GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE

V. THE DISTRIBUTION PATTERN OF CHIASMATA BETWEEN POLLEN MOTHER CELLS

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1. INTRODUCTION

A RECENT paper (Rees and Thompson, 1956) described the variation in chiasma frequencies in *p.m.c.* of rye inbred lines and their F_1 hybrids. It was shown that the average chiasma frequencies vary between lines and between F_1 families ; they are genotypically controlled. Not all the chiasma variation, however, could be ascribed directly to genotypic differences. Part of it is not heritable and depends on environmental factors affecting chiasma formation. Such variation was detected and measured in three ways, by comparing (1) genetically identical plants within a family, (2) *p.m.c.* within the same anthers, and (3) bivalents within *p.m.c.* While the variation measured at these three levels must be determined by environmental (including cytoplasmic) fluctuations between and within plants we found that the degree of variation, that is the response to the varying circumstances, depends on the genotype. In general this was greater in the inbred homozygous lines. We concluded that the amount of variation exhibited at one level, *e.g.* that of the plant, could be partly independent of that at another, *e.g.* the cell, and also partly independent of the mean chiasma frequency. Certain predictions follow from this conclusion ; in particular that the average amount of chiasma variation between *p.m.c.* within plants of one population could differ from that in another even where the average frequency of chiasma formation per plant is the same for both populations. Where populations are outbreeding differences of this kind would of course affect their gametic output in respect of recombination, and hence their properties of variability. Analyses making use of F_2 and other data enable us to test this prediction and to inquire further into the factors affecting the variation within individuals.

2. MATERIAL AND METHOD

The inbred lines used have been bred by self-pollination for more than twenty generations (*cf.* Rees and Thompson, *l.c.*). F_1's and F_2's were obtained from crosses

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between these lines. Chiasma frequency was scored in 20 *p.m.c.* in each plant, and for each plant two quantities were estimated :—

1. The *chiasma frequency*, found as the average per bivalent in the 20 cells.
2. The *cell variance* is measured by the variance—with 19 degrees of freedom—between the *p.m.c.* in chiasma frequency per bivalent.

The variation between cells could of course have been expressed as the standard deviation. We found in fact that calculations, whether based on variances or on standard deviations, gave essentially the same results.

TABLE 1

The averages of plant chiasma frequencies and cell variances in inbred, F_1 and F_2 families, with approximate standard errors. The numbers of plants scored are in brackets

	1954		1955	
	Chiasma frequency	Cell variance	Chiasma frequency	Cell variance
Lines				
P3 (9, 10)	1·85±0·028	0·141±0·049	1·91±0·024	0·202±0·028
P12 (10, 10)	1·71±0·055	0·226±0·086	1·78±0·085	0·240±0·091
F_1				
3×12 (8, 10)	2·07±0·034	0·148±0·054	2·07±0·025	0·100±0·032
F_2				
3×12 (33)			1·90±0·101	0·132±0·074
Other F_2's				
3×6 (15)			1·86±0·086	0·197±0·148
3×13 (20)			1·93±0·049	0·129±0·063
6×12 (20)			1·86±0·082	0·156±0·121
6×13 (29)			1·93±0·064	0·085±0·049
12×13 (15)			1·91±0·059	0·091±0·049

In this account we are concerned only with the variation between different genotypes and between cells within anthers. We have not considered the variation between plants of the same genotype or the variation between bivalents within cells.

Because individuals of different genotypes matured at different times not all plants were fixed on the same day. The effect of fixing on different days was, however, found not to influence significantly either of the characters scored. This was established by comparing different F_2 plants fixed over a period of about 5 weeks at intervals of about 5 days.

3. INBRED LINES AND F_1

Generally where the chiasma frequency is low, as for example in inbred plants, the variation between cells is high. In contrast high chiasma frequencies are associated with low cell variances in F_1 hybrids between lines (Rees and Thompson *l.c.*). The two characters therefore show a negative correlation. On the basis of evidence obtained chiefly by the analysis of a diallel cross we did, however, conclude in our previous work that the correlation between them is not complete. For further evidence concerning their inter-relations we therefore

sought data involving inbred and F_1 families which covered more than one season. It should be possible with such data to find out whether the characters reacted differently to different seasons and whether the relations between them were the same for all genotypes. We could, in other words, test for independence of response to environmental as well as to genotypic differences.

