GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE

I. INBRED LINES

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Received 26.v.54

I. INTRODUCTION

GENES within the nucleus are relatively unchanging, permanent hereditary units : they comprise the nuclear genotype. The chromosomes which bear them, on the other hand, change considerably in behaviour and appearance. In this respect the nucleus presents a phenotypic aspect. This nuclear phenotype changes not only during cell division : throughout the life history of any organism it is subject to the control and interaction of the genotype and the environment.

Few attempts have been made to study the genetics of chromosome behaviour. There are exceptions, notably the works of Darlington (1932) ; Beadle (1933) on maize ; Peto (1933) on *Lolium-Festuca* hybrids ; Maeda (1937), Emsweller and Jones (1945) on *Allium* species crosses ; Müntzing and Akdik (1948) and Prakken and Müntzing (1942) on rye.

The problem of genotypic control can be tackled in five ways, (1) by the study of hybrids, and in particular their derivatives (as in *Lolium-Festuca* and *Allium* above), (2) by investigating inbred lines produced from an organism that normally outbreeds (as in rye), (3) by practising selection for a chromosome character (which has not yet been attempted), (4) by establishing positive correlations between parents and offspring (Müntzing and Akdik, 1948), (5) by the study of genotypes unbalanced by supernumerary chromosomes, as in *Sorghum* (Darlington and Thomas, 1941).

Segregation for chromosome characters in the derivatives of a hybrid or inbred organism reflects genotypic control ; and the manner in which these characters segregate may be expected to reveal something of the nature of the control, *e.g.* whether monogenic or polygenic. Response to selection or the establishment of positive parent-offspring correlations for a particular chromosome character would also conclusively demonstrate genotypic control. Lastly, where extra chromosomes are used, both their influence and constitution can be determined, *e.g.* in *Sorghum* the supernumeraries which cause extra mitoses in the pollen are heterochromatic.

The following account concerns inbred rye, which is suitable

material for the purpose in that (1) the chromosomes are few ($2n = 14$), large and easy to handle, (2) the plants are economical to grow in terms of space and labour, and (3) under natural conditions the plants are virtually complete outbreeders.

A normal rye population is heterogenic by virtue of its outbreeding system: and its efficiency as a population will have been achieved by a selection for efficient heterozygotes. Experimental inbreeding gives rise to homozygous plants, a genetic condition which does not occur and hence is never exposed to selection under normal circumstances. In this enforced, unnatural, relatively homozygous state there is, theoretically, good reason to expect phenotypes that do not compare favourably with the population average; the heterozygous genetic balance that had been built up by selection has been replaced by a homozygous condition that, being untested, is genetically unbalanced (Darlington and Mather, 1949). In practice this has been confirmed by following the progress of inbreeding in morphological, and also cytological, characters. Lamm (1936) was the first to show that the chiasma frequency in p.m.c. of inbred plants is characteristically lower than in population plants. In addition a relatively high proportion of p.m.c. in the inbred plants are structurally abnormal at meiosis. The fertility of inbred plants is reduced in consequence.

Inbreeding experiments should, however, do more than reveal the deleterious effects on the chromosomes that are due to the breakdown of the genetic system. They should show segregation for characters that are genetically controlled. This segregation would lead to increasing diversity between inbred lines, and at the same time to a greater uniformity of the plants within lines. Where this can be demonstrated for a nuclear property, genetic control is proved. This is the principle underlying the experiments to be described.

Line differences in chromosome pairing and contraction are indicated by Lamm's results in 1936. In 1948 Müntzing and Akdik showed that in the second and third selfing generations there are significant differences between inbred lines for one of the chromosome characters which they studied, namely the average number of chromosome arms paired per cell at first metaphase of meiosis. They found no line differences for other characters at meiosis. It is evident, however, from the work of Prakken and Müntzing (1942) on neo-centric, or T-end, chromosomes, that line differences exist for other chromosome characters in rye. Inbred lines differ in the presence and in the intensity of neo-centric activity. While our chief aim is to inquire further into the effect of the genotype on chromosome behaviour in rye, the characters used for this purpose are in themselves instructive. They throw light on the causal sequences producing chromosome abnormalities, and they will therefore be described in some detail.

2. MATERIAL

The basic inbred lines described were taken at random from material kindly presented to the department by Professor Müntzing. They are derived from a population of the Swedish variety Stålråg, and their history is set out in table 1. Two pairs of inbred lines originated from two population plants (A and B) grown

TABLE 1

The history of the inbred lines (see text). A, B and C are the three population plants of the variety Stålråg, from which the lines were derived by self pollination

in 1926. The other line comes from a population plant (C) grown in 1928. All have been inbred by continuous self pollination. At Birmingham in 1948, the two pairs of inbred lines from A and B were split and sub-lines started from sister plants within each line.

3. METHOD

All the observations were made at meiosis in p.m.c., chiefly at first metaphase and anaphase. The chromosome characters followed will be described in the next section. Meiosis has been studied in five plants from each of the eight sub-lines derived from A and B, and in five plants in the line started from C. The fixations were made at intervals of about five days, from 17th June to 20th August 1952.

For cytological examination whole heads were fixed and mordanted in acetic alcohol containing a few drops of aqueous ferric chloride solution. After a week the heads were put into 70 per cent. alcohol and stored at 0° C. Squashing, and staining with aceto-carmine, then produced satisfactory preparations.

4. THE CHARACTERS

(i) Numerical

Chiasma frequency.—The scoring was made at metaphase in 20 p.m.c. in each plant. In squash preparations there is little difficulty in interpreting the bivalents, although some selection of the best cells has often to be made. The values are expressed as the average chiasma frequencies per cell.

