GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE

VIII. THE DISTRIBUTION OF CHIASMATA WITHIN POLLEN MOTHER CELLS

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Received 15.vi.64

1. INTRODUCTION

In order to achieve the regular disjunction at first anaphase of meiosis that is essential for fertility, the frequency and distribution of chiasmata must be such as to ensure that each pair of homologous chromosomes in a diploid species forms at least one chiasma. Such complete bivalent formation depends on three conditions.

1. An adequate mean chiasma frequency per cell.

2. A distribution of chiasmata between cells so that each cell has at least as many chiasmata as there are potential bivalents.

3. A distribution of chiasmata within cells such that each chromosome pair forms at least one chiasma.

The three conditions are to some degree obviously interdependent. For example, an adequate mean chiasma frequency is clearly a prerequisite for an efficient distribution of chiasmata between cells and within cells. The reverse, however, is not necessarily true. One can visualise situations where sterility could arise despite a high chiasma frequency per cell, due to a distribution of chiasmata within cells such that some chromosome pairs have inordinately high chiasma frequencies whereas others remain unpaired. This situation would require that variation in the distribution of chiasmata between bivalents is, to some degree, independent of the mean chiasma frequency.

Both the mean chiasma frequency and the distribution of chiasmata within cells are known to be genotypically controlled and there is, in fact, some evidence from work on rye that the two characters may vary independently of one another in response to changes in genotype (Rees and Thompson, 1956). The aim of the present work is to enquire further into the control over variation in chiasma distribution between bivalents within cells and, in particular, to produce evidence of such control leading to extreme sterility.

2. MATERIAL

Two kinds of rye material were used. The first consisted of plants from inbred lines (P_6 and P_{13}, see Rees, 1955) and the second of F_2 derivatives from a cross between *Secale dighoricum* and *Secale turkestanicum*. The reason for choosing this material was

that segregation, following forced inbreeding in the two inbred lines, and following extreme hybridisation in the F_2 derivatives, gives rise to a wide range of different genotypes which show considerable variation in chromosome behaviour at meiosis. Both kinds of material are therefore ideal for investigating the nature and the consequences of genotypic control. It is important to stress that neither in the inbred lines nor in the F_2 derivatives is there any indication of chromosome structural change which could affect the chiasma distribution within pollen mother cells (*p.m.c.*). F_1 hybrids between the lines as well as the F_1 species hybrid from which the F_2s were derived show normal behaviour and, in particular, a normal distribution of chiasmata between bivalents within *p.m.c.*

Cytological preparations were made in aceto-carmine or propionic-orcein following fixation in Carnoy's solution.

3. METHODS AND RESULTS

(i) *Inbred lines*

Previous evidence for genotypic control over the distribution of chiasmata between bivalents in rye *p.m.c.* was based largely on comparisons of the mean variances of chiasma frequency between bivalents

TABLE 1

Bivalent variances for each p.m.c. *chiasma frequency class in the two inbred lines*

Plant		P_6										Mean bivalent variance
		1	2	3	4	5	6	7	8	9	10	
P.m.c. chiasma frequency	10	0·285	0·619	0·619	—	—	0·619	0·396	0·285	0·396	0·507	0·466
	11	0·285	0·452	0·369	0·285	0·419	0·396	0·285	0·285	0·285	0·369	0·343
	12	0·239	0·239	0·239	0·239	0·306	0·364	0·239	0·306	0·239	0·239	0·265
	13	0·143	0·143	0·143	0·143	0·143	0·143	0·143	0·143	0·143	0·143	0·143
	14	0	—	0	0	0	0	0·084	0	0	—	0·0105