Adequate data were available for two lines, P3 and P12, and their F_1 hybrid, in 1954 and 1955. It will be recalled that the two seasons

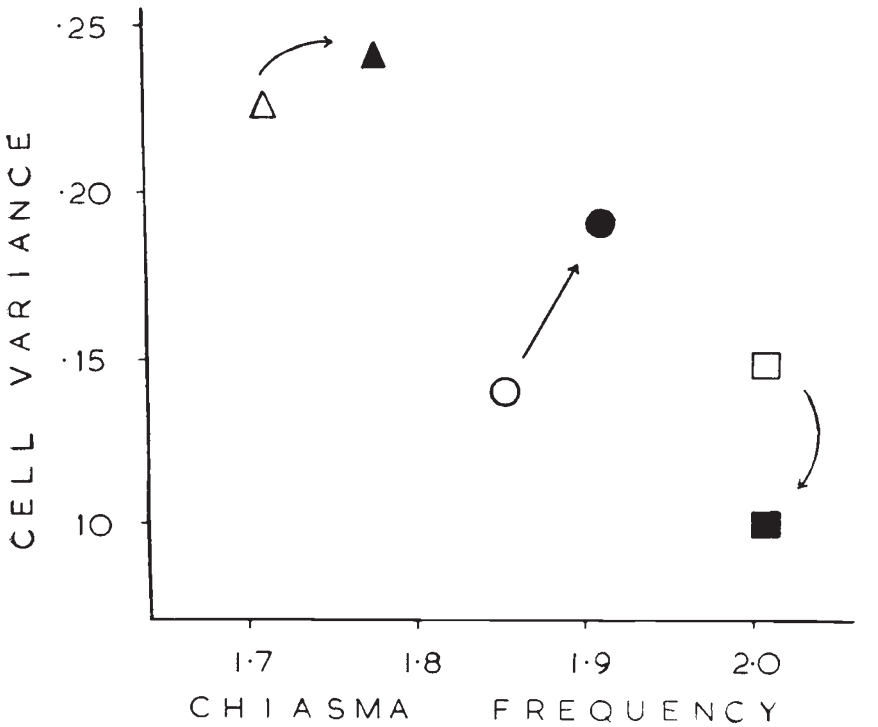

FIG. 1.—The mean chiasma frequencies of inbred and F_1 families plotted against their mean cell variances. Triangles = P12, Circles = P3, Squares = 3×12. Open symbols for 1954, blocked in for 1955. Explanation in text.

were very different, 1954 being wet and cool and 1955 hot and dry. From the plant chiasma frequencies and cell variances the averages for the lines and F_1 were calculated for both years. These, along with their standard errors, appear in table 1.

The variation between the families. In fig. 1 the family mean cell variances are plotted against the mean chiasma frequencies. The graph clearly reveals in the first place a general trend in both years, *viz.* a decreasing cell variance with increasing chiasma frequency. The regression (cell variance on chiasma frequency) of these family means is significant ($P = <0{\cdot}001$)—as shown in the analysis of variance (table 2) ; there is no doubt of a negative correlation.

The regression on chiasma frequency does not, however, account for all the variation in mean cell variances. This we conclude from

the fact that the item for residual variation is significant (P = <0·01). Two causes could account for this residual variation (1) a curvilinear relation between cell variance and chiasma frequency, or (2) some degree of independence between the two characters. Now there is no significant heterogeneity of regressions within the families and furthermore there is no suggestion of a significant difference between the joint regressions within families ($b = -0{\cdot}331$) and the regression of means ($b = -0{\cdot}306$). On the basis of these two facts we can rule out curvilinearity as contributing other than a negligible amount in this case to the residual variation. We therefore conclude, and confirm

TABLE 2

The joint regression analysis of cell variance on chiasma frequency in P3, P12 and their F_1 in 1954 and 1955

Item	SS	N	MS	VR	P
Within families					
Joint regression . . .	0·013	1	0·0134	3·38	0·20-0·05
Heterogeneity of regressions .	0·0054	5	0·0011	...	...
Between families					
Regression of means . .	0·0998	1	0·0998	25·12	<0·001
Residual variation . .	0·0482	4	0·0121	3·03	0·05-0·01
Error	0·1788	45	0·0040	...	...
Total . . .	0·3457	56			

our previous findings, that to some degree cell variances and chiasma frequencies vary independently of one another.

