, in normal compost with John Innes base fertiliser without *n* or *p* applications. The inherited percentage DNA increments were:

Pl	Pl x R	R x Pl	R
7.5	5.5	-1.1	-0.7

There is again a highly significant difference between the same two groups.

8.2 Fourth backcrosses

Reciprocal F_1´s from crossings made in 1964 were backcrossed to both parents in 1965, and the backcrossing continued to give the fourth backcrosses in 1968. In 1969 tests were made on the plasticity of the fourth backcrosses, Pl and R parent plants and F_1´s from more crosses between them made in 1968, using *n* and *p* specific inducing environments as before (Durrant and Timmis, 1973). The fourth backcross to Pl females, denoted Pl^5 x R, obtained by repeatedly pollinating Pl plants with plants of each generation, has a nucleus that is 97 per cent Pl. Applying Pl pollen to plants of each generation gives fourth backcross plants, R x Pl^5, also with 97 per cent Pl nuclei, but the difference is that any independent replicating particles in the Pl cytoplasm is preserved in Pl^5 x R but not in R x Pl^5. In backcrosses to R, in the same manner, both have nuclei that are 97 per cent R but any independent replicating particles in R cytoplasm are preserved in R^5 x Pl, but not in Pl x R^5.

Table 8.2 Amounts of nuclear DNA in Pl, R, reciprocal F_1 and fourth backcross plants grown in *n* and *p* inducing environments.

	n	*p*	*n* – *p*	Percentage difference
Pl	99.1	88.8	10.3	11.6
Pl x R	98.0	87.9	10.1	11.5
Pl^5 x R	96.9	85.1	11.8	13.9
Pl x R^5	94.9	93.3	1.6	1.7
R x Pl^5	92.0	90.8	1.2	1.3
R^5 x Pl	94.0	94.1	-0.1	-0.1
R x Pl	90.6	90.2	0.4	0.4
R	90.3	90.3	0	0

As before, the data in Table 8.2 divide sharply into two groups. In the first are the plastic types, Pl and Pl x R as expected and Pl^5 x R also expected since its cytoplasm is Pl and its nucleus is essentially Pl. In the non-plastic group are R, and R x Pl. But R x Pl^5 is also non-plastic although its nucleus is essentially Pl. And Pl x R^5 is non-plastic although the Pl cytoplasm line has been maintained over the backcross generations. Therefore the Pl nucleus and the Pl cytoplasm must be maintained together for plasticity.

Pl x R is plastic, but Pl x R^5 is not and the change from one form to the other occurs in the intervening backcross generations. The first backcross Pl x R^2 was tested with other types for comparison in a larger experiment with improved environments to determine whether the fall off in plasticity could be detected at this point. The percentages were

Pl	Pl^2 x R	Pl x R^2	R
18.1	19.1	15.0	2.5

There is a significant drop to 15 from 18 or 19 per cent. Evidently the change from plastic to non-plastic is gradual over the backcross generations. One interpretation is that the Pl cytoplasmic factor is self-replicating and maintained independently of the nucleus throughout the backcross generations to R male parents but it cannot confer plasticity in the absence of Pl nuclear factors. Another interpretation is that the Pl cytoplasmic factor is self-replicating but not autonomous, requiring the presence of Pl nuclear factors to multiply.

In addition to these cytoplasmic and nuclear factors which must be present for changes in amount of nuclear DNA to be induced by the environments there exist other chromosome regions in which the induced changes themselves occur. Pl plants must contain loci that are receptive to induced changes, but R plants must posses loci which, when R is crossed with Pl, are equally

receptive because the induced changes in DNA are of the same magnitude in Pl x R as in Pl, unless Pl compensates.. The average percentage differences here for Pl and Pl x R are 11.4 and 12.3. Royal was one of the varieties which, when crossed with L and S, showed diversion in plant weight apparently in response to the induced plant weight changes in L and S (section 7.2).

8.3 Crosses and backcrosses

The results of the crosses and backcrosses in Table 8.2 show that the plants are plastic if the maternal parent is Pl, non-plastic if it is R, apart from the Pl x R^5 noted above. More crosses were made in subsequent years between Pl, R and combinations of the backcrosses listed in Table 8.3 and nuclear DNA estimates made by Al-Saheal (1974, AU). The first four items are controls and confirm previous results. The third and fourth items are in each case another backcross generation where, for example, (R x Pl^5) x Pl is (R x $Pl^{6)}$ and therefore are non-plastic as was the 5th backcross before.

Table 8.3 Amounts of nuclear DNA in crosses and backcrosses grown in *n* and *p* inducing environments. Phenotypes of the plants crossed.

	n	*p*	*n* – *p*	Percentage difference	Phenotype
Pl	104.4	90.2	14. 2	15.7	Pl
R	99.3	100.1	0.8	-0.8	R
(R x Pl^5) x Pl	100.0	99.4	0.6	0.6	Pl x Pl
(Pl x R^5) x R	99.8	99.8	0	0	R x R
Pl x (Pl x R^5)	106.2	92.7	13.5	14.6	Pl x R
Pl x (R x Pl^5)	108.0	93.8	14.2	15.2	Pl x Pl
(Pl x R^5) x Pl	106.8	93.1	13.7	14.7	R x Pl

The fifth item re-introduces the Pl cytoplasm and is now essentially Pl x R and therefore is plastic as expected. Similarly the Pl cytoplasm is re-introduced in the sixth item which is now essentially Pl x Pl. The last item is plastic either because it has regained a 50 per cent Pl nucleus and/or it has enabled the Pl cytoplasmic factor to operate again.

The phenotypes (appearances) of the plants crossed are shown in Table 8.3. Some are deceptive with regard to their plasticity. The experiments began with the plastic flax variety,

Stormont Cirrus, Pl, and the non-plastic linseed variety, Royal, R. Now in one case, male and female plants which are both clearly Stormont Cirrus type, when crossed,

$$(R \times Pl^{5}) \times Pl,$$

produce non-plastic offspring. In another case,

$$(Pl \times R^{5)} \times Pl,$$

the offspring are plastic even though a Royal type plant is used as the female parent. But in another cross, not included in the above, of these same phenotypes, i.e. R x Pl, when the male parent was (R x Pl^{5}),

$$(Pl \times R^{5}) \times (R \times Pl^{5})$$

the offspring were non-plastic. So when Pl is maintained in R cytoplasm it appears to loose its potential for plasticity. Hence the capacity for plasticity of nuclear DNA amount cannot always be predicted from the phenotypes of the plants themselves. Stormont Cirrus plants can be obtained which are no longer plastic, and some plasticity can be introduced into linseed variety Royal plants.

SUMMARY: CHAPTER 8

Environmentally induced changes in DNA amount can be induced in the flax variety Stormont Cirrus (Pl), but not in the linseed variety Royal (R). In the F_1, only the Pl x R reciprocal cross is plastic so a cytoplasmic factor in Stormont Cirrus is required for plasticity.

Backcrossing for four generations to both parents shows that nuclear factors in Stormont Cirrus must also be present for plasticity. Plants which are apparently Stormont Cirrus type can be produced which are not plastic, and Royal type plants produced that have acquired some plasticity.

9 REVERSION

9.1 The effects of growing plants outside for the first five weeks for three generations.

Earlier chapters have described the induction and characteristics principally of three types of plants, the large stable genotroph, L, the small stable genotroph, S, both induced from the plastic genotroph, Pl, the original variety Stormont Cirrus, by growing Pl plants in different environments. With the aim of giving a coherent account the emphasis has been on the stability of L and S and the plasticity of Pl but as described in this and the following chapter these are not necessarily permanent attributes. It was shown in sections 5.6 and 6.7 that some changes in the amount of nuclear DNA and peroxidase activity in L and S could occur when they were grown in specified environments. Chapter 10 describes some loss of plasticity in Pl. Attempts were also made over the years to change L and S back to the original Stormont Cirrus variety, Pl, and to find out to what extent the induced changes in the different characters are associated. Do they result from a single initial genetic change or from several changes occurring together at the same time in each of the environments?