Plant		P_{13}										Mean bivalent variance
		1	2	3	4	5	6	7	8	9	10	
P.m.c. chiasma frequency	10	—	0·452	—	—	—	0·619	0·352	0·352	—	0·452	0·445
	11	0·285	0·285	0·507	0·396	0·285	0·285	0·285	0·341	0·452	0·730	0·385
	12	0·239	0·239	0·299	0·239	0·322	0·239	0·322	0·239	0·406	0·239	0·278
	13	0·143	0·210	0·346	0·191	0·171	0·227	0·366	0·143	0·352	0·227	0·238
	14	0	0·333	0·333	0·250	0	0	—	—	0·166	0·333	0·177

within the *p.m.c.* of different genotypes (Rees and Thompson, 1956). This kind of evidence, however, is not entirely satisfactory because it does not take into account the correlation between the bivalent variances and the *p.m.c.* chiasma frequencies. For example, a rye *p.m.c.* having 14 chiasmata characteristically contains 7 bivalents each with two chiasmata, with consequently, a bivalent variance of zero.

P.m.c. with thirteen chiasmata normally contain 6 bivalents each with two chiasmata, and one rod bivalent with a single chiasma, resulting in a higher bivalent variance. It is clear therefore that variation in bivalent variances between plants is often simply a reflection or even a consequence of a difference in chiasma frequency. What is required is a measure of variation in bivalent variance that is independent of *p.m.c.* chiasma frequency, so as to be able to determine whether cells in different genotypes with similar chiasma frequencies show differences in the manner of distribution of these chiasmata between the bivalents. For this purpose data were collected from 20 *p.m.c.* in each of 10 plants

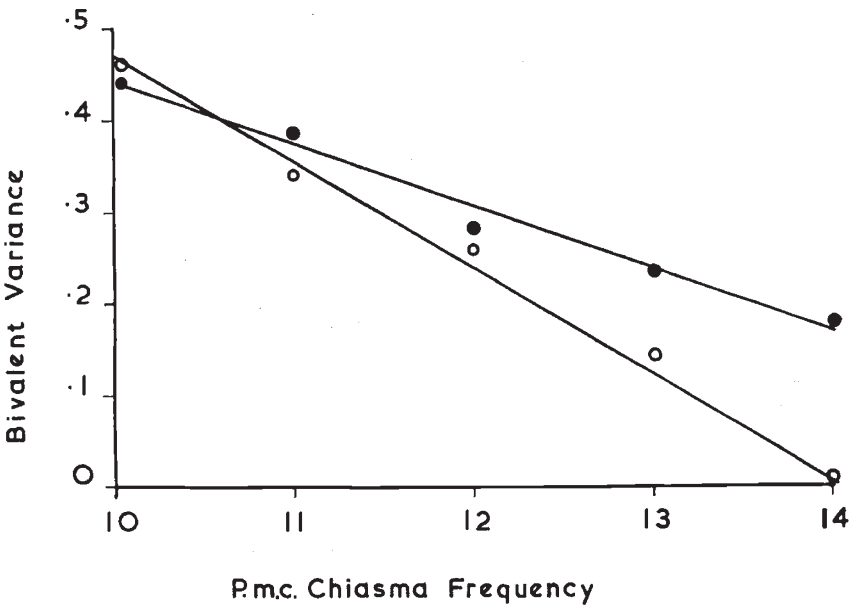


FIG. 1.—Regression of bivalent variance on *p.m.c.* chiasma frequency for the two inbred lines. Open circles are P_6, blocked circles are P_{13}.

from each inbred line. The bivalent variances were then calculated separately for *p.m.c.* with 10, 11, 12, 13 and 14 chiasmata in each plant. In this way the bivalent variances, and hence the pattern of distribution of chiasmata within *p.m.c.*, could be directly compared between *p.m.c.* of identical chiasma frequency in the two genotypes.

The data are presented in table 1 and are also plotted in the graph in fig. 1. It will be seen from the table and from the graph that in P_{13} the mean bivalent variances are, with one exception, higher for each chiasma frequency class than in P_6. This is confirmed by the joint regression analysis of bivalent variance on cell chiasma frequency (table 2), which shows that:

1. The joint regression is negative and highly significant ($P<0{\cdot}001$), confirming that the bivalent variance is to a large degree dependent on chiasma frequency.