Terminalisation.—It is usually possible to distinguish between the terminal and intercalary chiasmata with reasonable accuracy at metaphase, but the scoring is perhaps not as precise as for the frequency of chiasmata. The terminalisation values were obtained by expressing the terminal chiasmata per cell as a proportion of the total number of bivalent arms paired per cell. Such proportions give a truer

picture of terminalisation than when terminalisation is expressed in relation to total chiasmata. This is made clear in considering ring bivalents at metaphase with two chiasmata and three chiasmata. In each case the maximum number of chiasmata which could be terminalised is two. Yet, given maximum terminalisation in both types of bivalents, if this is represented in relation to total chiasmata the values for the two bivalents is different, 1 and $\frac{2}{3}$ respectively. When, on the other hand, the proportion is terminalised chiasmata to the total of arms paired, the two kinds of bivalents have the same value, 1; and this more correctly indicates the terminalisation

FIG. 1a, b, c, e.—First metaphase showing different degrees of chromosome pairing and coiling. c. Six univalents; nucleic acid starvation near to the chromosome ends in the two rod bivalents. d. First anaphase where the chromosomes are poorly coiled. Two univalents have divided late. $\times 1100$.

capacities of the two bivalents. The method is useful here where there is a large variation in the chiasma frequencies of the plants compared. The proportions derived in this way were then transformed to angular values so that the results could be treated by simple averaging (Mather, 1943).

(ii) Structural

Uncoiled chromosomes.—The degree of uncoiling varies (figs. 1b, c, e). For the purpose of classification only the more extreme cases of uncoiling (as in figs. 1c and e) were taken into account. Cells with uncoiled chromosomes form about one per cent. of the total in one

anther, side by side with normal cells. Their chiasma frequencies are characteristically lower than the average for the anther (graph 1). Poor coiling and poor pairing suggest a reduced precocity of meiosis in these cells.

Uncoiling, however, is not always associated with low pairing. The different kinds of asynaptic cells indicate, as might be expected, more than one cause of pairing failure.

In a few cells where uncoiling is extreme a number of chromosomes show nucleic acid starvation near to the ends (fig. 1c). These starved areas correspond to the heterochromatin mapped by Levan (1942) in haploid and also in cold-treated rye.

GRAPH 1.—Relationship between the chiasma frequencies of single uncoiled nuclei and the plant averages. The regression is significant at approximately the 5 per cent. level.

Bridges without fragments.—Anaphase bridges of this kind are the result of splitting errors. The regions at which the chromosomes stick at anaphase are invariably confined to the ends or near ends of the chromosomes (figs. 2a, b, c, f). This localisation of error may well be controlled by the centromere in the manner deduced from the behaviour of X-rayed chromosomes in *Scilla* (Rees, 1953). Alternatively, the separation errors may coincide with, and be determined by, the heterochromatin near the ends (*cf.* cold-treated *Trillium* root tips, Darlington and La Cour, 1940). In some plants as many as 16 per cent. of first anaphases are affected. Similar bridges are common in inbred maize (Blanco, 1948).

In all cases the anaphase sticking involves two of the four strands (figs. 2a, b). The cause may be a local failure of reproduction of the chromosome thread or its nucleic acid sheath, or an abnormal chiasma

formation (Darlington, 1949). Failure to split can cause breakage by tension (fig. 2c).

In one plant one case of intercalary sticking was seen at second anaphase. It is probable that terminal splitting errors may be responsible for other bridges found at the second division in this and other plants.

FIG. 2a, b, c.—Splitting errors at first anaphase. In c the tension due to the splitting error has resulted in breakage during separation. f. Intercalary splitting error at second telophase. d, e. Bridges and fragments at anaphase in P13. g. B' breaks in P13 bivalents at first metaphase. The acentrics remain attached by chiasmata. One break is interstitial, the others at or near the centromere. $\times 1100$.

Bridges with fragments at anaphase.—These are found in most plants (table 4). Rare bridges and fragments could possibly be explained by crossing over in segments heterozygous for inversions; for even

after prolonged inbreeding a small amount of residual heterozygosity might be expected.

In sub-line P₁₃, however, each plant has 20-50 per cent. of its p.m.c. containing from one to four acentric fragments with or without bridges at first anaphase (figs. 2*d*, *e*). If this is due to inversions it means either :—

- (i) high structural heterozygosity of the original population parent and intense selection against structural homozygotes, or
- (ii) an accumulation of inversions by breakage and reunion during the course of inbreeding, and their maintenance in the heterozygous state by selection against the survival of homozygotes.

On either view it has to be assumed that the inbred plants are heterozygous for considerable lengths of the chromosomes. Evidence of two kinds will be provided that inversion heterozygosity is not in fact a satisfactory explanation for the bridges and fragments in this sub-line.

1. Sub-line P₁₄ was derived from a sister plant to that from which sub-line P₁₃ was continued, only four generations before this analysis (table 1). Yet in P₁₄ 7 per cent. is the maximum frequency of cells found to contain fragments and bridges at anaphase, the average for the five plants being less than 6 per cent. If P₁₃ plants were inversion heterozygotes their persistence would have depended on the structural homozygotes being more or less lethal. There is no suggestion of lethality in P₁₄ plants, yet the scarcity of bridges and fragments indicates that they are structurally relatively homozygous.

2. Four F₁s from crosses between plants of sub-line P₁₃ and another line show fragments and bridges in a maximum of 2 per cent. p.m.c. Assuming P₁₃ plants to be heterozygous for a number of inversions, the F₁s would be expected to show good evidence of these.

In the face of these facts inversion crossing over appears inadequate to explain anaphase bridges and fragments in P₁₃.

“Intra-haploid pairing” could explain bridges and fragmentation (Darlington, 1937); but it is not likely in this case in view of the relatively high chiasma frequency in P₁₃ which indicates good pairing. It may well be that in other lines where pairing is poor, bridges and fragments do arise from this cause.

Splitting errors account for some (less than 5 per cent.) of the bridges and fragments at first anaphase. The remainder, it is suggested, are due to a genetically controlled chromosome breakage (with or without reunion) at first prophase of meiosis, the acentric fragments being held in the bivalents until anaphase by virtue of coiling (*cf.* X-rayed meiosis in *Locusta*, White, 1937) and chiasmata. Reductional and equational separation for the points of sister reunion would give second and first division bridges respectively. Prophase breakage is

in fact detectable at metaphase in about 1 per cent. of P13 bivalents (fig. 2g).*

On this view, too, a divergence is indicated between sub-lines P14 and P13, but it need not be of the same order implicit in assuming that the bridges and fragments were due to structural heterozygosity. Indeed the divergence, with respect to this one character, could be due to the segregation of a single gene difference when the sub-lines were separated. It would of course imply heterozygosity of the parent from which the sub lines arose, or possibly, mutation subsequent to splitting the sub-lines.