L and S are maintained each generation year after year in a general environment in which plants are reared in a greenhouse in pots or trays for about the first five or six weeks then placed outside where they remain, or transplanted into the field. This is referred to as the T_1 environment. Another environment used in the following experiments was the same except the pots were placed outside immediately the seed was sown so that at no time did the plants receive the benefit of greenhouse temperatures. This is environment T_2.

In the first experiment, replicated lines of L and S plants were grown in T_1 and T_2 in 1965 and again in 1966. Their progeny were grown in the field in the general environment T_1 in 1967, forty plants of each of the four combinations of L and S with T_1 and T_2. Ten plants of each of the four combinations were sampled for DNA estimations by Jones (1967, AU). The difference in DNA amount between L and S dropped from about 15 to 5 per cent in the progeny of plants grown for two generations in T_2 but the difference in plant weight held constant with L about two and a half times the weight of S (Table 9.1). The decrease is not due to the DNA of either genotroph increasing or decreasing, but to both converging towards an intermediate value, towards the same amount as in Pl, the original variety, Stormont Cirrus.

Table 9.1 Plant weights (g) and nuclear DNA amounts (arbitrary units) in 1967 of the progeny of L and S plants grown in all previous generations in a greenhouse for the first five weeks, (i.e. in a general environment), T_1, and the progeny of plants grown outside for two previous generations in 1965 and 1966, T_2. After two generations outside the difference in DNA amount between L and S has dropped from about 15 per cent to 5 per cent, but the difference in plant weight has not changed significantly. (Durrant and Jones, 1971).

		DNA		Plant weight	
			Difference		Difference
T_1	L	48.2	14.8 %	61	154 %
T_1	S	42.0		24	
T_2	L	45.8	5.3 %	65	171 %
T_2	S	43.8		24	

This is one experiment lasting only three years and though there were two ancestral lines and several plants in each line in each combination the results are not independent in the sense that the same ancestral lines were carried through over the years. Hence a larger experiment was begun according to a pattern allowing independent comparisons within and between generations, where for example plants in their second generation in T_2 were descended from a different lineage of plants from those in the first generation in T_2 and plants in their third generation T_2 from a different lineage from those in the second generation T_2. The amounts of DNA were measured by Joarder (1973, AU) in one set of plants beginning with the first generation in T_2 in 1968 and another set beginning in 1971. At the same time he measured the DNA of the 1968 set which had been transferred to T_1 for one generation after the three generations in T_2 to check if the changes brought about by T_2 would be maintained when the plants were returned to the T_1 general environment (Joarder, Al-Saheal, Begum and Durrant, 1975).

Fig.9.1 confirms the previous results and shows that taking the plants one more generation in T_2, to give a total of three generations in T_2, reverts L and S totally to a common DNA value, half way between L and S, which is the same amount as in Pl (section 5.1, Table 5.1), from which L and S were induced. The difference of 16 per cent nuclear DNA between L and S, which had been maintained throughout the ten or more generations, and years, grown in a general environment with the first five weeks each year in a greenhouse, consistently reverts when grown outside from

sowing. Furthermore the reversion can be stopped at any point by growing the plants in the greenhouse again so that plants containing any DNA amount within the proscribed limits can be maintained indefinitely over generations. There is no reversion in plant weight. L plants and S plants containing the reverted amounts of DNA look exactly the same as the original L and S plants. Hence it is necessary here to distinguish those that have been outside for three generations as L_3 and S_3, and the original genotrophs as L_0 and S_0.

Evans (1968, AU) showed that the difference in nuclear DNA between L_0 and S_0 was associated with a parallel difference in nuclear dry mass (section 5.1). Timmis (1971, AU) made nuclear dry mass and protein measurements on L_0, S_0, L_3, and S_3 and found they reverted in parallel with the nuclear DNA. Therefore none of these environmentally induced nuclear changes are responsible for the environmentally induced changes in plant weight, unless small undetected differences remain, or they initially triggered other changes.

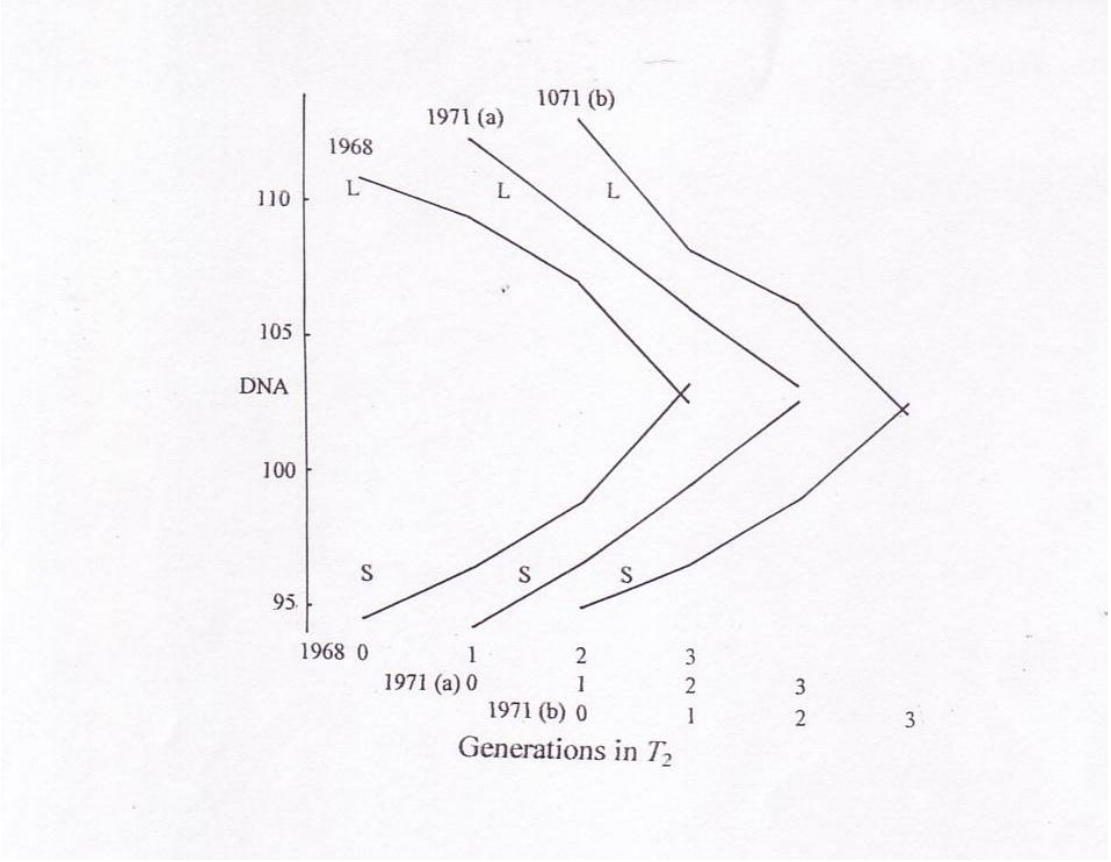
Table 9.2 Nuclear DNA amounts (arbitrary units), ribosomal gene numbers, plant weights (g) and plant heights (cm), of original genotrophs, L_0 and S_0, after three generations grown outside a greenhouse, L_3 and S_3, and after six generations outside, L_6 and S_6. Percentage increases of L over S. (a) Joarder, 1973, AU, (b) Timmis and Ingle, 1975. (c) Cullis, 1976. (d) Ghogain *et al*., 1982.

	DNA	Ribosomal genes			Plant weight	Plant height
	(a)	(b)	(c)	(d)	(d)	(d)
L_0	112	3287	2370	2118	169	24.0
	19%	58%	65%	60%	76%	21%
S_0	94	2073	1430	1320	96	19.8
L_3	103	3064	2440	2304	170	23.8
	0%	35%	53%		70%	16%
S_3	103	2269	1600	(900)	100	20.5

The other important nuclear difference between L and S, noted in section 5.8, was in the number of ribosomal genes, L having about 60 per cent more than S. There was a suspicion that this difference, which would not be detected by Feulgen photometry, could be responsible for the differences in plant weight, in which case one would expect the difference in number between L_3 and S_3 to be no different from that between L_0 and S_0, i.e. about 60 per cent, since there had been no

reversion of plant weight. Estimates from three laboratories (Table 9.2), by Timmis and Ingle, Botany Department, Edinburgh University; Cullis, John Innes Institution; and Ghogain, Byrne and Timmis, Botany Department University College, Dublin, are variable, and there is one exceptionally low value (bracketed). At least after three generations in T_2, a substantial difference in number of ribosome genes between L and S remains, together with the difference in plant weight. Only the difference in nuclear DNA amounts, and associated nuclear dry mass and protein content, between L and S have reverted.