2. The heterogeneity of means item is significant ($P<0{\cdot}01$),

showing that the bivalent variances also differ between *p.m.c.* of the different genotypes independently of the variation in *p.m.c.* chiasma frequency.

3. The heterogeneity of regressions item is significant at the 1 per cent. level, that is the slopes of the two regression lines are significantly different. There is a strong indication, therefore, that the *rate* at which the bivalent variance changes with chiasma frequency varies between genotypes.

From these data on inbred lines there is therefore good evidence for genotypic control over the variation in chiasma distribution between bivalents within *p.m.c.* This variation, it was stressed, is to some degree independent of *p.m.c.* chiasma frequency. The implications of such control are of some importance. As was indicated earlier it is

TABLE 2

Regression analysis of bivalent variance on p.m.c. *chiasma frequency for the two inbred lines*

Item	SS	N	V	VR	P
Joint regression . . .	0·1607	1	0·607	507·58	0·001
Heterogeneity of regressions .	0·0092	1	0·0092	28·42	0·01
Heterogeneity of line means .	0·0087	1	0·0087	27·47	0·01
Error	0·0019	6	0·000316	—	—
Total	0·1805	9	—	—	—

clear that an extreme asymmetry in the distribution of chiasmata between bivalents could well lead to sterility despite an adequate mean chiasmata frequency. In neither of the inbred genotypes is the distribution such as to cause extensive univalent formation in *p.m.c.* with reasonably high chiasma frequencies. There is, consequently, comparatively little gamete sterility. The following data from F_2 plants, however, demonstrate the extreme consequences of a disruption in the distribution pattern of chiasmata between bivalents at meiosis. In the F_2 material, as with inbred lines, the variation is inferred to be genotypically determined.

(ii) *The F_2 derivatives*

Chiasma distribution. Data from 100 *p.m.c.* from each of two F_2 plants, A_1 and A_2, from a cross between *S. dighoricum* and *S. turkestanicum* are presented in table 3 and in fig. 2. These two plants have very similar mean *p.m.c.* chiasma frequencies. A comparison of within cell variances between A_1 and A_2 shows, however, that the distribution of chiasmata between the bivalents within cells is very different in the two genotypes. In A_1 the distribution is very much like that found in the inbred lines. In marked contrast the bivalent variances in A_2

for each *p.m.c.* chiasma frequency class are very much higher (see table 3 and fig. 2). This difference in distribution is made particularly clear when we compare the chiasma frequencies of the individual

TABLE 3

Bivalent variances for each p.m.c. *chiasma frequency class in* A_1 *and* A_2

	P.m.c. chiasma frequency													
	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A_1	—	—	—	0·81	0·89	0·37	0·35	0·34	0·28	0·14	—	—	—	—
A_2	1·48	—	2·06	1·57	1·43	1·81	1·33	1·28	1·50	1·69	1·60	1·58	1·95	2·24

bivalents in *p.m.c.* of identical chiasma frequency in the two genotypes (see tables 4*a* and 4*b* and fig. 3). For example, A_2 *p.m.c.* with 14 chiasmata have on average 0·86 bivalents with 4 chiasmata, 1·57 with