Before attempting to explain the genetical basis for this independence it is first necessary to inquire further into the variation in expression of the two characters in relation to changing environment and genotype. It is clear from these and earlier data that differences in both chiasma frequency and cell variance are genotypically determined. The present results also reveal that seasonal differences must affect their expression. Thus in P3 both chiasma frequency and cell variance are significantly higher in 1955 than in 1954 (P = <0·01).* The same is true for P12 although the increase in neither character is significant. In the F_1 the chiasma frequency remains constant, whereas the cell variance is seen to be significantly *lower* in 1955 (P = <0·01). It is in fact evident not only than an environmental as well as a genetic component contributes to the variation but also that the characters responded differently to environmental (seasonal) change in different genotypes.

* For these and similar comparisons the error sums of squares are based on the variation within the particular pair involved. This is more appropriate than an over-all sum of squares because different family means have widely and significantly different standard errors (see table 1).

The question to consider now is whether the variation in chiasma frequency and cell variance that is independent of their overall negative correlation is to be attributed to genotypic or to environmental causes. We shall see that both causes most probably are responsible. For example, the effect of season is to increase significantly *both* the chiasma frequency and the cell variance in P3 in 1955 in comparison with 1954 (fig. 1). These changes in the two characters due to season are *positively* correlated : they are independent of the negative correlation which applies to them generally. Two facts suggest that the varying inter-relations of chiasma frequency and cell variance depend also on the genotype, (1) the cell variance in P3 is lower than in 3×12 in 1954, despite the markedly lower chiasma frequency of P3 : (2) in 3×12 the cell variance, as in the inbred lines, is different between years, whereas (*cf.* the inbred lines) the chiasma frequency is unchanged. It must be noted, however, that these last two comparisons, in one respect, do not provide entirely satisfactory evidence. In the first place, although the cell variance of P3 in 1954 is lower than that of 3×12 it is not significantly so, and in so far as the mean cell variances are not known exactly the true means could, unlikely though it may be, show a negative correlation with the chiasma frequency. Secondly, while the estimated mean chiasma frequencies are the same for the two years in 3×12 the true means might possibly not be. More evidence confirming the role of genotype in controlling the inter-relations of the two characters is therefore desirable. Such evidence will be presented in the later sections.

The variation within families. The analysis of variance provides overall no evidence of a correlation between chiasma frequency and cell variance within the families considered as a group—the item for the joint regression is not significant. When, however, regressions are calculated for the families individually a significant negative correlation is found within P12. There is no indication from the analysis that the regressions within the families are significantly different from one another, and no evidence therefore in these results to suggest that genetic differences between the families affect the correlations within them.

4. PARENTS, F_1 AND F_2

In 1955, 33 plants of an F_2 derived from a cross between P3 and P12 were fixed over the same period as the parent lines and the F_1. We have investigated the relations between chiasma frequency and cell variance in these four families and made comparisons between them. In particular, we sought evidence of genotypic variation affecting the relations between the characters. This has been done, as in the previous section, by means of a joint regression analysis of cell variance on chiasma frequency in the parents, F_1 and F_2. The analysis is given in table 3.

Within families. In the inbred lines and F_1 the variation is of course non-heritable, whereas the F_2 includes a heritable component in addition. Within the families, as the analysis shows, there is, overall, a correlation between the characters; the joint regression is significant ($P = <0{\cdot}001$) and it is negative. There is no evidence that the regressions within the homogeneous families (parents and F_1) are different from that within the F_2. Neither, as shown earlier, are the regression slopes within the homogeneous families significantly different from one another. Thus, from the comparisons of the regressions within families we can demonstrate no variation that could be attributed to genotypic differences between them.

Between families. Evidence of genetic differences affecting the correlation between cell variance and chiasma frequency comes from

TABLE 3

The joint regression analysis of cell variance on chiasma frequency in P3, P12, their F_1 and F_2 in 1955

Item	SS	N	MS	VR	P
Within families					
Joint regression . . .	0·1119	1	0·1119	42·39	<0·001
Heterogeneity of regressions .	0·0088	3	0·0029	1·11	...
Between families					
Regression of means . .	0·0810	1	0·0810	30·68	<0·001
Residual variation of means .	0·0607	2	0·0303	11·49	<0·001
Error	0·1452	55	0·0026		
Total . . .	0·4076	62			

a consideration of the variation between the means of the families. The regression of means is significant ($P = <0{\cdot}001$), and negative, but, as shown in the analysis, there is again a significant variation in cell variance that is independent of the regression on chiasma frequency ($P = <0{\cdot}001$). Since there is again no indication of curvilinearity, we conclude that to some degree the cell variance varies independently of the chiasma frequency, and must, furthermore, be genotypically determined.