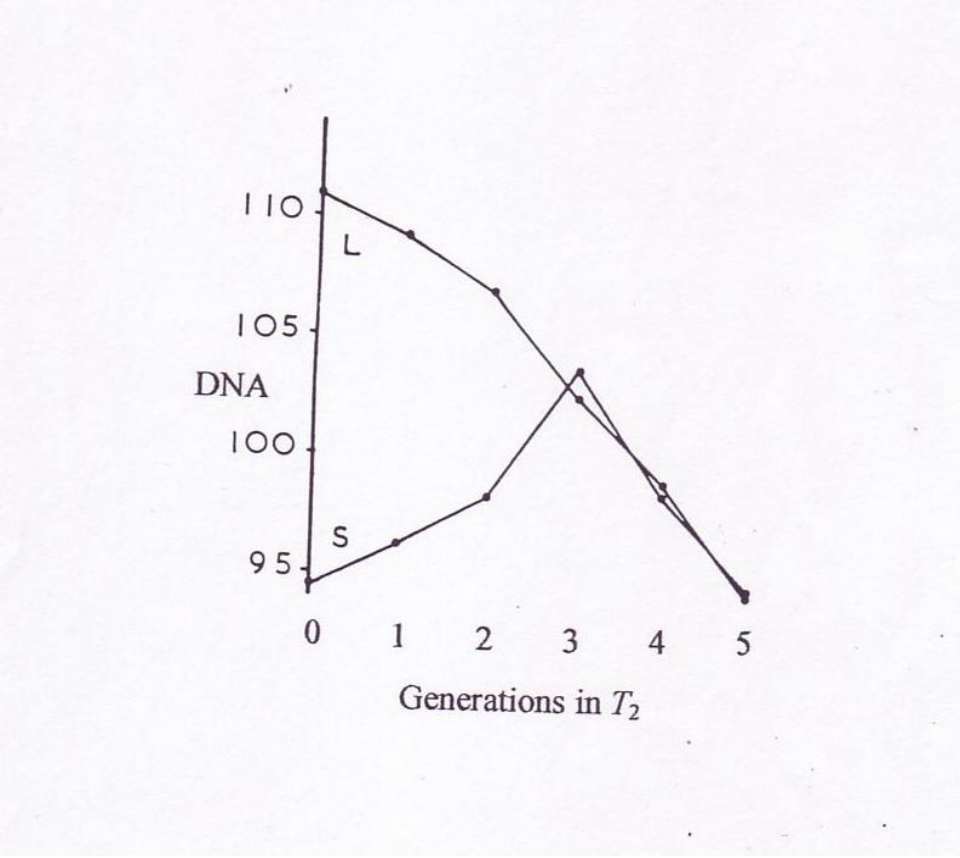
Fig.9.1. Amounts of DNA in L and S genotrophs grown outside from sowing to maturity (T_2 environment) for 0, 1, 2, 3 generations. The graphs are displaced for clarity. 1971 (a) is a repeat experiment of 1968 with different lines. In 1971 (b) all 1968 lines were returned to a common general environment for one further generation before analysis. (Joarder *et al*., 1975.).



9.2 The effects of growing the plants outside for six generations

After growing the plants outside for three generations the induced changes in nuclear DNA amount in L and S revert to the same amount as in the original Stormont Cirrus variety, Pl. This has no effect on the associated induced difference in plant weight but apparently has a variable effect on the number of ribosomal genes. One might expect that once having reverted, L_3 and S_3 would remain in this reverted state if grown in the same outdoor conditions for more generations. This may occur initially but the main response is a gradual reduction in DNA amount in both L and S. This is shown by Joarder`s (1973, AU) measurements of the progeny of plants grown outside for one to five generations, in Fig. 9.2. Typically the DNA amounts are almost identical in L and S after three generations outside, yet up to this point the induced plant weight difference between them remains.

Fig. 9.2 Amounts of DNA in L and S genotrophs grown outside from sowing to maturity (T_2 environment) for 0 to 5 generations.



At the sixth generation outside there was a change (Table 9.2). The nuclear DNA in L_6 and S_6 now practically the same is additionally accompanied by a drop in plant weight, plant height and number of ribosomal genes in L_6 to about the same values as in S_6. Furthermore hairless septa, *hh*, in the capsules of L_0 change in L_6 to hairy septa, *HH*, a character only shown previously by S in this pair of genotrophs. In fact it appears that L has changed to S in plant weight, height, DNA amount, number of ribosomal genes and in the *H,h* character. The amount of nuclear DNA in S increased at first up to the third generation in T_2 then declined in parallel with L.

Overall there is a good correlation between ribosomal gene number and plant weight and plant height, and no correlation with nuclear DNA amount. Ghogain, Byrne and Timmis (1982) reported a significant association between ribosomal gene number and root growth rate, plant weight and total shoot length. But they also say they found that rRNA gene number changes and associated changes in the transcription of pre-rRNA appear to be relatively unimportant in regulating the accumulation of rRNA. More important were post transcriptional controls permitting greater accumulation of rRNA in L. In other words it seems that the induced changes in plant weight should be referred back to other factors in the genome.

More recently other lines of L and S have been maintained outside which show no sign of L changing to S though nuclear estimations have not been made on them. Furthermore, as described in the next chapter, Pl has always been kept out of doors, from sowing to seed set, for 50 generations and has shown no outward sign of large changes in plant weight under these conditions.

Joarder (1973, AU) crossed reciprocally L_0, S_0, L_6 and S_6 in all combinations and measured their amounts of nuclear DNA in the first generation (F_1). Crosses between L_0 and S_0 gave DNA amounts the same as their mid-parent value, i.e. the contributions of the parents were additive as expected from previous estimates (section 5.3). L_6, S_0 and S_6 have the same plant weights, the same nuclear DNA amounts and the same number of ribosomal genes. On these criteria S_6 is no different from S_0 despite an increase and decrease in DNA over recent generations in T_2; and L_6 is no different from S_o although previously it was the L_0 genotroph. Therefore one would expect the DNA in the F_1 of their reciprocal crosses to be the same as in their parents, whereas they gave a highly significant deviation of 4.1 from the mid parent value (Joarder, 1973),

$L_6 \times L_6$	$L_6 \times S_6$	$S_6 \times L_6$	$S_6 \times S_6$
95.1	98.3	100.2	95.2

which is almost half way back from the mid-parent value to the full DNA amount in L_0. So L_6 is not entirely the same as S_6 though they look the same. Hidden changes mediated by the environment occur which are not apparent from observations on the plants.

The obvious difference in the environment of the plants grown outside compared with those grown in a normally heated greenhouse for the first five weeks from sowing is the lower temperatures. Plants taken from the greenhouse after five weeks may be up to 15 cm high with strong side shoots. At this time those outside consist of no more than a centre shoot of 2 to 3 cm plus the cotyledenous leaves. Associated environmental differences, day length and summer temperatures for example could be partly responsible for inducing changes. The growth and development stages of the plants grown outside experience longer days, and flower primordia are formed on less developed plants.

SUMMARY: CHAPTER 9

After growing L and S genotrophs outside from sowing for three generations instead of in a greenhouse for the first five weeks each generation, the 15 percent difference in nuclear DNA amount disappears, the DNA of both reverting to an intermediate amount which is the same as in the original Stormont Cirrus variety from which they were induced. At this point there is no reversion in the induced plant weight changes which therefore appear to be independent of nuclear DNA amount.

If the L and S genotrophs are grown outside for a further three generations the DNA amount in both drops to about the same amount as in the original S genotroph. In L this is accompanied by a drop in plant weight, height, number of ribosomal genes and a change from hairless septa, *hh*, to hairy septa *HH*. So in practically all characteristics, though not in crosses, L has changed to S. Overall there is a good correlation between induced plant weight and number of ribosomal genes.

