DEVELOPMENTAL VARIATION IN CHROMOSOME BEHAVIOUR

H. REES

*Agricultural Botany Department, Institute of Rural Science,
University College of Wales, Aberystwyth*

and

BERYL NAYLOR

ARC Unit of Biometrical Genetics, Genetics Department, University of Birmingham

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1. INTRODUCTION

CHANGES which initiate anatomical and physiological differentiation during development also affect chromosomes, whose form and behaviour in consequence may vary between cells of different tissues. A familiar and extreme example is the difference between the chromosomes of the salivary gland cells and those, a hundred times smaller, of nerve ganglion cells in *Drosophila* larvæ. Differences may sometimes be detected also between component cells within tissues. Thus in the nurse cells of *Drosophila* ovaries a proportion of cell nuclei are polyploid, the remainder diploid (Hertwig, 1935). Chromosome variation therefore provides an additional and useful means for investigating the patterns of cell variation arising during development, especially within tissues where the differences in cell anatomy and physiological activity are otherwise difficult or impossible to detect. In the following account two characters, chiasma frequency and the timing sequence of cell divisions, are investigated at meiosis in the pollen mother cell (p.m.c.) tissue of rye anthers. The aim was, first, to determine whether definite patterns of variation for the characters could be distinguished within the anthers and, second, to inquire into the relations between them.

2. CHIASMA FREQUENCY

(i) *Method*

To determine the variation along its length each anther was cut transversely into four equal sections—numbered 1 to 4 from base to tip. Where metaphases were available in all sections the chiasma frequency was then scored from a sample of cells in each. The plants used were from five inbred, homozygous, lines (see previous papers, e.g. Rees, 1955) and F_1 hybrids between them.

(ii) *The results*

Table 1 gives the chiasma frequencies per section in one anther from each of 14 inbred plants and 12 F_1 hybrids. The results for each genotype are represented also in fig. 1.

In this graph a definite pattern of variation within the anther is indicated, the chiasma frequency tending to decrease from one end to the other.

A more detailed analysis of the data was desirable and this was achieved by fitting polynomial regressions. In the analysis of variance

TABLE 1
Chiasma frequencies per section in rye anthers.
Twenty cells were scored in each section

Plant	Section			
	1	2	3	4
P_1^1	253	252	252	251
P_1^2	239	248	247	250
P_6^1	239	250	241	244
P_6^2	252	236	246	237
P_6^3	255	257	246	254
P_7^1	252	240	231	249
P_7^2	244	244	241	229
P_7^3	251	245	235	250
P_{12}^1	242	247	245	240
P_{12}^2	252	240	238	240
P_{12}^3	255	252	237	259
P_{17}^1	249	247	238	242
P_{17}^2	230		244	236
P_{17}^3	247	246	237	231
1×6^1	287	282	281	275
1×6^2	285	285	283	281
1×6^3	280	275	278	279
1×6^4	283	277	278	284
1×6^5	285	282	280	278
1×6^6	271	273	275	267
1×7^1	283	282	282	279
1×7^2	281	282	279	281
1×7^3	286	282	280	277
1×7^4	282	269	273	269
1×7^5	274	280	271	269
1×7^6	276	276	277	277

of the regressions, which is given in table 2, the degrees of freedom for the linear, quadratic and cubic regressions were split into components depending on heterogeneity between genotypes and between plants within genotypes. The conclusions may be summarised as follows:—

1. There is a significant difference between the mean chiasma frequencies of genotypes—due, chiefly, to the heterosis for this character displayed by the F_1 .

2. The significant mean linear regression confirms the gradient in chiasma frequencies from the base to the tip of rye anthers. A relation is clearly established between the chiasma frequency of p.m.c. and their location within the anther.

3. There is no significant heterogeneity for the linear regression between inbred or outbred genotypes. Within genotypes however it will be observed that the heterogeneity between plants is significantly

FIG. 1.—The distributions of chiasma frequencies in p.m.c. in sections of rye anthers.

larger for inbred than for F_1 families. Since plants within families are genetically identical it must be concluded that the pattern of developmental variation in the inbred, homozygous, individuals is less uniform, less consistent, than in the heterozygotes (cf. Rees and Thompson, 1956).