Another comparison suggests more precisely how this variation could have arisen. When the regression of cell variance on chiasma frequency is calculated for the pooled data from the parents and the F_1, we should, subject to error variation, obtain a good estimate of the change of cell variance over the range of chiasma frequencies exhibited by the three genotypes. The F_2 chiasma frequencies, as a result of recombination in F_1, and also of environmental fluctuations, cover a considerable part of this range. If we assume no heritable variation affecting the correlation between the characters, we should expect the F_2 chiasma frequencies to be associated with cell variances

not significantly different from those in parent and F_1 plants with comparable chiasma frequencies. The regressions for the F_2 and for the parents and F_1, are plotted in fig. 2. The regression co-efficients are not significantly different, *i.e.* there is no evidence that the rate of change of cell variance on chiasma frequency is different in the two groups. The graph does, however, suggest that for a given

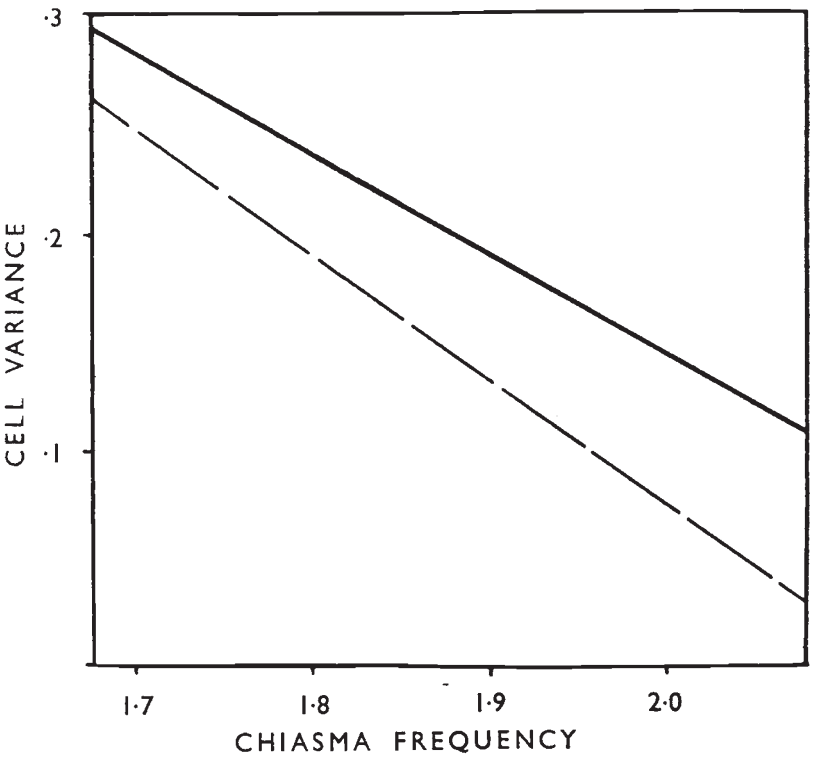

FIG. 2.—The regressions of cell variance on chiasma frequency in plants of P3, P12 and their F_1 (solid line) and their F_2 (broken line) in 1955. For the same chiasma frequencies the F_2 cell variances are lower.

chiasma frequency the cell variance in F_2 is lower. An analysis of variance shows the difference to be significant ($P = <0{\cdot}001$). It may well be, therefore, that one generation of inbreeding by self pollination, which reduces the mean chiasma frequency considerably, has a disproportionately smaller effect on the cell variance (which increases with inbreeding, *cf.* Rees, 1955).

5. A COMPARISON OF F_2 FAMILIES

In so far as genetical adjustments in chiasma frequency and cell variances could be expected in natural populations, they would be effective in relation to recombination and the release of heritable

variation in heterogenic populations of outbreeding species. In order to investigate somewhat comparable populations in rye we grew six F_2 families in 1955. These were derived from self-pollinating the F_1's from a diallel cross involving lines P3, P6, P12 and P13 (see Rees and Thompson *l.c.*). They include the 3×12 F_2 described in the previous section. Altogether 132 plants were scored and the data are presented in table 1.

As for the previous data, we need to consider and compare the rate of change of cell variance on chiasma frequency within and between the different families. Once again the comparisons were made by a joint regression analysis of variance (table 4).