10 MORE INDUCED CHANGES

10.1 Changes in the plasticity of the Stormont Cirrus variety, Pl.

Previous chapters have reported on the inherited changes induced in the plastic variety Stormont Cirrus in the 1950s and later, and on the characteristics of the two stable types, the L and S genotrophs. It seems from this account that inducing inherited changes is an easily repeated process, whereas the real situation is different. The variable nature of the process appeared initially with the induction of plants of different weights from parents grown in different combinations of fertilisers in 1954. The largest and smallest of the environmentally induced plants were stable; they could not be changed by further fertiliser treatments, and were labelled L and S stable types. Those of intermediate weight could be changed by more inducing environments and were labelled plastic, i.e. like the original variety Stormont Cirrus, Pl, from which they were all induced. Hence plastic and stable types appeared side by side in the field and whether a plant was plastic or stable depended on the environment of the previous generation.

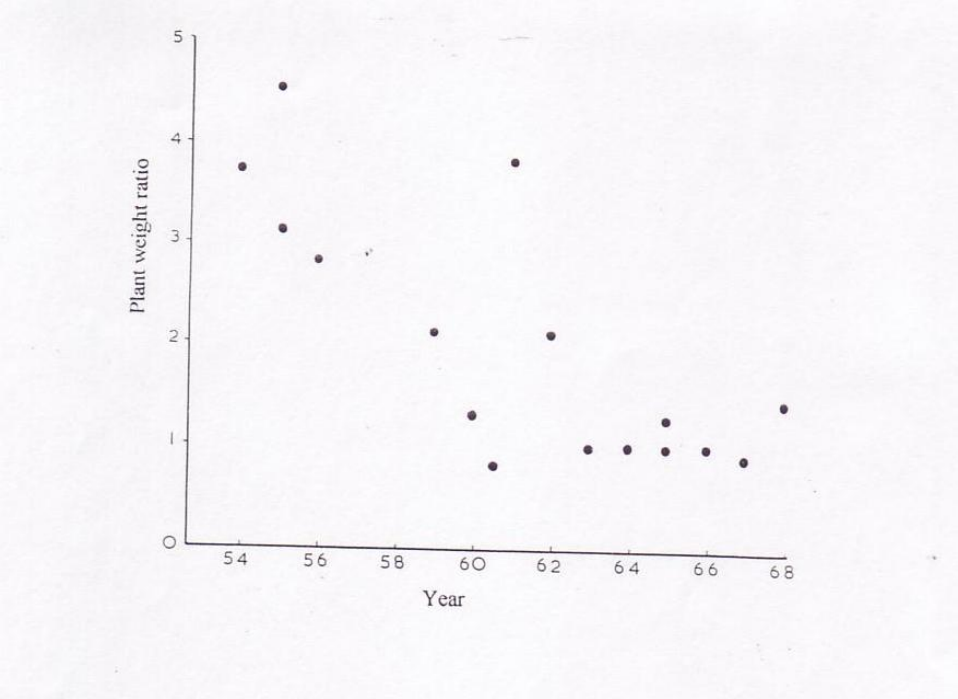
If there were no permanency in the changes there would be no evidence for environmentally induced genetic change. But if the original plants lose their plasticity and become stable in the induction process, then over generations and environments it would seem all would eventually become stable types. The *rationale* of environmentally induced inherited changes was queried earlier since it would seem that induced changes would be modified or cancelled in turn by the environment of the next generation. In fact plants change from plastic to stable, and from stable to plastic, over generations in the appropriate environments. From an experimental point of view if plants are found to be plastic, for what ever reason, then it would be important to multiply them each generation in an environment which does not induce inherited changes, or at least does not destroy their plasticity. In other words the possession of the plastic character depends on both the genotype and ancestral environments of the plant.

It was clear in the later 1950s that because in some environments plants of the original variety, Stormont Cirrus, lost their plasticity and apparently no further changes in plant weight could be induced them, whereas in other environments they remained plastic, it was necessary to have regard to the environments in which the plants were grown when multiplied from generation to generation. From an assessment of the early induction results, the best environment for this purpose seemed to be one where the plants were not reared in the greenhouse at any time but kept outside from sowing to seed set, and in compost of only moderate fertility. Hence rather than transplant

them into the field, several plants were grown per 17 cm pot containing compost made from soil in the field to which a small quantity of compound fertiliser was added in solution. The plants were tall and healthy and appeared no different from the originals. Their plant weight relative to the weights of L and S, their interaction with the environment, nuclear DNA content and *H,h* capsule character were all unchanged and it was assumed this environment was suitable for maintaining plasticity. But it is now clear that hidden inherited changes occur from generation to generation without any change in the outward appearance, i.e. phenotype, of the plants.

Despite these precautions, whether due to this environment or because they would have changed in any case, the Stormont Cirrus plants lost some of their capacity for inherited change in plant weight when grown in inducing environments similar to those originally used. The results of a number of induction experiments are gathered together in Fig.10.1. They are not strictly comparable since they were done in different years and different environments, and are shown as the ratio of the largest mean plant weight induced, e.g. by nitrogen fertiliser, divided by the smallest mean plant weight, e.g. induced by phosphorus fertiliser. The rapid fall off coincides with the multiplication of the variety although outwardly the plants looked the same. This was not an end point for induction experiments with this variety because the plants had not lost their plasticity entirely for plant weight nor for environmentally induced changes in other characters. For example, the full 16 per cent difference in nuclear DNA was induced in Stormont Cirrus plants many times in later experiments, and in crossing and backcrossing experiments with a non-plastic linseed variety Royal, 1968 to 1974 (Chapter 8). These plants still have plastic characteristics and continue to be referred to as Pl. These results are in accord with the conclusion in Chapter 9 that the observed induced differences in amount of nuclear DNA were not responsible for the induced differences in plant weight, although there could remain small undetected differences in DNA that were responsible.

Fig. 10.1 Ratios obtained by dividing the weights of the larger plants by the weights of the smaller plants in the first or second generations following inducing treatments applied to Stormont Cirrus plants, Pl. During selfing and multiplication over generations, Stormont Cirrus plants have apparently lost their plasticity for induced changes in plant weight from about 1960 onwards. (Durrant, 1971).



Assessing whether a plant has, has lost or has gained, plasticity is made more difficult by the problem of determining what environments induce change, and establishing whether changes have been induced. They need not be of the magnitude of a hundred per cent or more, described earlier, which for obvious reasons have been concentrated on. Smaller changes could occur of 10 or 20 per cent which in plant breeding or agriculture would be important. Such changes are more troublesome to establish within the environmental variation of the field which is compounded by comparing plants which may have differences due to maternal effects. They should be taken at least to another generation. Large established inherited differences in plant size may not show up when plants are grown in some environments and smaller differences may be more easily hidden.

Sometimes one may be alerted to an induced change in plant weight by a change in another character. For example, a change occurring in the capsule character *H,h* of plants receiving inducing treatments, which can be checked on the plants as they ripen (section 6.1), is usually associated with an induced change in plant weight. In 1972, relatively longer stamens were noted in Pl plants that had been grown in the field instead of in pots, the *an* character (section 6.6). The plants were subsequently found in later generations to be nine centimetres shorter and 15 per cent heavier than

the pot plants. On the other hand associations between various characters, plant weight, height, nuclear DNA, ribosomal genes, *H,h*, cannot always be expected, and those that appear can be broken later. Cullis (1981) at the John Innes Institute, Norwich, noted that changes in the rDNA amount were not always associated with changes in peroxidase enzymes.

The remainder of this chapter describes changes that would not normally be predicted from the results of earlier experiments.