4. Neither the mean quadratic nor cubic regressions are significant.

It would appear however from fig. 1, particularly in respect of inbred lines, that there is variation in chiasma frequency distribution that is independent of and additional to that accounted for by the linear regression. Thus section three in anthers of inbred plants tends to have disproportionately low chiasma frequencies.

Further evidence for this additional variation comes from the analysis itself. The mean squares for heterogeneity within inbred and F_1 families for both the quadratic and cubic components are significantly

TABLE 2
Polynomial regression analysis of variance—chiasma frequency against anther sections

Item		N	MS	VR	P
Linear	Joint regression	1	264.69	10.61	0.001-0.01
	Het. between inbreds and F_1 . .	1	0.47		
	Het. between inbred genotypes .	4	23.46		
	Het. between F_1 genotypes . .	1	1.50		
	Het. within inbred genotypes .	9	45.74		
	Het. within F_1 genotypes . .	10	11.60		
			24.94		
Quadratic	Joint regression	1	60.01	2.103	0.10-0.20
	Het. between inbreds and F_1 . .	1	33.58		
	Het. between inbred genotypes .	4	27.90		
	Het. between F_1 genotypes . .	1	0.52		
	Het. within inbred genotypes .	9	51.09		
	Het. within F_1 genotypes . .	10	10.77		
			28.53		
Cubic	Joint regression	1	7.63		
	Het. between inbreds and F_1 . .	1	29.82		
	Het. between inbred genotypes .	4	17.69		
	Het. between F_1 genotypes . .	1	3.04		
	Het. within inbred genotypes .	9	61.02		
	Het. within F_1 genotypes . .	10	6.78		
			28.82		
Mean chiasmata	Between inbreds and F_1	1	30,556.73	117.25	
	Between inbred genotypes . . .	4	158.14		
	Between F_1 inbreds	1	28.52		
	Within inbred genotypes	9	93.07		
	Within F_1 genotypes	10	65.75		

different. They are larger between inbred than F_1 plants. This variation within inbred families also, of course, confirms the earlier conclusion of the developmental instability of the homozygotes as compared with the heterozygotes.

Having established a definite distribution pattern for chiasma frequency within anthers it is worthwhile now inquiring, as far as possible, how, during development, it originates. In *Allium* Darlington and Haque (1955) have demonstrated that localised abnormal chromosome behaviour, including failure of metaphase pairing, is associated with abnormal *dyschronisation* in the division of p.m.c. In view of this it was worthwhile inquiring whether "normal" differences in the timing of cell divisions can be correlated with the developmental variation in chiasma frequency. In the next section therefore, data are considered with regard to the timing of cell divisions in the four sections of the anther.

3. THE TIMING OF MEIOSIS

To begin with the problem was to determine whether or not divisions in the four sections were synchronised. Data for this purpose were available for six anthers of a P_{12} plant (P_{12}^4) scored in 1954. The p.m.c. of each section were classified according to their stage of

TABLE 3
The distribution of meiotic stages in anther sections of P_{12}^4

Anther	Section	No. of p.m.c.			“M.I.” (in angles)
		At stages earlier than metaphase	At metaphase	Later than metaphase	
1	1	0	35	19	56
	2	0	7	48	76
	3	0	14	42	70
	4	1	57	12	50
2	1	0	50	9	50
	2	0	28	48	65
	3	0	50	4	47
	4	7	42	2	42
3	1	1	48	1	45
	2	0	54	10	50
	3	10	56	0	41
	4	19	49	0	37
4	1	1	74	0	45
	2	0	74	1	46
	3	6	64	0	43
	4	33	20	0	26
5	1	0	30	0	44
	2	0	34	0	44
	3	3	28	0	42
	4	19	20	0	31
6	1	0	20	26	63
	2	0	11	37	71
	3	0	25	31	61
	4	3	24	30	60

meiosis. The results for three classes of cells, (1) those at first metaphase, (2) at stages earlier and (3) at stages later than first metaphase are given in table 3.