Errors at the pre-meiotic mitosis.—Pre-meiotic disturbances have been inferred in inbred and population rye by Müntzing and Akdik (1948), Müntzing and Prakken (1941). The consequences of such irregularities have been studied in some detail at meiosis in one of our inbred lines.

Where pre-meiotic abnormality does occur it affects about one per cent. of the p.m.c. In these cells a number of extra chromosomes or fragments accompany the normal seven pairs. The extras range from one acentric fragment (fig. 3c) to many centric fragments and even complete extra bivalents and univalents (fig. 3h). All are enclosed in one cell wall. The presence of extra unbroken chromosomes and centric fragments indicates that a spindle abnormality accompanies breakage, so that the cell wall formed does not equally separate daughter nuclei. Cell walls occasionally fail to form independently of breakage. Then the p.m.c. are fully binucleate. But when this happens, one nucleus can often be distinguished from the other (fig. 3h): at metaphase its chromosomes are less contracted and more faintly stained and have fewer chiasmata. Although the two nuclei share the same spindle, no multivalents occur, so that pairing must take place within separate nuclear membranes. This is true also where the extra chromosomes do not make up a full set, and micronuclei can in fact be seen separate from the normal nucleus at pachytene and early diplotene (figs. 3a, b).

Like the chromosomes of many extra complete nuclei, those of micronuclei can clearly be distinguished at metaphase from the normal set, and for the same reasons (figs. 3c, d, e). Thus even though the extra chromosomes, whether they are complete or broken, reach a metaphase condition simultaneously with those of the normal nucleus, their reduced coiling and staining, and comparative asynapsis indicate a delay at earlier stages. This in fact is what happens (figs. 3a, b). At diplotene and diakinesis the extra chromosomes and fragments are at a stage corresponding to pachytene. This initial delay must be followed by great acceleration in the division cycle of a micronucleus to enable its chromosomes to coil and be ready to move on to the spindle at the same time as those of the normal nucleus in the same cell. Initial delay of retarded nuclei followed by an acceleration of the division process has been inferred by Darlington

* Details of this breakage will be published in a later paper.

and La Cour (1945) in X-rayed *Tradescantia* pollen grains, and observed in p.m.c. of *Scilla* that were asynaptic and showed chromosome breakage (Rees, 1952). In *Scilla* it was suggested that the retarded nuclei were stimulated to division by the actively dividing healthy nuclei.

FIG. 3.—Extra chromosomes and fragments in p.m.c. of P4 and P5. *a, b.* Retarded micronuclei at diplotene. In *b* the extra chromosome shows pairing at three places, including ends, between its own segments. *c.* Extra, thin acentric fragment at metaphase. *d.* Extras are univalents or fragments. *e.* Extra bivalent, univalents and fragments. *f.* Extra piece retarded at metaphase. *g.* One micronucleus retarded, the contents of another in a more advanced state of coiling on the metaphase plate. Note starved heterochromatin in the latter. *h.* Two complete nuclei in one p.m.c. One is distinguishable from the other, being less densely stained, relatively uncoiled and having only four chiasmata. $\times 1100$.

nuclei surrounding them. The same can be inferred for the micronuclei in rye p.m.c. It is all the more likely in this case since the healthy and sick nuclei share the same cytoplasm, whereas in *Scilla* and *Tradescantia* they occupied different cells.

Delay followed by acceleration is true of most but not all micronuclei. In three out of about a hundred abnormal metaphases, the contents of the micronucleus have not caught up with the chromosomes of the normal nucleus. They still appear as early prophase chromosomes (fig. 3f). The question arises why some micronuclei can reach a metaphase condition and others not. The cytoplasms of different cells containing micronuclei might differ in constitution. Fig. 3g, however, shows both kinds of behaviour in the same cell. Evidently the explanation must be sought inside the micronuclei themselves.

The absence of a centromere in the contents of a micronucleus does not appear to prevent it from reaching a "metaphase" condition (e.g. fig. 3g). Neither does the absence of a nucleolus; the majority of diplotene micronuclei lack one, yet the majority reach "metaphase". There is evidence (John Innes Report, 1951) that *Vicia* and *Hyacinthus* micronuclei, produced by chemical and X-ray treatment respectively, keep going when they contain both a nucleolus and a piece of heterochromatin. In rye the nucleolus is ruled out, and it may be therefore, that heterochromatin is the essential constituent. Whether all the "successful" micronuclei contain heterochromatin is not known. That many do so can be seen where it shows up as starved bands (fig. 3g).

The metaphase configurations in cells with broken chromosomes are explained by chromatid (B') breaks, with or without reunion, at the pre-meiotic mitosis. As the breakage occurs a division before the cells are examined, it is perhaps not surprising that the breakages seen are of the B' type. If B'' (chromosome) breaks occur, both daughter nuclei will be deficient and less likely to undergo another cell division.

To account for the inclusion of unbroken chromosomes and centric chromosome fragments in micronuclei, it was suggested earlier that a spindle abnormality generally accompanies the breakage at the pre-meiotic mitosis. Since the micronuclei are found in cells in which the main nuclei are perfectly normal, one pole must have been a proper one, the other disturbed, and probably split. This suggests a gradient within the cell such that the cytoplasm at one end is actively different from that at the other end. Now, two complete nuclei in one cell, it will be remembered, are often phenotypically different at first metaphase. This is precisely what would be expected if a cytoplasmic gradient of the kind suggested exists. Such a gradient could account for the invariable delay of micronuclei at the early prophase of meiosis. The micronuclei are derived from the postulated disturbed pole of the cell. Their behaviour, which is similar to that of one of the two complete nuclei in binucleate cells, may reflect the persisting influence of their cytoplasmic environment in the parent cell. Intra-cell cytoplasmic gradients that differentiate nuclei are, of course, well known, particularly in pollen grains (La Cour, 1949; Darlington and Mather, 1949).