10.2 Experiments in controlled environment rooms in the Earhart Laboratories.

The first of these concerns experiments done in the Earhart Laboratories, California Institute of Technology where temperature and nutrients could be controlled. The plants were grown in half-pint pots containing a mixture of gravel and vermiculite in a 16 hour day, 8 hour night, in two temperature regimes; 27° C during the day, 15° C during the night (27/15), and 19° C during the day and 11° C during the night (19/11). Sown in November 1961 they received the following treatments. For the nitrogen fertiliser treatment the plants received 0.05 per cent ammonium sulphate solution (*n*) in the morning for four days a week, otherwise water or one-fifth strength Hoaglands solution morning and afternoon, in the 27/15 regime. The phosphorus treatment was the same except 0.2 per cent triple superphosphate solution (*p*) was applied instead. Control plants were grown in either 19/11 or 27/15 regime, but with water in the morning and half strength Hoaglands in the afternoon (*Ho*). There were three plants per treatment each in a separate pot. This is the parental (C_0) generation.

Table 10.1 Weights (g) at Aberystwyth of C_2 generations of parent and F_1 plants, C_0 and C_1, grown at different temperatures and with different nutrients in the Earhart Laboratories. There is a large inherited difference due to temperature, the 19/11 regime inducing a smaller plant. (Durrant, 1971).

C_0	27/15 *n*	27/15 *p*	27/15 *Ho*	27/15 *Ho*	19/11 *Ho*	19/11 *Ho*
C_1	27/15 *Ho*	27/15 *Ho*	27/15 *n*	27/15 *p*	27/15 *n*	27/15 *p*
C_2	55	62	56	48	26	23

Seed from C_0 was sown in April 1962 to give the C_1 generation which was grown entirely in 27/15 regime but the nutrients were switched round, those which received *Ho* now received *n* or *p*, and those which received *n* or *p* received *Ho*, as shown in Table 10.1. All seed from C_1 was harvested in July 1962, and the C_2 generation grown in a general environment at Aberystwyth in 1963. No inherited changes were induced by the treatments *Ho*, *n* and *p* treatments but there was a large significant ($P < 0.1\%$) inherited difference due to temperature, the C_2 27/15 plants being twice the size of the C_2 19/11 plants. The plant weights (g) of fourth generation plants grown alongside plants of Stormont Cirrus, Pl, in 1966,

27/15	27/15	19/11	19/11	
n	*p*	*n*	*p*	Pl
212	208	87	78	207

show the difference was due to the lower temperature reducing plant weight, the higher temperature having no effect.

This change was as much due to other conditions in the growth rooms as to 19/11 itself, because Stormont Cirrus plants sown in 19/11 in June instead of November were unchanged. This was probably because, although the ambient temperature was controlled, the amount of daylight and radiant heat was greater in summer at the time of the earlier application than in winter when the later application induced the lower plant weights.

10.3 Induced changes transmitted by crosses

L plants were grown in *npk* and *nk* inducing environments and crossed with Pl plants grown in a control environment, i.e. the usual multiplication environment for Pl. Table 10.2 shows that changes were induced in L which were transmitted to the crosses. On average L parent plants grown with *p* gave crossed progeny in subsequent generations, C_1 and C_2, 34 per cent larger than those grown with *npk*. The difference was highly significant and was the same whether L was used as the male or female parent. But the response to the fertilisers was different from those in earlier experiments because previously *npk* always induced a larger plant than *p*. Nuclear changes were again involved since reciprocal crosses between plants from different treatments had the same plant weight. In parallel experiments no permanent changes were induced in progeny of L parents grown in the *npk* and *p* environments and Pl itself showed only a marginal significant difference.

Table 10.2 Mean plant weights (g) in C_1 and C_2 generations of crosses between L and Pl plants. The Pl parent plants were grown in control environment but L parent plants were grown in *npk* and *p* environments. Induced changes are transmitted via the L male and female gametes in crosses with Pl. (Durrant, 1971).

	L(*npk*) x Pl	Pl x L(*npk*)	L(*p*) x Pl	Pl x L(*p*)
C_1	37.7	32.3	48.4	41.5
C_2	38.0	32.8	49.7	46.6

Changes were also induced at the same time at the *h* locus described in sections 6.1 to 6.5. Pl and S are *HH*, (hairy septa) and L is *hh* (hairless). In the *npk* L parental environment the crosses L x Pl and Pl x L (*hh* x *HH* and *HH* x *hh*) gave 10 *H* and 38 *h* plants in C_1 when they should all be phenotypically *H*. Changes are induced in L that effect plant weight and *H,h* but these are only effective when L is made heterozygous with Pl. Thus L plants induced originally from Pl retain some capacity for induced changes which are only revealed on crossing to Pl, even though Pl may have lost some plasticity.

Similar experiments at the same time with S in place of L were equivocal, a large difference occurring in plant weight in C_1 in the same direction as with L but almost disappearing in C_2, although some anomalous *h* phenotype plants appeared in C_2.

10.4 A pre-induction environment.

A pre-induction environment was used in a final experiment which began in 1993. Normally all sowings are made during the last week of March, denoted E_1, for plants in the greenhouse, T_1, and those outside T_2. For the pre-induction environment, sowing time was delayed by about six weeks, to about the second week in May, an environment which none of the genotrophs, L, S and Pl, had previously experienced, at least in the course of the experiments reported here.

Fig.10.2 shows the regime for the L genotroph which previously had always been grown in a general environment, T_1E_1. Two lines were grown for three generations, 1993 to 1995, in the late sowing environment, T_1E_2. They were also split and transferred to outside late sowings, T_2E_2, for two generations, 1994 to 1995. Controls were maintained in their usual general environment, T_1E_1. Two lines of the S genotroph received the same regime. The genotrophs Pl and Plf (section 6.6) were treated similarly except, since these were normally gown outside, T_2E_1, the reverse situation applied

in that lines were transferred to inside sowings. The late sown plants had longer days and higher temperatures than those sown at the normal time.

In 1996, seed from the 1995 plants were sown in normal inducing environments, T_1E_1 with *n* and *p* treatments, similar to those described in earlier chapters and a control environment which was essentially a general environment (Fig.10.2). A few plants of each line (C_0 generation) were grown to collect seed to monitor any induced changes in the following generation., but changes in the capsule character, *H,h*, were found in some of the treated plants themselves. They occurred only in the L and S genotrophs and only in those plants whose ancestors had been late sown (Table 10.3), i.e. only those which received the pre-induction environment. Consequently some *n* and *p* inducing treatments were applied in 1997 to some additional S plants which were the progeny of T_1E_2 1994 plants. These conformed to the same pattern where *n* induces $H \rightarrow h$ changes and *p* induces $h \rightarrow H$ changes. In other words, *n* ensures plants have hairless capsules and *p* ensures that plants have hairy capsules.

Fig.10.2 Environments in which the L genotroph was grown in years 1991 to 1996. The progeny of plants grown in a general environment in 1991 were grown for three generations from 1993 to 1995 in a late sowing environment, E_2.
Progeny of 1993 plants were also grown outside the greenhouse for two generations, T_2, in the late sowing environment.
Progeny of 1991 plants were maintained in a general environment for two generations. The progeny of all 1995 plants were grown in *n* and *p* inducing and control environments in 1996.