TABLE 4

The joint regression analysis of cell variance on chiasma frequency in six F_2 families grown in 1955

Item	SS	N	MS	VR	P
Within families					
Joint regression . . .	0·2906	1	0·2906	56·1	<0·001
Heterogeneity of regressions .	0·0201	5	0·0040	...	...
Between families					
Regression of means . .	0·1169	1	0·1169	22·58	<0·001
Residual variation of means .	0·0439	4	0·0110	2·12	0·10-0·05
Error	0·6215	120	0·0052		
Total . . .	1·0930	131			

It will be seen firstly that the joint regression within families is significant, the cell variance being negatively correlated with the chiasma frequencies. Secondly, there is no significant heterogeneity of the regressions in the different families, and no evidence therefore that the rate of change of cell variance on chiasma frequency varies with the different genotypes.

We see also, from the analysis, that the regression of the means of the families is significant ($P = <0{\cdot}001$). The correlation is negative and there is no doubt that families with the higher chiasma frequencies tend to have lower cell variances. We can next look for evidence of variation in the characters that is independent of this correlation. From these F_2 data the evidence is not so conclusive as from the results previously analysed. The mean square for the residual variation of means is not significant. It does, nevertheless, approach the 5 per cent. level of significance, and we can, in view of our previous evidence, reasonably assume that this in part reflects independent variation of the two characters. An examination of fig. 3, in which the mean chiasma frequencies of the families are plotted against the mean cell variances, supports this assumption. Thus it will be seen that each of two pairs of families have virtually identical chiasma

frequencies, 6×12 and 3×6 with means of 1·86 and 6×13 and 3×13 with means of 1·93. We note, however, that the mean cell variances of the families within each pair differ considerably, despite the similarities in their chiasma frequencies. Between 6×13 and 3×13 the difference in cell variances, which are 0·085 and 0·129 respectively, is highly significant ($P = < 0{\cdot}01$). This kind of variation between populations is exactly what we should expect where the genotype can bring about independent adjustments in cell variances and chiasma

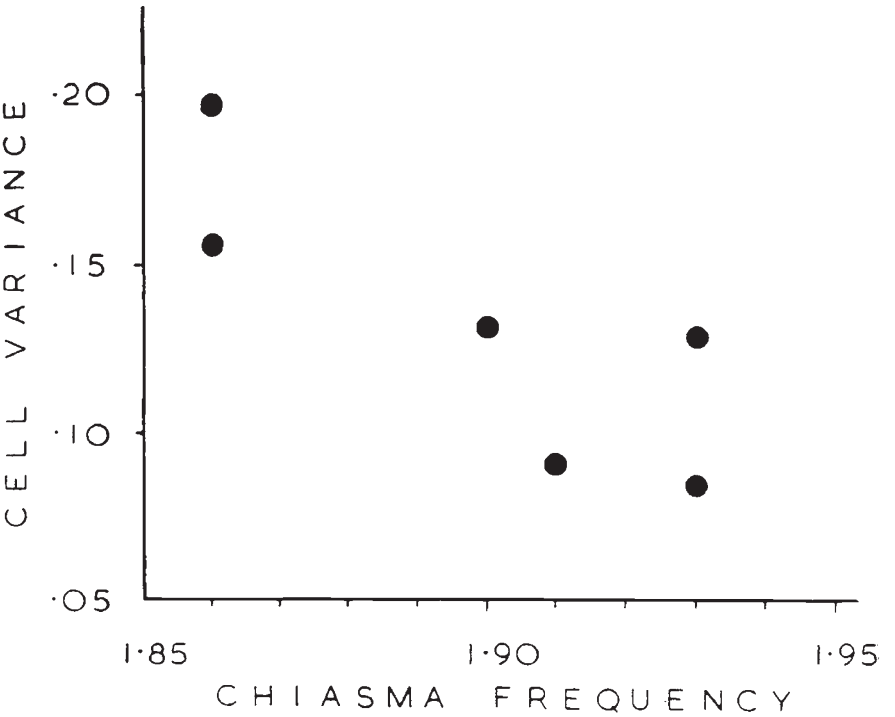

FIG. 3.—The mean chiasma frequencies of F_2 families plotted against their mean cell variances. The two families with means of 1·93 have significantly different cell variances.

frequencies. In these populations the amount of recombination is the same but the pattern of distribution within plants different.