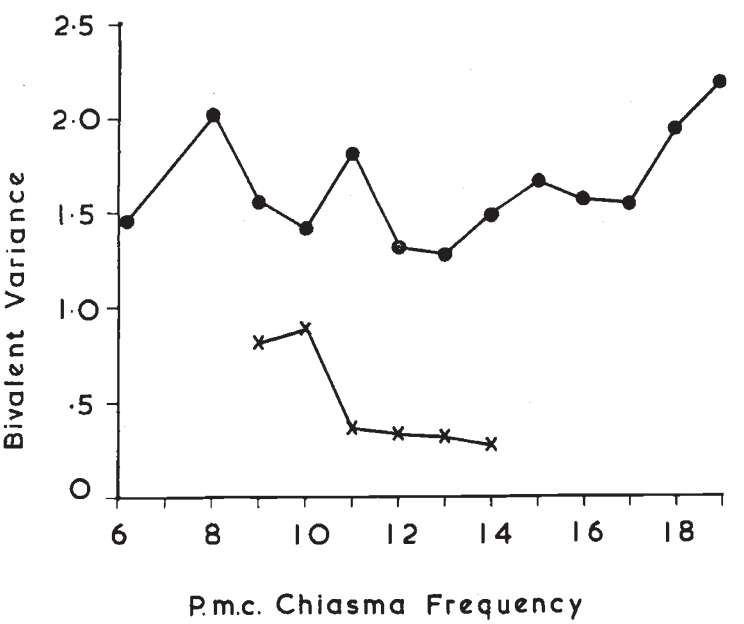


FIG. 2.—Bivalent variances plotted against *p.m.c.* chiasma frequency. Crosses are A_1, blocked circles are A_2.

3, 2·07 with 2, 1·71 with 1 and 0·79 with no chiasmata. In A_1, on the other hand, corresponding *p.m.c.* have on average 0·84 bivalents with 3 chiasmata, 5·32 with two and 0·84 with one. None of the A_1 *p.m.c.* with 14 chiasmatas contain bivalents with 4 chiasmata, nor do they contain univalents.

TABLES 4*a* and 4*b*

Mean frequencies per cell of various bivalent types, for each p.m.c. *chiasma frequency, in* A_1 *and* A_2

TABLE 4*a*

		P.m.c. chiasma frequency													
		6	7	8	9	10	11	12	13	14	15	16	17	18	19
A_1 Chiasmata per bivalent	0	—	—	—	2·0	1·33	0·23	0·068	0·03	0	0	—	—	—	—
	1	—	—	—	2·0	2·0	2·53	2·14	1·45	0·84	0	—	—	—	—
	2	—	—	—	3·0	3·0	4·23	4·52	5·0	5·32	6	—	—	—	—
	3	—	—	—	0	0·66	0	0·275	0·5	0·84	1	—	—	—	—
	4	—	—	—	0	0	0	0	0	0	—	—	—	—	—

TABLE 4*b*

		P.m.c. chiasma frequency													
		6	7	8	9	10	11	12	13	14	15	16	17	18	19
A_2 Chiasmata per bivalent	0	4·0	—	2·75	2·33	1·57	1·57	1·31	0·66	0·79	0·69	0·06	1·0	1·0	1·0
	1	1·0	—	2·56	1·66	2·43	2·29	1·92	2·17	1·71	1·57	1·30	0·57	0	0
	2	1·0	—	0·75	2·0	1·71	1·29	1·54	2·28	2·07	2·0	1·70	1·57	2·0	2·0
	3	1·0	—	0	0·66	1·0	1·29	1·92	1·5	1·57	1·78	2·30	2·43	2·0	1·0
	4	0	—	1·0	0·33	0·29	0·57	0·3	0·44	0·86	0·93	1·10	1·29	2·0	3·0

(iii) *Fertility*

Univalent formation is, of course, a most important cause of gamete sterility. It will be seen from table 5 that the univalent frequencies in A_1 and A_2 are markedly different. In fact, in A_1 fewer than 10 per cent. of *p.m.c.* contain univalents, whereas 75 per cent. of *p.m.c.* in A_2 contain one or more pairs of univalents. Both plants, it will be recalled, have very similar mean chiasma frequencies. The different patterns of chiasma distribution exhibited by the two plants, however, result in a striking difference in univalent formation and, in consequence, in gamete fertility. In this respect the comparison between A_1 and A_2 demonstrates most forcibly the importance of an effective control over the distribution of chiasmata between bivalents. This control, it is inferred, is exercised by the genotype. Its breakdown in F_2 derivatives such as A_2 is, of course, to be expected following hybridisation between species and the subsequent segregation of novel, unadaptive gene combinations.