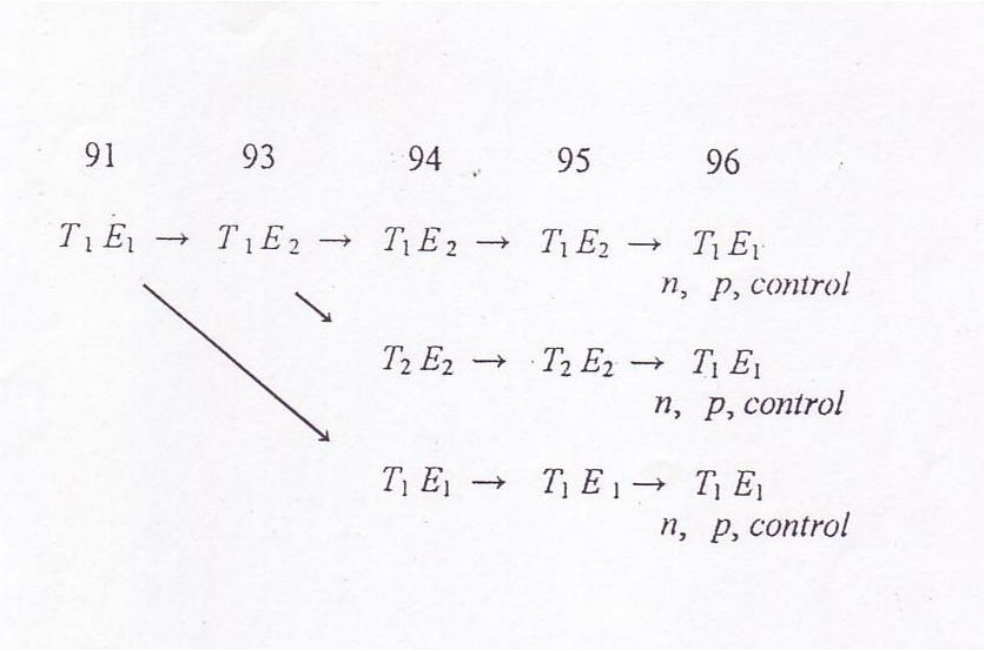


Table 10.3 Changes are induced in the capsule character, *H,h,* of L and S genotrophs whose recent ancestors were late sown (E_2) in May (instead of March, E_1, not shown) irrespective of whether the ancestors were grown inside (T_1), or outside (T_2), a greenhouse for the first five weeks from sowing. An *n* environment induces $H \rightarrow h$ changes in *H* plants; a *p* environment induces $h \rightarrow H$ changes in *h* plants. No changes occur in plastic plants (Pl), or in any plants whose ancestors were sown at the usual earlier time in March.

Origin Fig. 10.2	Genotroph	*n* treatment	*p* treatment
$T_2\ E_2$	L	*h*	$h \rightarrow H$
1995	L	*h*	$h \rightarrow H$
	S	$H \rightarrow h$	*H*
	S	$H \rightarrow h$	*H*
	Pl	*H*	*H*
	Plf	*H*	*H*
$T_1\ E_2$	L	*h*	$h \rightarrow H$
1995	L	*h*	$h \rightarrow H$
$T_1\ E_2$	S	$H \rightarrow h$	*H*
1994	S	$H \rightarrow h$	*H*

These changes have occurred in the treated plants themselves, i.e. the C_0 generation, and from past results it was presumed that such changes would be carried through to later generations and probably be accompanied by inherited changes in plant weight. Seed taken from the C_1, C_2 and C_3 generations grown in successive years was sown collectively in a general environment in year 2000 giving plant weight and height data for C_2, C_3 and C_4 generations. Their mean values in Table 10.4, taken over the three generations, two lines each and replicates, show that *p* treatment has induced huge, highly significant increases in weight and height in L, whereas *n* treatment had no effect. A consequence of applying the *p* treatment to L plants whose ancestors received the late sown pre-induction environment was to induce plants 80 percent larger than L plants whose ancestors had not received the late sown pre-induction environment (Plate 7). There were no significant differences between the three generations with regard to these new induced changes i.e. they were fully maintained up to the fourth generation at least, and they all had hairy capsules.

Table 10.4 Overall mean plant weights (g) and heights (cm) in the summed C_2, C_3 and C_4 generations of L and S T_2E_2 plants grown in *n*, *p* and control environments in 1996.
n compared with control has had no effect; *p* has induced large inherited increases in weight and height in L, but smaller increases in S.

		n	*p*	*control*	
Plant weight	L	114	198	110	$P < 0.1$ %
	S	70	90	67	*not sig*
Plant height	L	76	92	73	$P < 0.1$ %
	S	65	74	68	$P < 5$ %

Plate 7. Four genotrophs grown in 2006. From left, L, Pl, S and Lp. The newly induced type, Lp, from L, is heavier than the others but also taller than the original Stormont Cirrus plant, Pl.

Differences among the S plants were much smaller and are barely significant although they conformed to the same pattern as L. One prediction was not realised. None of the S plants retained the induced change $H \rightarrow h$ change, i.e. they had all reverted back to hairy septa. *H*, so all plants derived from these pre-induction treatments now had hairy capsules. This can be compared with the large difference in plant weight induced in the progeny of S grown with *p* and crossed with Pl which also disappeared in later generations (section 10.3).

Another comparison can be made with the reversions described in Chapter 9. When grown outside (at the usual sowing time) the amount of nuclear DNA in S first increases to a mid-value then decreases to approximately its original value, retaining hairy capsules throughout (Fig. 9.1, 9.2). The amount of nuclear DNA in L decreases to the mid value but thereafter continues its decline to about the same amount as in S. At the same time its plant weight drops to about the same as S and its capsule character changes from $h \rightarrow H$. So here too all plants have hairy capsules. The original variety Stormont Cirrus has hairy capsules. From these results it appears that changes are less easily induced in S than in L. In other experiments there were, as expected, no significant differences between descendents of plants grown in T_1E_1 and T_2E_1, i.e. sown at the usual time, when similarly treated with *n*, *p* and control environments.

As described earlier (chapter 3) the original induction treatments in the 1950s and onwards induced L with greater plant weight, and S with smaller plant weight, than Pl, the original flax variety Stormont Cirrus, but neither was as tall as Pl. No induced type had since been obtained even equalling the height of Pl. But here, experiments comparing the heights (cm) of the new, large L plants, denoted Lp, induced by the *p* treatment, with the existing genotrophs (Plate 7) showed it was taller than any of these, notably taller than Pl:

L	S	Pl	Lp
106	94	113	120

So the recent environments have induced an inherited increase in height as well as plant weight of a flax variety, in so far as Pl is still representative of Stormont Cirrus.

The ‘pre-induction environment’ of later generation sowings is something of a misnomer because it itself is an environment which has induced inherited changes allowing fertilisers applied in a later generation grown at the earlier sowing time to induce inherited changes in plant weight and height. Furthermore, the fact that the changes are induced in L and not in Pl, and doubtful in S, means that earlier environments inducing L from Pl, and/or the many generations in their respective environments in which they have been bred over years, are also factors determining whether an inherited change occurs.

SUMMARY: CHAPTER 10

Some later experiments induced unpredicted induced changes. Stormont Cirrus (Pl) plants were maintained over generations and years in an environment thought not to induce inherited changes, so as to preserve their plasticity, yet after about six years they lost plasticity for large inherited differences in plant weight when grown with different fertilisers, but they remained plastic for DNA amount.

In the Earhart laboratories, growing Stormont Cirrus plants at a lower temperature induced a smaller plant whereas *n* and *p* treatments applied at higher or lower temperatures had no effect.

L genotroph plants grown with *npk* and *p* fertilisers transmitted changes to the progeny when crossed to Stormont Cirrus, *p* inducing a larger plant than *npk*, the reverse of their previous effects.

L genotroph plants were sown at a later time, in May instead of March, for three generations and then grown in *n* and *p* environments. This pre-induction treatment of late sowings resulted in *p* inducing an even larger L plant which was also taller than Stormont Cirrus plants. It is the largest plant obtained yet, in weight and height. No induced changes appeared in any plants whose ancestors had not been grown from late sowings.

11 REVIEW

Changes are induced in several characters

The several characters in which inherited changes have been identified are plant weight, plant height, the capsule character *H*,*h*, nuclear DNA amount, number of ribosomal genes, a floral character *an*, and various phytohormones and isozymes. The observation that several characters change at the same time, induced by the same treatment, in so far as they have been studied, does not mean a single chromosomal factor is involved, nor that they are due basically to a single induced genetic change. In fact all the data support the notion that several chromosome loci have responded to the environments. Although one would expect some association between plant weight and height, plant growth producing a heavier plant being reflected to some extent in a taller plant, their correlation here is weak. In the initial experiments different fertilisers produced heavier plants and lighter plants from the original variety, Stormont Cirrus, but all were shorter than Stormont Cirrus. Increase in height per unit increase in weight due to fertilisers applied directly to the plants is four times greater than increase in height per unit increase in weight due to inherited effects of fertilisers, over the same plant weight range. Height bears no clear relationship with weight in linkage with *H,h* nor in the reversion of their induced changes in crosses with other varieties. This is not to say that the environment cannot induce a correlated change, as described in a later experiment.