Mobile chromosome ends.—The property of active movement of certain chromosome ends in rye has been dealt with at great length by Prakken and Müntzing (1943), Ostergren and Prakken (1946)

FIG. 4.—Neo-centric activity in P4 and P5. *a*. Prometaphase with the neo-centric ends of one bivalent directed to one pole. *b*. Co-orientation between neo-centric ends and centromeres of the same chromosome in a bivalent at prometaphase. *c*, *d*. Neo-centrics at first metaphase. *e*. First anaphase with neo-centric ends leading to the poles. *f*. Second anaphase with four neo-centric ends. *g*. P bivalents in P13. All are of the kind with sub-terminal centromeres. On the extreme left is one with a proximal and a distal chiasma in each arm. $\times 1100$.

and Kattermann (1939). Similar cases are known in maize (Rhoades and Vilkomerson, 1942), and in *Bromus* species hybrids (Walters, 1951, 1952).

Active ends are to be seen at both meiotic divisions in sub lines P₄ and P₅ (figs. 4a-f). As Prakken and Müntzing point out, it is mainly the short arms of the chromosomes that show such activity (fig. 4d). Where they occur the neo-centric ends are most often found in one pair of chromosomes only, but in one plant they were seen in five pairs of the same cell.

Diffusion of substances secreted at the centromere to the ends of the chromosome has been given as the cause of neo-centric activity (see Rhoades, 1951). The physiological implications are discussed at length by Darlington (1951).

Proximal chiasmata: P. bivalents.—As a rule few chiasmata form near to the centromeres of rye bivalents. The differential distance (Mather's *d*, 1937) is long. In some plants, however, at first metaphase as many as one fourth of the p.m.c. have one bivalent (henceforth P bivalents), rarely two, in which chiasmata are found in roughly the first proximal quarter of the chromosome arms. This feature is confined to the three bivalents with centromeres relatively sub-terminal (fig. 4g).

Proximal localisation would result from an alteration in the points of initial contact between pairing homologues; from distal, as in normal rye bivalents, to proximal in P bivalents (*cf.* Frankel, Darlington and La Cour, 1940). Distal as well as proximal contact is indicated in more than 90 per cent. of P bivalents where a second chiasma forms towards the end of the long arm. That P bivalents are always of the sub-terminal centromere kind demonstrates how the differential distance can vary independently within a chromosome set. Evidence will be provided for the genetic control of this character. Genetic control of the localisation of chiasmata is, of course, well known in *Allium* (Emsweller and Jones, 1945, and others), and in one species, *Allium nutans*, localisation has appeared, as in rye, by inbreeding (Darlington, 1937).

Out of 29 p.m.c. with one P bivalent, five showed the P bivalents lying off the metaphase plate. No cases of non-disjunction were found at anaphase, so that the P bivalents rarely fail ultimately to congress in time for proper separation. Their relative slowness in moving on the spindle indicates, however, that the close proximity of the chiasmata to the centromeres interferes with movement. This interference is comparable to the case in spiders (Revell, 1947). There the bivalents at meiosis move on to the metaphase plate in a sequence which is inversely related to the differential distance.

Giant cells.—Coenocytic cells occur in three lines. They represent less than one per cent. of p.m.c. and contain on average about six nuclei. The coenocytes result from cell wall failures, beginning two or three mitoses before the first meiotic division. Similar giant cells were found by Peto at meiosis in *Lolium-Festuca* hybrids (1933).

Evidence of spontaneous breakage: Acentric fragments at first metaphase.—These have been found (other than where they result from pre-meiotic breakage as described earlier) in rare single cells in very few anthers.

Bivalent interlocking was also noted.

5. METRICAL AND MERISTIC CHARACTERS

There is an important distinction between the characters listed as structural and those listed as numerical. This is made clear on considering pre-meiotic errors and chiasma frequency respectively as representatives of the two groups.

Where pre-meiotic errors occur the majority of p.m.c. within a plant anther are perfectly normal, the minority abnormal, *i.e.* showing extra chromosomes or fragments. The intra-plant variation is discontinuous, and two classes of cells exist side by side. This character, like others in the structural group, can be described as meristic.

In contrast, the intra-plant distribution of chiasmata per cell is a continuous one around the mean value. There is no sharp distinction between cell classes with few and with many chiasmata. The same holds for terminalisation values. These characters can be described as metrical. In the strictest sense, of course, chiasma frequency shows meristic variation in that there are many classes of cells in one anther, but as the differences between the cell classes are so small, the characters can conveniently be treated as metrical.

Since p.m.c. within an anther are genetically identical, the variation between them must be non-genetic, being due presumably to minute differences in conditions within the anther. As we have seen, there can be variation of more than one kind. There is continuous variation around the mean for metrical characters. For meristic characters the p.m.c. comprise two categories, normal and abnormal. The control of meristic characters is evidently explained by a threshold type of reaction that is common for structural abnormalities at meiosis (*cf.* Darlington and Upcott, 1941). The threshold determines whether the development of the p.m.c. will be phenotypically normal or abnormal. All the p.m.c. in the structurally abnormal plants are genetically potential variants from normal. Whether this potentiality is realised in any cell is decided by its immediate environment within the anther. Beyond a certain range of conditions it will be abnormal, within this range, normal.

There may evidently be an important difference between meristic and metrical characters in their response to selection. For metrical characters most p.m.c. will reflect more or less closely the genotypic potentialities of the individual. Where, for any reason, there may be a selection for or against the mean value of the character this selection will be effective in most of the cells. Response will be relatively quick. Contrast this with meristic characters, such as pre-meiotic errors. Here the control is such that the genetic potentiality for abnormality is realised only in a small proportion of p.m.c. Since selection acts on phenotypes, it is clear that whatever the advantage of the character in question, response to selection will be less effective because the phenotype of the majority of the cells in no way reflects their genetic difference from normal.

6. ANALYSIS OF THE INBRED LINES

(i) *Environmental variation*

Since the fixations for meiosis were extended from 17th June to 20th August, fluctuations in the weather may account for part of the variation observed in chromosome characters. There is no satisfactory way of eliminating this variation when, as in this instance, the plants are grown out of doors. The plants were sown at the same time and in so far as the different rates of maturity of the inflorescences, and

GRAPH 2.—The distribution of mean chiasma frequencies in the inbred lines. Each pair of sub-lines bracketed together was derived from the splitting of an inbred line four years previous to the analysis. Note similarity between members of pairs of sub-lines.

consequently the dates of fixation, affect the behaviour of chromosomes, the variation from this cause is in a sense heritable because the rate of development of the inflorescences is itself genetically controlled. This kind of variation is, however, as we shall see, of little importance in comparison with the variation due to genotypic control exercised more directly at the p.m.c. level. Nevertheless, an estimate of the amount of variation due to the effects of fixing on different days is desirable. This has been made, for chiasma frequency, by comparing plants from an F_3 generation that were fixed on seven separate days covering most of the fixation period, *viz.* 17th June

to 27th July.* An analysis of variance showed that the effect of fixing the plants on these separate days contributes very little to the total variation in chiasma frequency. It is not significant. On the strength of this evidence the method adopted will be based on the assumption that where differences can be shown between lines for nuclear characters they can be accepted with confidence as being predominantly genetic in determination.