6. THE PHYSIOLOGICAL MECHANISM

One further aspect of the variation in cell variance and chiasma frequency needs to be considered, *i.e.* the physiological mechanism governing the relations between them. In our previous work we concluded that the negative correlation found between the two characters within inbred lines was determined as follows :

1. There is an upper limit to the number of chiasmata per cell within an anther. The upper limit was deduced from investigating the distributions of *p.m.c.* chiasma frequencies within anthers (see also Mather, 1936).

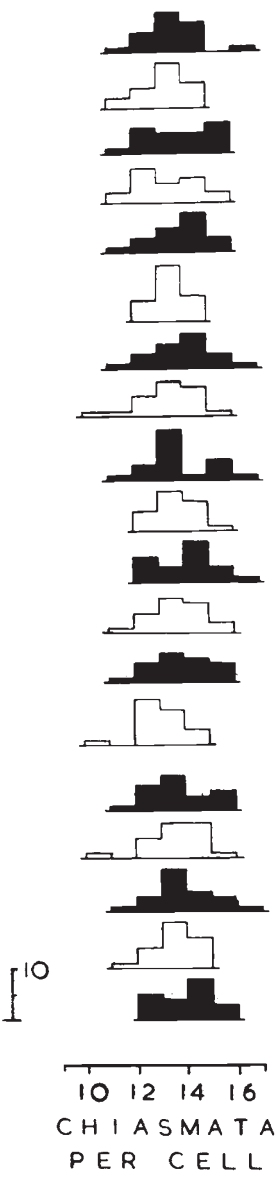

FIG. 4.—The distribution of chiasma frequencies in 20 *p.m.c.* in P3 plants grown in 1954 and 1955. Except for alternation of plant distributions from different years the arrangement of histograms is random. Histograms for 1955 are blocked in. The upper limit per cell is higher in 1955.

2. It follows that the greater the cell variance the more cells will deviate from this limit. The result will be lower chiasma frequency, and *vice versa* ; hence the negative correlation.

It was also suggested that one way in which the cell variance could change independently of the chiasma frequency in these lines was by alteration of the upper limit. Using our present data, a comparison of the chiasma frequency distributions of P3 plants in 1954 with those in 1955 (fig. 4) indicates that the upper limit can indeed alter ; it is higher in 1955 than in 1954. The increase in the upper limit would explain, on our view, why the average chiasma frequency of P3 in 1955 is higher, despite an increase in cell variance.

The negative correlations within the F_2 families are essentially similar to those within inbred lines. They can be explained on the basis of the same upper limit mechanism, although within the F_2 the variation in part would be heritable variation. Further, the partial independence of chiasma frequency and cell variance in the F_2, as in the inbred lines, could be equally well accounted for by changes in the upper limit. While the change in upper limit described in P3 was the result of environment, there is every reason to suppose that a similar phenotypic change in the F_2 could be determined by the genotype.

7. SUMMARY AND CONCLUSIONS

Previous work has shown that the average plant chiasma frequency is genotypically controlled, and also that the genotype influences the amount of non-heritable variation in chiasma frequency between *p.m.c.* within a plant. The investigations described confirm these results. They also show that both characters respond to environmental (seasonal) changes, and that the response may be different in different genotypes.

Generally in rye plants with high chiasma frequencies the variation between *p.m.c.* is relatively small, and *vice versa*. This negative

correlation may be due to some degree of pleiotropy or to linkage of genes controlling the two characters. Evidence is produced that the correlation is not, however, invariable : it can be altered by environmental and genetic changes. The evidence may be summarised as follows :

1. In an inbred line seasonal change increases *both* the chiasma frequency and the cell variance (which measures the variation between *p.m.c.*). These changes are *positively* correlated. In this case the increased average chiasma frequency is associated with a higher " upper limit " to the chiasma frequency per *p.m.c.* within an anther.
2. In an F_1 family grown under the same conditions the cell variance decreased significantly, whereas the chiasma frequency remained constant. Compared with the inbred parent lines and their F_1 the comparable chiasma frequencies of the F_2 were associated with significantly lower cell variances.
3. Two F_2 families with identical mean chiasma frequencies differed in their cell variances. Another pair of families with the same chiasma frequencies also differed considerably in cell variance, although not significantly in this case.

We conclude, therefore, that even within a species the pattern of recombination, particularly in respect of the relation between the average per individual and the variation between cells within individuals, may be adjusted genotypically. It is important to know whether, and under what circumstances, the patterns of recombination both between and within individuals vary between natural populations. There is already some evidence from *Drosophila* populations (Carson, 1954) that the average varies from one locality to another.

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