Plant weight and nuclear DNA amount initially appeared to be correlated giving the notion that here was evidence that the induced differences in nuclear DNA amount were responsible for the induced differences in plant weight. In reciprocal crosses between L and S (the large and small genotrophs environmentally induced from the flax variety, Stormont Cirrus) the nuclear DNA amounts and plant weights were intermediate between those of L and S. There was a correlated response in nuclear DNA amount in the F_4 generation of crosses between L and S after selection for high and low plant weight. In the F_4 of reciprocal crosses of L to Pl and S to Pl there was no reversion or conversion in nuclear DNA amount or in plant weight. There were correlated changes in nuclear DNA amount and plant weight in crosses of L and S with another variety, Liral Monarch. Even so these are not critical observations because, for example, given the differences in plant weight and DNA amount between L and S both could independently give mid-parent values in reciprocal crosses between the two genotrophs.

More changes are induced later

A different situation emerges when the plants are grown outside. The DNA amounts in L and S converge to the point where there is no detectable difference, yet the plant weight difference between L and S is as large as before, destroying any idea that the plant weight difference is caused by this large DNA difference, though a small undetected difference in DNA could still be present. When the plants were grown outside for a further three generations L and S continue to contain equal amounts of DNA but both drop to a value virtually the same as in the original S genotroph. At the same time L plant weight also drops suddenly to the same as that of S, i.e. L is now practically indistinguishable from S in plant weight and DNA amount; in fact phenotypically it is no longer L, but S. Evidently induced changes can occur independently in plant weight and DNA amount but additionally other factors operate at other times to effect changes in both, without implying that one causes the other.

The number of ribosomal genes also showed a good correlation with plant weight, L having about 60 per cent more than S. Grown outside for three generations, there was very little change, though the results were variable, so at this point the association between ribosomal gene number and plant weight is maintained, although the difference in total DNA between L and S had disappeared. Grown for a further three generations outside, at the 6th generation the number of ribosomal genes in L, as with the other two characters, plummets to the same number as in S. So the eventual result of growing L and S outside, in this sequence of experiments at least, is to turn L into S, in plant weight, DNA amount and number of ribosomal genes. It is a slow process though, six generations over six years. Yet this does not certify a direct causal link between any of these characters. Ghogain *et al.* (1982) found for example that differences in ribosome gene number do not affect the amount of their products in the cell and that other factors are probably involved.

Two characters which are associated, and in fact show genetic linkage, are plant weight and a gene. *H,h,* determining presence or absence of hairs in the capsules, in which changes are also induced by the environment. S has hairy capsules, *H*, and L is hairless, *h*. Plant weight and *H,h* are both unstable in the heterozygotes of crosses between L and S, with positive and negative associations, giving a range of variation in hair number and plant weight. This implies there is a particular chromosome region where the environment induces changes which affect both plant weight and the *H,h* gene. After six generations outside, *h* in the L plants changes to *H* in company with the other characters, plant weight and DNA mentioned above, completing the phenotypic change L → S. Nevertheless there are underlying differences between L (now phenotypically S) and

S, because they interact to give more than an additive amount of nuclear DNA in reciprocal crosses between them.

Fertilisers generally do not have specific effects in inducing inherited changes

Nitrogen, phosphorus and potassium fertilisers do not always have specific effects in inducing inherited changes. In the 1954 treatments, *npk* induced large plants, *nk* small plants, which suggests that the inclusion of *p* was responsible for the large plants. But *n* applied by itself also induced large plants, while *p* by itself induced no change. Thereafter for many years *n* consistently gave large plants and *p* small plants, the latter probably due to lower *pH* compost. In the Earhart experiments there was no significant difference between *n* and *p* in their effect on plant weights but there was a significant difference due to different temperature regimes. L plants grown with *p* and crossed with Pl gave larger plants in the F_1 and F_2 generations than L plants grown with *npk* and similarly crossed. More recently, *p* induced even larger and taller plants when applied to L plants which had been sown later in the year in previous generations, whereas *n* had no effect.

Environments are not exactly reproducible each year but such differences cannot be responsible for the reversal of fertiliser effects of this magnitude. The reason must be because the genetic constitution, the genome, changes. The activities of the many genes interact one with another and with the environment, and overall produce a favourable working genome for the growth of the organism. The induced changes in several characters and the broad range of repetitive DNA sequences suggest that the genome as a whole co-operates in the induction of the environmentally induced changes and their favourable growth patterns. It seems there are broadly three kinds of environmental effects. There is the obvious one where fertilisers induce big differences in plants weight. These effects are consistent and repeatable provided they are applied to similar plants. Another are the progressive changes that occur over several generations when plants are grown in another environment. And a third is the response to fertilisers when plants are grown in a different ancestral environment. All three are in fact dependent on the ancestral environments of the plants that are exposed to the inducing environments at any one time. Plants or species grown in new locations are most likely to experience changes in temperature or day length which are important agents in inducing inherited change.

Changes in the genome

It is clear the induced inherited changes are not due to rarely occurring classical gene mutations arising from chemical or physical mutagens, or from uncorrected errors in chromosome replication, which frequently have lethal or debilitating effects. None of the increases or decreases in plant weight or height induced by the environments here appear to have had any bad effects on the plants, nor any impairment of their health. Instead the genetic changes induced by the environments are likely to be due to the inheritance of changes in the expression of genes due to their activation or repression by some means, i.e. epigenetic changes. Normally changes in gene expression during the development and differentiation of an organism are not transmitted to the progeny, each individual beginning life in a relatively undifferentiated state, but in some cases changes in the genetic material, or in its expression, occur which are inherited.

In the flax experiments the environments could have mobilised transposable elements which are small reiterated DNA sequences which move from place to place on the chromosomes. Where they become newly attached they can affect the expression of genes and produce visible mutations. The environments could have altered the distribution of heterochromatic regions where the chromosomes are more tightly coiled so that genes near or contained cannot be expressed. These position effects can have significant effects on the organism. Changes of this kind can also result in a reversal of the original changes, which would not occur had they been due to classical mutations. Other genetic mechanisms associated with the manipulation and control of gene activity are transpositions, slippage during replication, unequal crossing over between chromosomes, conversion, amplification and, specifically, methylation which switches genes off.

Methylation

Inherited epigenetic changes due to methylation have been known in animals for a long time. Mammalian females have one of their two X chromosomes in each cell methylated. The chromosome is condensed, heterochromatic, and its genes inactivated, so effectively the female has only one X chromosome in common with the male. Genes on the other chromosomes may also be methylated (or imprinted). Some are methylated by the male, others by the female, parent. Since the reproductive cells in animals are tucked away at an early age, unlike those in plants exposed to the environment in the meristematic dividing tip of the growing shoot, it would seem unlikely that the environment of animals could have an effect on imprinting, or initiate imprinting. Yet there is evidence of inherited epigenetic effects in mice by methylation brought about by chemicals There is

also evidence this may be achieved by changes in nutrition in early life, and there are suggestions that environmentally induced methylations may be responsible for some recent trends noted in human populations.

Of interest here is the possibility they may play a part in the induced changes in flax, although there is no direct information on their role in the induction of the large and small genotrophs. Fieldes (1994) at the Wilfrid Laurier University, Waterloo, Ontario, decreased height and flowering time by applying the demethylating agent 5-azacytidine. These changes were inherited through to the second generation implying they were due to hypomethylation, i.e. a decrease in methylation, brought about by the agent. Later Fields with co-workers Schaeffer, Krech and Brown (2005) measured the methylation and compared the amounts in the induced early flowering lines with the original lines. They found the decrease in flowering time was associated with a decrease in methylation which was inherited for at least nine generations and concluded they had induced inherited epigenetic changes in phenotype by reducing the level of methylation.