TABLE 2

The average chiasma frequencies in 45 inbred plants. Each pair of sub-lines bracketed together is derived from an inbred line that was split four years previous to the analysis (see table 1)

Population ancestor	C	A				B			
Lines and sub-lines	P ₂	P ₄	P ₅	P ₇	P ₈	P ₁₃	P ₁₄	P ₁₇	P ₁₈
5 plants in descending order of chiasma frequency	14.55 14.30 14.30 14.10 12.50	12.50 12.15 11.90 11.00 10.15	12.35 12.15 11.80 10.80 9.90	13.10 12.45 12.35 12.35 12.20	12.80 12.10 12.05 11.70 11.50	13.85 13.80 13.40 13.30 13.15	14.25 13.70 13.30 13.05 12.95	12.00 11.75 11.65 11.45 11.00	12.25 11.65 11.50 11.40 10.65

(ii) Numerical characters

Chiasma frequency.—The average frequency per cell is given for each plant in table 2. Five lines are represented, four of them by a pair of sub-lines each. The data are also represented in graph 2.

Below is an analysis of variance for differences between lines and between sub-lines within lines.

Item	SS	N	MS	VR	P
Between lines	39.874	4	9.969	23.238	0.001
Sub-lines within lines	0.594	4	0.149	...	...
Error	15.439	36	0.429	...	...
Total	55.907	44	...	...	...

The differences in chiasma frequencies between lines are highly significant ($P = <0.001$) when compared with the differences between individuals within lines. There is no doubt that the lines differ genetically in the control of this character.

* F_3 plants for the purpose of this estimation have three important advantages: (1) they are partly inbred, (2) they comprise a number of diverse families. They should therefore react to environmental changes more like the inbred lines than population plants used as a control. (3) The maturing of plants within F_3 families is not uniform to the same extent as in most of the inbred lines. Consequently, F_3 fixations on any one day, in contrast to those of the old inbred lines, tend to be a random sample of inbred plants from more than one family. Hence environmental variation can be more efficiently separated from the heritable and estimated.

There is no evidence of significant differences between the sub-lines within lines. At the time of splitting into sub-lines the four lines had undergone twenty-three generations of selfing. If there were significant differences between the sub-lines derived from any one line it would suggest some residual heterozygosity at the time of splitting. There is no evidence of this for the genes controlling chiasma frequency.

TABLE 3

*The average terminalisation per cell in the inbred lines, expressed in angular values.
The order of plants is the same as for table 2*

Population ancestor	C	A				B			
Lines and sub-lines	P ₂	P ₄	P ₅	P ₇	P ₈	P ₁₃	P ₁₄	P ₁₇	P ₁₈
Average terminalisation	56	57	55	58	55	52	63	61	62
	53	54	56	57	57	54	60	64	61
	60	60	57	60	58	51	59	60	64
	59	54	56	58	56	51	61	65	64
	59	63	58	61	60	53	67	63	66

The analysis of variance follows :—

Item	SS	N	MS	VR	P
Between lines	253.459	4	58.865	10.742	0.001
Sub-lines within lines	249.285	4	64.821	11.829	0.001
Error	197.287	36	...	...	...
Total	692.031	44	...	...	...

Terminalisation.—The values were first calculated as proportions of terminalised chiasmata to the total number of bivalent arms paired per cell. The proportions were then converted to angular values. For each plant, the average value per cell is calculated from 20 p.m.c. They are shown in table 3 and graph 3.

Differences between lines are again highly significant, but so also are differences between sub-lines within lines in this instance. The significant difference within lines is accounted for by sub-lines P₁₃ and P₁₄ (see graph 3). When the sum of squares for their differences (and the one degree of freedom relating to it) are taken away, the differences within lines are not more than would be expected by chance. The new mean square for within lines is then 4.737, a value smaller than the error mean square. The sum of squares for the difference in terminalisation between P₁₃ and P₁₄ is 245.075, the Variance ratio 44.722, and the probability for getting such a large difference by chance less than 0.001.

From this analysis of terminalisation values there is (1) clear evidence for genetic differences between lines, (2) evidence of a divergence between sub-lines P₁₃ and P₁₄ which suggests some residual heterozygosity of the line from which they were derived four generations earlier. Earlier, a genetic divergence between P₁₃ and P₁₄ was indicated with respect to anaphase bridges and fragments : this supports the view that the line at the time of splitting was heterozygous. Such heterozygosity could result from gene mutation, or

GRAPH 3.—The distribution of mean terminalisation values in the inbred lines. Note the difference between P₁₃ and P₁₄. No differences are indicated between members of other sub-lines.

from intense selection against homozygotes, or both. Residual structural heterozygosity in P₁₃ was earlier considered to be extremely unlikely.

(iii) Structural characters

The distribution of abnormal chromosome characters in the inbred lines is shown in table 4. The method of demonstrating genotypic control will be essentially the same as for the numerical characters, namely to compare the variation between individuals within lines with the variation between lines.

Within lines.—It is clear from the table that plants within lines, and within sub-lines, are phenotypically heterogeneous for many of

these characters. Not only do plants within sub-lines and lines differ in that some do and others do not show a particular character ; there may also be marked differences in the expression of this character. For instance, in sub-line P₅ four plants out of five have p.m.c. with neo-centric ends at meiosis, the other has not. Of the plants with neo-centrics one has two, sometimes three, neo-centric end bivalents in some of its cells, whereas the others have not more than one in any p.m.c. Further, there is variation in the intensity of neo-centric activity. In some plants, the neo-centric activity is confined to a small proportion of p.m.c. and is so weak as to be hardly distinguishable ; in others it is strongly expressed in a high proportion of the cells.