(iv) *Stability*

The variation in distribution of chiasmata between bivalents in *p.m.c.* of different genotypes may quite properly be regarded as

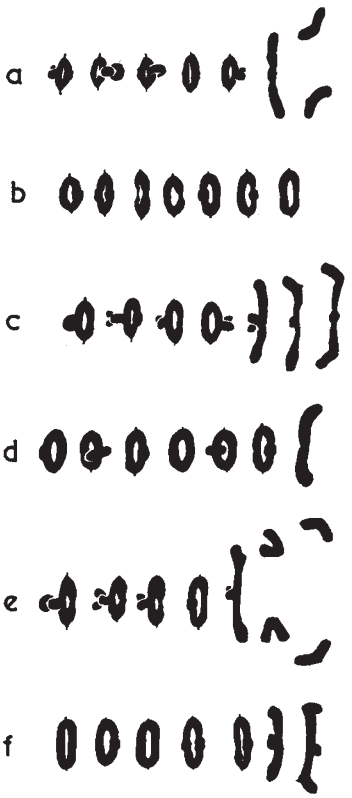


FIG. 3.—First metaphases in *p.m.c.* of A_1 and A_2 with 14 (*a*, *b*), 13 (*c*, *d*) and 12 (*e*, *f*) chiasmata. *a*, *c* and *e* are from A_2, *b*, *d* and *f* are from A_1. Note the difference in the distribution of chiasmata between bivalents in A_1 and A_2 *p.m.c*