Chiasma frequencies also were scored in these anthers and are given in table 4.

For analysis of the timing data a time scale, the *metaphase index* (M.I.), was calculated as follows :—

$$\frac{100 - a + b}{2}$$

where a is the percentage of cells at stages earlier than first metaphase and b is the percentage of cells at stages later than first metaphase.

Because they are based on percentages the M.I. estimates were subsequently transformed to angular values for statistical analysis, and these angular values are also presented in table 3. Sections with low

TABLE 4
*Chiasma frequencies per section in anthers of P₁₂⁴.
Ten cells were scored in each section*

Anther	Section				Total chiasmata
	1	2	3	4	
1	121	115	110	116	462
2	113	115	120	115	461
3	115	116	117	116	464
4	122	121	113	117	473
5	119	115	113	120	467
6	118	118	109	116	461
Total	708	700	682	700	

M.I.s, where cells at first metaphase will be amongst the first to reach that stage, will be less advanced in division than those with high M.I.s, where most cells will have already progressed beyond metaphase. It

FIG. 2.—The M.I. distribution in anther sections of P₁₂⁴ (see text).

must, of course, be borne in mind that a difference in M.I. does not necessarily imply that p.m.c. proceed through prophase to metaphase at different rates; rather that they may do so at different times.

By means of this arbitrary time scale we can now inquire in some detail into the sequence of meiotic divisions in different regions of the

anther. It will be immediately apparent from the table that timing is variable between sections. It will be seen furthermore that the variation in timing is consistent between anthers; 2 is the most advanced, 4 the least and 1 and 3 are intermediate. The general pattern is illustrated in fig. 2.

TABLE 5

Polynomial regression analysis—M.I. against anther sections

Item	N	MS	VR	P
Between anthers in mean M.I.	5	479.54		
Linear { Joint regression	1	399.68	37.11	0.01
{ Het. between anthers	5	10.77		
Quadratic { Joint regression	1	477.04	13.65	0.05-0.01
{ Het. between anthers	5	34.94		
Cubic { Joint regression	1	63.08	2.82	
{ Het. between anthers	5	22.37		

A statistical analysis, by means of polynomial regressions as for chiasma frequency, shows that the linear and quadratic components are significant whereas the cubic regression mean square is not (table 5). The analysis confirms the relation between the timing of meiosis in p.m.c. and their distribution. It shows too that while the basal region is in general more advanced than the tip the timing gradient is not a simple, that is a linear, function of distance—as indeed the graph indicates.

One notes in relation to the above that the vascular system of the anther (fig. 3) is such that the probable order of supply of nutrients and other substances to the sections is 2, 1 and 3, 4—the order of increasing distance from the point of entry of the vascular bundle. It is not unreasonable to suppose therefore that stimulation causing the onset of meiosis, being in the same section order, is provided by a substance diffusing inwards along the vascular strand. Such a substance would not necessarily act immediately but perhaps, through the medium of the cytoplasm, over a period of one or more cell generations.

It has now been established that both in the timing of their meiosis and in the frequency of chiasmata which they form during meiosis the p.m.c. vary in quite well defined developmental patterns within the anthers. In the following section we shall consider whether there is evidence for a causal relation between the two.

FIG. 3.—The vascular supply to a rye anther.

4. TIMING AND CHIASMA FREQUENCY

The average chiasma frequency distribution in P_{12}^4 (see fig. 4) is in good agreement with the 1958 data for inbred lines generally and for

FIG. 4.—Chiasma frequencies per cell in sections of P_{12}^4 anthers.

P_{12} in 1958 especially. We note in particular the corresponding low values in section 3.

The relation between chiasma frequencies and M.I. in P_{12}^4 has been investigated at two levels, the anther and the sections. The results were as follows.

FIG. 5.—Chiasma frequencies plotted against M.I.s of anthers of P_{12}^4 .