TABLE 4

The distribution of structural abnormalities at first metaphase and anaphase in the inbred lines. Five plants from each sub-line were scored for the metaphase characters. Where fewer than five plants were scored for anaphase characters this number is given in brackets. The figures in the columns represent the number of plants in the appropriate sub-line in which a particular abnormality appeared

Lines and sub-lines	P ₂	P ₄	P ₅	P ₁₃	P ₁₄	P ₁₇	P ₁₈	P ₇	P ₈
M ₁ —									
Neo-centrics . . .	...	5	5	...	...	...	...	...	...
Pre-meiotic breakage	...	4	4	...	...	...	...	...	...
P bivalents . . .	4	...	...	5	1	...	1	...	...
Uncoiling . . .	3	3	3	4	3	1	...	1	...
Accentrics . . .	...	...	...	4	1	1	...	...	...
Giant cells . . .	1	1	...	3	...	...	...	...	...
Interlocking . . .	2	...	4	1	...	...	...	2	...
A ₁ —									
Bridges . . .	3 (4)	4 (4)	2 (4)	5	3 (4)	4 (4)	4	3 (4)	4 (4)
Bridges and ff. . .	4 (4)	4 (4)	2 (4)	5	2 (4)	3 (4)	5	3 (4)	4 (4)

Phenotypic heterogeneity of this order within an inbred line, however, does not necessarily mean that genotypic differences occur within it. Indeed heterogeneity may be expected for the structural characters in view of the threshold type reactions, described earlier, that control them. The threshold differentiates genetically equivalent nuclei within the same anther, and also causes differences between anthers. For example, one plant, P₄⁴, showed distinct neo-centrics in two anthers, none in another anther. All three anthers were fixed on the same day. In another plant, there were pronounced but rare neo-centrics in one anther, none in two others. These three anthers also were fixed at the same time. Evidently where this kind of variation exists between genetically identical cells, and anthers, of the same plant, it is not surprising to find phenotypic differences between plants of the same sub-line. In other words, the qualitative phenotypic differences are not in every case reflections of genotypic differences.

Between lines and sub-lines.—Bearing this limitation of classification in mind, genotypic differences between lines can be concluded where a character is restricted to, or significantly more frequent in, particular lines.

It is clear from table 4 that neo-centric activity and pre-meiotic errors in our sample are restricted sharply to P₄ and P₅. There is, too, undoubted heterogeneity with respect to P bivalents; they are found mainly in P₁₃ and P₂. Genotypic line differences for these three characters can be concluded with confidence. In sharp contrast some other characters, like splitting errors, are widespread throughout all lines and sub-lines. Considering the characters one by one, the probability of the lines being significantly heterogeneous for any one of them can be found by using a $2 \times n$ contingency table (see Mather, 1943). The analysis, in view of the smallness of the numbers involved, is not entirely satisfactory, but is nevertheless the most reliable in the circumstances. The analysis is given in full for neo-centrics (table 5).

TABLE 5
The contingency table for the distribution of neo-centric activity in the inbred lines

Lines and sub-lines	P ₂	P ₄	P ₅	P ₇	P ₈	P ₁₃	P ₁₄	P ₁₇	P ₁₈	Total
Neo-centrics	...	5	3	...	...	...	...	...	...	8
No neo-centrics	5	...	2	5	5	5	5	5	5	37
Total	5	5	5	5	5	5	5	5	5	45

The "between line heterogeneity" $\chi^2_{[4]}$ (there being five lines) is 34.053 and is highly significant ($P = < 0.001$).

The heterogeneity $\chi^2_{[4]}$ for differences between sub-lines within lines can be calculated by subtracting the $\chi^2_{[4]}$ for lines (34.053) from the $\chi^2_{[8]}$ for the nine sub-lines (36.791). This gives 2.738 for $N = 4$ and is not significant ($P = 0.7-0.5$). It is concluded, therefore, that, (1) lines are significantly heterogeneous with respect to neo-centrics, and (2) sub-lines within lines are not heterogeneous.

Shown overleaf (table 6) are the heterogeneity χ^2 s and probability values for lines, and sub-lines within lines, for the "metaphase" structural characters. There is no suggestion of qualitative difference between lines for the anaphase characters and a test of significance is unnecessary.

The table shows that there are undoubted differences between the lines for the characters neo-centric ends, pre-meiotic errors and P bivalents (the probability in each case being less than 0.001). Line differences for acentric fragments and uncoiling are also significant, at the 0.01 level. The probabilities (0.2 to 0.1) are not significant for giant cells and interlocking.

For differences between sub-lines within lines there is significance at the 0.02 level with respect to interlocking. This is almost entirely attributable to the difference between P₄ and P₅. Their difference contributes a $\chi^2_{[1]}$ of 10.000 to the heterogeneity χ^2 of 13.125 for the sub-lines within lines. When this $\chi^2_{[1]}$ of 10.000 is removed, the $\chi^2_{[3]} = 3.125$ for the remaining sub-lines within lines and is not significant ($P = 0.5-0.3$). It is possible that P₄ and P₅ are genotypically divergent, but the possibility, in the absence of other evidence, must be treated with some caution. For P bivalents, although the $\chi^2_{[4]}$ for sub-line heterogeneity is not significant (9.203, $P = 0.10-0.05$), the individual heterogeneity $\chi^2_{[1]}$ for P₁₃ and P₁₄ is significant (9.148, $P = 0.01-0.001$). In view of the evidence already put forward indicating a divergence between these sub-lines, their heterogeneity with regard to P bivalents may well be genotypic. The heterogeneity χ^2 s for the other characters are compatible with genotypic uniformity within lines.