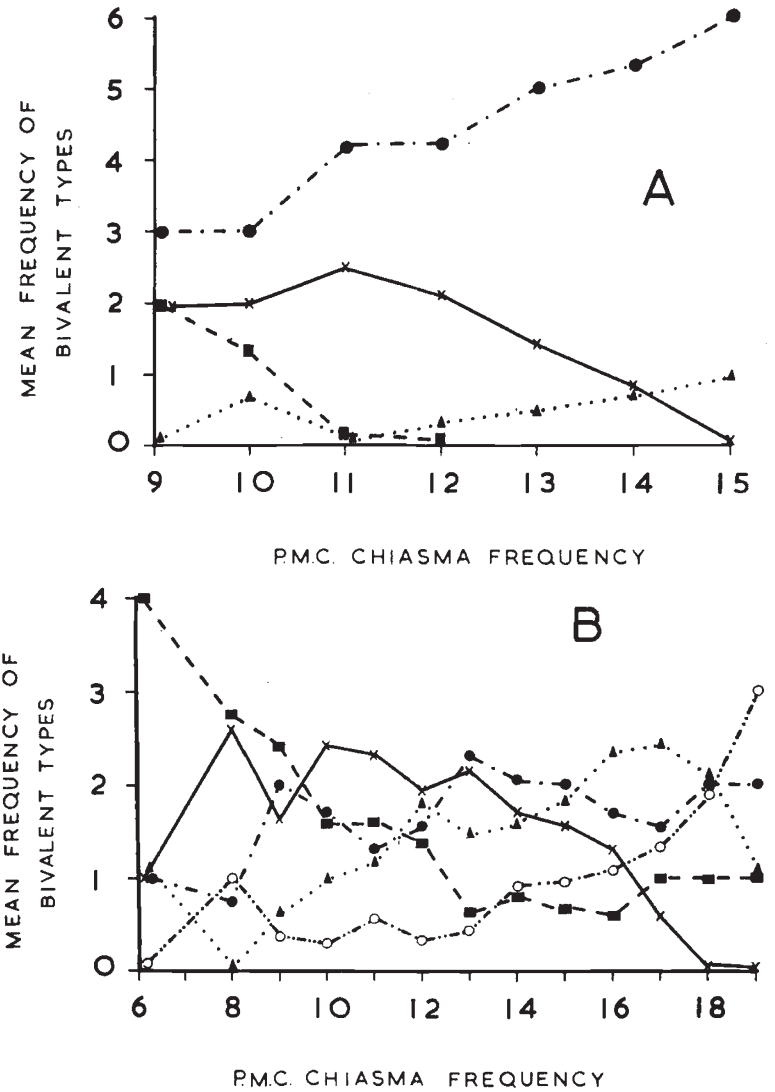
TABLE 5

The distribution of p.m.c. *with varying numbers of univalent pairs in* A_1 *and* A_2

		No. of pairs of univalents in *p.m.c.*				
		0	1	2	3	4
p.m.c. Frequency (per cent.)	A_1	90	8	2	0	0
	A_2	25	49	20	5	1

reflecting variation in developmental stability or homoestasis (Rees and Thompson, 1956). Thus the asymmetrical pattern of chiasma distribution found in A_2 indicates extreme developmental instability in chromosome behaviour, a property not uncommon among unbalanced genotypes of hybrid origin or, for that matter, among genotypes produced by forced inbreeding (see Rees, 1961).

Instability in development at the chromosome level is manifested in other ways. When we compare the range of *p.m.c.* chiasma frequencies found in A_1 and A_2 (see table 3) it will be seen that in the



FIGS. 4A and 4B.—Mean frequencies of bivalent types plotted against *p.m.c.* chiasma frequency for A_1 (A) and A_2 (B).

■ – – – ■ Univalents.
● –·–·– ● Bivalents with 2 chiasmata.
o –·– o Bivalents with 4 chiasmata.
× ——— × Bivalents with 1 chiasma.
▲ ····· ▲ Bivalents with 3 chiasmata.

latter there is a much wider spread, from *p.m.c.* with 6 chiasmata to *p.m.c.* with 19 chiasmata. A_1, in contrast, has a much smaller range of *p.m.c.* chiasma frequencies, from 9 to 15. This kind of developmental variation between genotypes is common and has been described in

detail elsewhere (Rees and Thompson, 1958). It is worth noting, however, that the high variance of *p.m.c.* chiasma frequencies in A_2 must contribute in part to gamete sterility in that many of the *p.m.c.* with very low chiasma frequencies inevitably contain univalents. Although A_1 has a similar average *p.m.c.* chiasma frequency, fertility is not materially affected since comparatively few A_1 *p.m.c.* fall into this very low chiasma frequency class.

The chiasma frequency data from A_1 and A_2 also exhibit another, more subtle, kind of instability. This concerns the pattern of change of bivalent chiasma frequencies in relation to the chiasma frequencies of *p.m.c.* It will be seen from fig. 4A that in A_1 there is a regular variation in the frequencies of different types of bivalent dependent upon the chiasma frequencies of *p.m.c.* For example, univalent frequencies increase with decreasing *p.m.c.* chiasma frequencies whereas bivalents with three chiasmata, as we should expect, become more frequent as the *p.m.c.* chiasma frequency rises. What is particularly striking is that the pattern of change for the various types of bivalent is regular and consistent. Fig. 4B shows a very different state of affairs in A_2. Here, it will be observed, there is a highly irregular relation between bivalent type and *p.m.c.* chiasma frequency. The relation is, at best, obscure. The developmental pattern so clearly displayed in A_1, which indeed is typical of most rye plants, has been disrupted. This disruption in pattern in A_2 reflects in a novel fashion the instability of developmental control imposed upon chromosome behaviour at meiosis.