Anthers. In graph 5a the total chiasmata scored in each P_{12}^4 anther is plotted against the total M.I. of each. Transformation of the data with the aid of *logs* straightens this curve (5b) and permits a

simple test of significance by means of a linear regression analysis of variance. The regression is significant at the 0.01 level. Metaphases in the more advanced anthers, it is concluded, have lower chiasma frequencies than in the less advanced. Put in another way, the chiasma frequencies are higher in p.m.c. which come into metaphase first.

The correlation between timing and chiasma frequency is, of course, exactly what would be expected on the basis of the precocity theory, (Darlington, 1940). While compatible with this theory the evidence however is not entirely conclusive because, as mentioned earlier, it is not known whether the chromosomes in p.m.c. at metaphase at different times proceed through meiosis, particularly chromosome pairing, at different rates.

TABLE 6

Analysis of variance of mean chiasma frequencies in P_{12}^4 (see text).

Item	SS	N	MS
Between anthers .	24.50	5	4.9
Between sections .	60.50	3	20.16
Residual . .	167.50	15	11.16
Total .	252.50	23	

Sections. If p.m.c. dividing early have higher chiasma frequencies than those dividing later it might be expected that sections with low M.I.s, with the first crop of p.m.c. at metaphase, would have higher chiasma frequencies than those with high M.I.s.—where p.m.c. at metaphase would represent the late dividing cells in that region. While such a relation is not ruled out it is clear from a comparison of graphs 3 and 4 that the chiasma frequency variation between sections within an anther is by no means directly or completely dependent on timing. In these graphs it will be seen that the high and low P_{12}^4 chiasma frequencies do not correspond with the low and high M.I.s for sections. Another cause of chiasma frequency variation between sections is indicated.

A clue to one additional factor controlling the chiasma frequency variation between sections is provided by a straightforward analysis of variance of the chiasma frequencies in P_{12}^4 (table 6). It will be seen that the mean square for variation between sections is 4.1 times greater than the mean square for variation between anthers. While not significant at the 5 per cent. level ($P=0.1-0.05$) the much smaller variation between anthers, as compared with that within, suggests a limit to the number of chiasmata per anther and, further, the possibility of competition for chiasmata between sections. Competition for chiasmata within cells is, of course, well established by earlier work (e.g. Mather, 1936).

Evidence confirming and at the same time indicating the nature of the competition between sections is provided by further comparisons between timing and chiasma frequency within anther sections. In fig. 6 section chiasma frequencies are plotted against section M.I.s for

FIG. 6.—Section chiasma frequencies plotted against section M.I.s in six anthers of P12⁴. Note alternation of chiasma frequencies in relation to timing.

each anther separately. It will be observed that the chiasmata/M.I. curves are characteristically "zig-zag". The chiasma frequencies alternate or, rather, are compensating between successive sections. The compensation it will be observed relates to the distribution of sections with respect to their timing succession, not directly to their spatial distribution. Thus when the chiasma frequencies of the first two sections to divide are high and low respectively that of the third

will be high, the fourth low ; or alternatively the sequence may be low, high, low, high.

It will be recalled that substances were inferred to diffuse into the anther in order of sections 2, 1 and 3, 4. Given that a substance limiting chiasma frequency and itself of limited amount is supplied to sections in this order the compensation effect can be explained where excessive absorption by one region curtails the supply to the next and vice versa. Whether timing and chiasma frequency are influenced by the same substance or by different substances acting independently cannot be established by the present data.

5. SUMMARY AND CONCLUSIONS

Differences in chromosome behaviour between cells are shown to be a useful index of the developmental variation occurring within a tissue. For two characters investigated in rye p.m.c., namely chiasma frequency and the timing sequence of meiotic divisions, well defined patterns of variation are demonstrated within anthers. Variation in both characters is probably influenced by the system of distribution of nutrient or other substances to the anther during its development.

The pattern of developmental variation in chiasma frequency is more consistent in anthers of heterozygous than homozygous plants.

Timing and chiasma frequency are related ; p.m.c. reaching metaphase early have higher chiasma frequencies than those at metaphase later.

Another factor affecting chiasma frequency variation within an anther is competition for chiasmata between different regions.

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