TABLE 6

The χ^2 and probability values for heterogeneity between lines, and sub-lines within lines, for metaphase structural characters in the inbred lines. ($N = 4$ in all cases)

Character	Between lines		Between sub-lines within lines	
	χ^2	P	χ^2	P
Neo-centrics . . .	34.053	< 0.001	2.738	0.7-0.5
Pre-meiotic errors . . .	34.054	< 0.001	0	...
P bivalents . . .	22.802	< 0.001	9.203	0.1-0.05
Acentric fragments . . .	15.577	0.01-0.001	8.654	0.1-0.05
Uncoiling . . .	13.750	0.01-0.001	1.250	0.9-0.8
Giant cells . . .	6.526	0.2-0.1	10.124	0.05-0.02
Interlocking . . .	6.875	0.2-0.1	13.125	0.02-0.01

The lines can also be differentiated on a quantitative basis with respect to these structural characters. Thus 3 per cent. to 27 per cent. of p.m.c. in P₁₃ and P₁₄ plants have P bivalents. Elsewhere they occur in less than 1 per cent. of p.m.c. In the case of anaphase bridges and fragments, although they appear in all lines and sub-lines, their frequencies differ. In P₁₃ plants 100 first anaphases contain, on average, 37 fragments (never less than 20). In the 40 other inbred plants the average for 100 cells is much less than 10. In P₁₃ it has been inferred to be the result of a chromosome breakage not due to inversion crossing over, whereas inversion crossing over, and intra-haploid pairing, probably account for the comparatively rare anaphase fragments in other inbred lines.

The following conclusions can be drawn for the structural characters in the inbred lines :—

1. There are highly significant differences between the lines for (a) neo-centric ends, (b) pre-meiotic errors, (c) P bivalents.

2. There are probable line differences with respect to, (a) uncoiling, (b) acentric metaphase fragments.

3. Line differences are also indicated by variation in the frequencies of occurrence of (a) P bivalents, (b) anaphase fragments. The cause of anaphase fragments also is probably different in at least one of the sub-lines, P13.

4. There is no evidence of heterogeneity for (a) giant cells, (b) bivalent interlocking, (c) splitting errors (anaphase bridges without fragments).

The pattern of abnormalities.—Table 4 shows not only that particular abnormal characters are more, or less, frequent in some lines than in others. It shows also how each line tends to have its own particular pattern of abnormalities. Thus metaphase of meiosis in P7 and P8 is relatively regular, other than for occasional uncoiling and interlocking. In P4 and P5 meiosis is irregular in various ways; so, also, in P13 and P14 but in clearly different ways. The different patterns emphasise, first, the divergence between lines, and, second, that the irregularities, following the breakdown of the efficient control of meiosis by inbreeding, occur in many combinations, any one of which is characteristic of one inbred line.

7. THE BREEDING SYSTEM, THE GENOTYPE AND THE CHROMOSOMES

The results show that chromosome behaviour at meiosis in rye is undoubtedly subject to control by the genotype. This has been demonstrated for chiasma frequency, localisation and terminalisation, and for a number of structural abnormalities.

From the continuous variation shown for chiasma frequency (*cf.* Müntzing and Akdik, 1948) and terminalisation (see tables 2 and 3, and graphs 2 and 3) it would appear that the control of both characters is polygenic. This does not mean that major gene differences governing pairing do not occasionally arise in a rye population. An "asynaptic" gene has in fact been inferred by Prakken (1943). For some structural characters, pre-meiotic errors and neo-centric activity, the pronounced qualitative differences between lines could mean that few genes are involved. Alternatively, these characters may be manifested only by genotypes with a limited number of the possible polygenic combinations that emerge with inbreeding.

Structural abnormalities, and also poor pairing, are rare in population plants and in F_1 s (Thompson, unpub.) as compared with inbred plants. Hence, whatever the precise genetic mechanism, their control is closely influenced by, and related to, the mating system, and, consequently, to the amount of heterozygosity. An outbreeding rye population by virtue of the high average heterozygosity of its members is genetically balanced, and buffered against these disadvantageous irregularities. This genetically heterozygous buffer has

no doubt been built up, encouraged by selection, under natural conditions of outbreeding. Its breakdown coincides with the breaking down of the normal outbreeding system. With increasing homozygosis the abnormalities emerge, their particular form and distribution, as we have seen, being governed by segregation so that, although in the inbred plants in general the chromosomes work less efficiently, in families and in lines they may be less efficient in different ways.

While differences between p.m.c. nuclei in different lines are genotypic, variation between p.m.c. nuclei within a plant is a result of divergence in development of genotypically similar nuclei. Local environmental variations, acting we may suppose, through the medium of the cytoplasm, stimulate the divergence; frequently, by virtue of threshold type reactions, into two sharply distinct classes of nuclei. Thus, intracellular cytoplasmic gradients, it was earlier suggested, are associated with pre-meiotic errors, initiating differentiation into normal and abnormal daughter nuclei at the pre-meiotic mitosis. Cytoplasmic gradients could equally well account for differentiation between p.m.c. normal and abnormal for other structural characters. The origin of such a gradient would be partly a property of the genotype, as well as the result of the environmental variation within the anther.

We have seen that p.m.c. nuclei at the same stage of development may be differentiated spatially within the anther. Differentiation in time also affects the action of genes causing abnormality. Whereas the nuclear abnormalities in inbred plants arise mainly at meiosis, P₄ and P₅ nuclei regularly go wrong at an earlier stage of development, the pre-meiotic mitosis. Gene action determining the nuclear phenotype then is dependent on the cytoplasmic environment of the nucleus, which changes in response to external stimuli, and in the normal course of differentiation during development.

A vital aspect of the genetics of chromosome behaviour is the relationship between chiasmata and genetic variability in a population. At the nuclear level the adjustment of chiasma frequency and localisation controls recombination, thereby regulating the release of genetic variability. Frequency and localisation of chiasmata, we know, are genetically determined. More knowledge of the details of their inheritance and, in particular, of their behaviour under selection, is a necessary future step to a better understanding of the genetic system in a population.

Finally, the investigation of the variation in chromosome phenotypes between individuals, between tissues, between cells within individuals, and even within nuclei offers a unique opportunity of defining more clearly the relations between genotype and phenotype, not only in heredity, but also in development.

8. SUMMARY

1. Meiosis in inbred rye is less efficient than in population rye. Inbred lines differ with respect to the following nuclear characters in the anthers :—

- (i) Frequency, terminalisation and localisation of chiasmata.
- (ii) Neo-centric activity.
- (iii) Pre-meiotic errors.
- (iv) Chromosome breakage at first prophase.

2. Variation between inbred lines indicates genotypic control of the nuclear phenotype. Chiasma frequency and terminalisation are polygenically controlled.