Other genetic factors

The results with 5-azacytidine are suggestive but do not show that methylation contributed to the changes induced by growing the flax plants in more natural environments. If methylations had been induced these would not be the only response because as described earlier, changes are induced in total amount of nuclear DNA and in number of ribosomal genes. Cullis and others in the Biology Department of the Case Western Reserve University, Cleveland, Ohio, have studied the nuclear changes. In a summarising article Cullis (2005) confirms that variation occurs over the whole range of repeated sequences, spread throughout the genome, through the accumulation of changes during the induction process, and much of this variation is of a kind already present in other flax and linseed varieties. Timmis and Ingle (1974) concluded that DNA differences represented a wide range of the flax genome. Schneeberger and Cullis (1991) found identical variation, produced from a repetitive ribosomal gene cluster, in four kinds of S genotrophs (induced from Stormont Cirrus, from Liral Prince, and after changes in original L and S genotrophs). Cullis, Swami and Song (1999) reported on the wide distribution throughout the genome of induced changes. Oh and Cullis (2003) made further studies on differences between Stormont Cirrus and some of the derived genotrophs and their possible association with structural changes in DNA. Y Chen, Schneeberger and Cullis (2005) found a DNA fragment which is inserted during the induction of some of genotrophs. It is not found in Stormont Cirrus but occurs naturally in other flax and linseed varieties.

Ancestral environments

Returning to the subject of the importance of ancestral environments, this was first evident in the early experiments of the 1950s. Several types were induced from the original Stormont Cirrus variety. Some were stable and no further changes could be induced in them at that time whereas others were plastic and large differences again could be induced in them. Those plants whose parents had been grown without fertilisers, or with *k* or *p* were plastic, the others were not. It is one reason why changes can be induced in some plants and not in others. This raises the question as to why plants grown from the samples of Stormont Cirrus and Liral Prince seed from the Plant Breeding Station at Stormont in Northern Ireland were plastic. On receiving them at Aberystwyth they were sown on 1st April, 1953, and the plants kept outside. Seed taken from them were used for the first induction experiment in 1954 and they proved to be plastic. Either they were plastic in any case because of the conditions in which they were grown in Northern Ireland, or they became plastic because of the new conditions they experienced at Aberystwyth. What can be said is that, during multiplication over several years at Aberystwyth in the ongoing multiplication environments, Stormont Cirrus plants gradually lost their plasticity for the induction of large differences in plant weight, but continued to retain plasticity for differences in amount of DNA.

Another example was the effect of growing plants in a late sowing environment for two or three generations. Subsequent generations of L genotroph plants grown with a phosphatic fertiliser inherited large induced increases in plant weight and height, whereas there was no change in plants similarly treated whose ancestors were sown at the usual time.

Growing the L and S genotrophs outside from sowing instead of the first five weeks in the greenhouse each generation induced a progressive change in DNA amount and then in number of ribosome genes with a cumulative effect from one generation to the next, as mentioned above. At the sixth generation the large L genotroph suddenly changed to a plant which to all appearances was the same as the smaller S genotroph. Previously the L genotroph had been grown for at least 45 generations in a general environment. Suppose its origin were unknown and it was therefore assumed to be an ordinary flax variety and it was decided to grow it in another environment comparable with growing outside. After a few generations for no apparent reason the plants would change and became less than half the expected size. Unknown to the grower, changing to a different environment induced a gradual change in the genome and a different crop. This would normally be judged to be due to the direct effect of the environment or, if progeny tests were made, to selection of particular types in a heterogeneous crop by the new environment. Inherited changes may have

been directly induced by past climate changes aiding and abetting mutation and selection in promoting evolution.

Inherited changes that have been induced can be repeated provided similar environments are used and seed from the same samples are used. Even growing the seed on for one generation, if only for multiplication purposes, can reduce, alter or nullify the induction because a hidden change could have occurred or the environment was not suitable. On the other hand it is not possible to predict what environments will induce change, and the kind of change, and they may occur in the genome unseen and unrecorded. In fact it seems the genome is always changing, being in a continuously unsettled, or restless state. Which leads to the earlier cited problem, if environmentally induced changes are inherited how is it they do not disappear in the environments of subsequent generations? The answer is that plastic plants can become stable, and stable plants can become plastic. Consequently the environment can induce some plant types which may persist in some environments for scores of generations whereas in other environments they may revert or change to other types. In the meantime hidden changes may continue in the genome. Different types can appear, disappear or change, without the burden of severe mutations nor the necessity for outcrossing to provide additional variation, but they would at the same time be subject to natural selection.

Other species

It would be unrealistic to presume flax to be the only species to have plastic characteristics., as it would be to expect all species to be so endowed. Large induced changes may be uncovered in other species but smaller changes are probably more likely. A clue may be if a crop appears to have changed in some way when grown in another area. Or there might be pronounced heritable variation in progenies of single plants in an inbreeding population. Flax is an annual and an inbreeder, propagating by selfing, and its varieties are presumed to be virtually homozygous. To induce convincing inherited changes in an outbreeding species is practically impossible, unless one obtained a doubled up haploid, without instituting a rigorous inbreeding programme to clear out the 'genetic load' and obtain some semblance of homozygosity. An attempt began in 1969 using the common red campion, *Silene diocia*, a perennial dioecious species, i.e. one with male (stamens) and female (styles) parts on different plants, so that it is an obligate outbreeder. This means it can only be inbred by sib (brother/sister) mating which is much slower than selfing. Plants taken in 1969 from two sites at Cwm Mabws, a valley 10 miles south of Aberystwyth, were sib-mated, i.e. crosses

made between male and female plants grown from seed from the same capsule of a parent plant., and several inbred lines grown and maintained each generation at the same sites as their respective parent plants. Plants of two such lines, one from each site, which had been sib-mated for 15 and 16 generations were cloned, grown in supposed inducing environments with fertilisers and crossed in 1989, 1990. In 1992, F_2 plants whose ancestors had received nitrogen fertilisers were 45 per cent heavier than those which received no fertiliser. Four independent sets of plants gave the same results which were highly significant and the treated male and female parents contributed equally. Although these probably were environmentally induced inherited changes there was still 4 per cent heterogeneity in the sib-mated plants, calculated from the Fibonacci series, not including small sample effects or selection of favoured heterozygotes, so this conclusion is suspect.

No such problems exist in experiments carried out in the Genetics Department, Birmingham University, on inducing heritable changes in *Nicotiana rustica*. This natural outbreeder had earlier been inbred for experimental purposes, and it was further subjected to 20 generations of strict inbreeding by selfing in the Genetics Department where pure breeding lines were used for biometrical studies. Hill (1965) obtained significant differences in flowering time and plant height in the F_2 generation of one of three tested *Nicotiana* lines after growing the parent plants in a greenhouse in soils which had been treated with different combinations of nitrogen, phosphorus and potassium fertilisers. Hill and Perkins (1969) showed the differences persisted undiminished to the fifth generation though there were interactions with the environment over years. Three of the induced types were selected for biometrical analysis in a diallel cross by Perkins, Evelyn Eglington and John L Jinks. Their inducing environments and plant heights (cm) were

p	*nk*	*nil*
88	111	107

Like flax, the induced changes were mainly additive in their effects, and like flax there was also F_1 instability which differed in the reciprocal crosses. Using the same induced lines, Eglington and Moore (1973) and Moore and Eglington (1973) concluded there were sites on many chromosomes that had undergone heritable change. In an analysis of the cumulative data of the induced lines, Towey and Jinks (1976) showed the induced changes could be placed in three groups and, like flax the three genotrophs of flax, L, S, and Pl, the more characters studied the more distinctive phenotypes emerged. Therefore a significant feature of these environmentally induced inherited changes in flax and *Nicotiana* is that they are not confined to one or two sites but are spread over the genome as perhaps might be expected since the genome is an on-going, interacting set-up, where

genes are continually being switched on and off, and where no one gene can truly be independent of others. Genetic analyses by biometrical methods normally used in the analysis of genetic continuous variation were thus used here on inherited epigenetic changes in *Nicotiana*, and perhaps epigenetic changes may also be present in other data and other species customarily analysed by biometrical methods.

A comment on Lysenko's genetics

A question sometimes asked is, do these results support T D Lysenko`s theories on inheritance? The short answer is no because he interpreted inheritance in all plants as a consequence of their nutrition encompassing all aspects of their environment, which led to strange claims of crop improvements, ignoring the guiding principles of present day genetics and cytology. The theories of Lysenko were rejected. This was however many years ago and there may have been some caught up in the debacle who believed they were at least partly right. Without their acknowledging and allowing for the role of chromosomes in inheritance it is not possible to construe their results, but some might have been interesting.

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