3. A divergence is shown between two sub-lines taken off a line that had been inbred by selfing for more than 20 generations. This is explained either by residual heterozygosity of the parent line, or by mutation.

4. Pre-meiotic errors, found in one line, are confined to about 1 per cent. of p.m.c. within an anther. The abnormal cells each contain a micronucleus, or occasionally a complete nucleus, in addition to the normal nucleus. The chromosomes of micronuclei are frequently broken; they are invariably retarded during first prophase; and they have a reduced chiasma frequency. At the end of prophase their division cycle is accelerated.

5. The origin and behaviour of the micronuclei are inferred to result from intracellular gradients in the cytoplasm of abnormal pre-meiotic cells. The gradients initiate differentiation between normal and abnormal cells, as well as between normal and abnormal daughter nuclei within the cells. Since, initially, antecedents of p.m.c. within an anther are genetically identical, variation between p.m.c. reflects environmental fluctuations affecting development.

It is a pleasure to thank Professor Mather for his advice. Also I am pleased to thank Mr B. I. Hayman for his help with statistical matters, and Mr J. B. Thompson for the use of some of his data.

9. REFERENCES

- BEADLE, G. W. 1933. Further studies in asynaptic maize. *Cytologia*, 4, 269-287.
- BLANCO, J. L. 1948. Abnormalidades meioticas en relacion con la consanguinidad en "*Zea mays*" L. *Cons. sup. inv. cient. Mision Biol. Galicia*. Madrid.
- DARLINGTON, C. D. 1932. The control of the chromosome by the genotype and its bearing on some evolutionary problems. *Am. Nat.*, 66, 25-51.
- DARLINGTON, C. D. 1937. *Recent Advances in Cytology*. London: Churchill.
- DARLINGTON, C. D. 1949. The working units of hereditary. *Hereditas*, Suppl. Vol., 189-200.
- DARLINGTON, C. D. 1951. Mendel and the Determinants. *Genetics in the 20th Century*. Macmillan: New York.
- DARLINGTON, C. D., AND LA COUR, L. F. 1940. Nucleic acid starvation of chromosomes in *Trillium*. *J. Genet.*, 46, 180-267.

- DARLINGTON, C. D., AND MATHER, K. 1949. *The Elements of Genetics*. London : Allen and Unwin.
- DARLINGTON, C. D., AND THOMAS, P. T. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. *P.R.S.*, B, 130, 127-150.
- DARLINGTON, C. D., AND UPCOTT, M. B. 1941. Spontaneous chromosome change. *J. Genet.*, 41, 297-338.
- EMSWELLER, S. L., AND JONES, H. A. 1945. Further studies on the chiasmata of the *Allium cepa* $\times$ *A. fistulosum* hybrid and its derivatives. *Am. Jour. Bot.*, 32, 370-379.
- FRANKEL, O. H., DARLINGTON, C. D., AND LA COUR, L. F. 1940. The causal sequence of meiosis. *J. Genet.*, 41, 9-64.
- John Innes Horticultural Institution Annual Report, 1951.
- KATTERMANN, G. 1939. Ein neuer Karyotyp bei Roggen. *Chromosoma*, 1, 284-299.
- LA COUR, L. F. 1949. Nuclear differentiation in the pollen grain. *Heredity*, 3, 319-338.
- LAMM, R. 1936. Cytological studies on inbred rye. *Hereditas*, 31, 217-240.
- LEVAN, A. 1936. Die Zygologie von *Allium cepax fistulosum*. *Hereditas*, 21, 195-214.
- LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. *Hereditas*, 28, 177-211.
- MAEDA, T. 1937. Chiasma studies in *Allium fistulosum*, *Allium cepa* and their F_1 , F_2 and backcross hybrids. *Jap. Jour. Genet.*, 13, 146-159.
- MATHER, K. 1937. The determination of position in crossing-over. II. The chromosome length-chiasma frequency relation. *Cytologia*, pp. 514-526.
- MATHER, K. 1943. *Statistical Analysis in Biology*. London : Methuen.
- MÜNTZING, A., AND AKDIK, S. 1948. Cytological disturbances in the first inbred generation of rye. *Hereditas*, 34, 485-509.
- MÜNTZING, A., AND PRAKKEN, R. 1941. Chromosomal aberrations in rye populations. *Hereditas*, 32, 473-494.
- OSTERGREN, G., AND PRAKKEN, R. 1946. Behaviour on the spindle of the actively mobile chromosome ends of rye. *Hereditas*, 32, 473-494.
- PETO, F. H. 1933. The cytology of certain intergeneric hybrids between *Festuca* and *Lolium*. *J. Genet.*, 28, 113-156.
- PRAKKEN, R. 1943. Studies of asynapsis in rye. *Hereditas*, 29, 475-495.
- PRAKKEN, R., AND MÜNTZING, A. 1942. A meiotic peculiarity in rye, simulating a terminal centromere. *Hereditas*, 28, 442-482.
- REES, H. 1952. Asynapsis and spontaneous chromosome breakage in *Scilla*. *Heredity*, 6, 89-97.
- REES, H. 1953. Centromere control of chromosome splitting and breakage. *Heredity*, Suppl. Vol., 236-245.
- REVELL, S. H. 1947. Controlled X-segregation at meiosis in *Tegenaria*. *Heredity*, 1, 337-347.
- RHOADES, M. M. 1952. Preferential segregation in maize. *Heterosis* ; Iowa.
- RHOADES, M. M., AND VILKOMERSON, H. 1942. On the anaphase movement of chromosomes. *Proc. Nat. Acad. Sci.*, 28, 433-436.
- WALTERS, S. M. 1951. Spontaneous chromosome breakage and atypical chromosome movement in meiosis of the hybrid *Bromus marginatus* $\times$ *B. pseudolaevipes*. *Genetics*, 37, 8-25.
- WALTERS, S. M. 1952. Atypical chromosome movement in meiotic anaphase of *Bromus pitensis* $\times$ *B. marginatus*. *Am. Jour. Bot.*, 39, 619-625.
- WHITE, M. J. D. 1937. The effects of X-rays on the first meiotic divisions in three species of Orthoptera. *P.R.S.*, B, pp. 124-196.