(v) *Mechanism*

The abnormal chiasma distribution within A_2 *p.m.c.* can be interpreted in one of two ways. It may be that *particular* chromosome pairs in A_2 *p.m.c.* have consistently high or low chiasma frequencies. The alternative explanation is that the asymmetrical distribution of chiasmata is in no way related to *particular* chromosomes. If it were possible to distinguish between the 7 rye bivalents at metaphase I of meiosis it would be an easy matter to ascertain which of the above two alternatives applies. This unfortunately cannot be done. At diplotene, however, we can recognise and score chiasmata in one of the chromosomes, chromosome *V*, which organises the nucleolus (see Lima de Faria, 1952).

With this information on the chiasma frequency of *V* in A_1 and A_2 we can go some way towards determining whether the abnormally distributed chiasmata in A_2 *p.m.c.* relate to specific chromosomes or not. If not, we should expect the chiasma frequency of *V* relative to the chiasma frequency of the other bivalents to be similar in A_1 and A_2. A disproportionately high, or low, chiasma frequency in *V*, in A_2, on the other hand, would indicate that the abnormal distribution of chiasmata is in fact related to particular chromosomes.

Chiasma frequencies of chromosome *V* from 20 *p.m.c.* at diplotene in A_1 and A_2 are given in table 6. Alongside are the average *p.m.c.*

chiasma frequencies estimated from metaphase I in the two plants and in the last column of the table the ratios of average chiasma frequency in *V* to the average *p.m.c.* chiasma frequency are given for the two plants.

TABLE 6

Relation between the chiasma frequency of bivalent V *and the* p.m.c. *chiasma frequency in* A_1 *and* A_2 *(see text)*

		Chiasmata in *V*			Average chiasma frequency of *V*	Average *p.m.c.* chiasma frequency	Ratio
		0	1	2			
Frequency	A_1	0	17	3	1·15	12·75	1·15/11·6
	A_2	3	17	1	0·950	14·9	0·95/13·95

We note from these data:

1. In both A_1 and A_2 the average chiasma frequency of *V* is less than 1/7th of the average *p.m.c.* chiasma frequency ($P = <0·05$ and $<0·001$ in A_1 and A_2 respectively). This result is not surprising in view of the shorter length of chromosome *V* as compared with the other chromosomes.

2. The ratios of average chiasma frequency in *V* to the average *p.m.c.* chiasma frequency are very similar in A_1 and A_2 and statistical analysis shows they are not significantly different ($P = 0·1$). There is no evidence therefore that the abnormal distribution of chiasmata in A_2 results from a disproportionate excess or deficiency of chiasmata in particular chromosomes.

We can therefore conclude that both in A_1, and in A_2, variation in bivalent frequency is to some extent related to the variation in length of chromosomes within the complement. More important, in this present context, the unusually high bivalent variance of A_2 is not, apparently, achieved by consistently high chiasma frequencies in *particular*, *e.g.*, long chromosomes and consistently low chiasma frequencies in particular, *e.g.*, short, chromosomes.

To what extent the variation in chiasma distribution is determined, in this case, by the pairing behaviour of chromosomes or by factors affecting chiasma formation subsequent to pairing is not known.

4. DISCUSSION

It is well established that variation in the chiasma frequency of bivalents within cells at meiosis is determined by variation in chromosome length (*e.g.* Mather, 1938), and also, by chromosome structural change (*e.g.* Jain and Bose, 1960). The present work confirms that, over and above these structural factors, the genotype itself exercises

considerable control upon the distribution of chiasmata between bivalents. The evidence for genotypic control and the consequences of such control may be summarised as follows:—

1. The distribution of chiasmata between bivalents varies between *p.m.c.* of identical chiasma frequencies in different inbred genotypes.

2. Extreme differences in chiasma distribution between bivalents within *p.m.c.* of identical chiasma frequencies are displayed by two F_2 derivatives of a cross between *Secale dighoricum* and *Secale turkestanicum*. While the average *p.m.c.* chiasma frequency is similar in the two plants one of them A_2, shows a wide range of bivalent chiasma frequencies within cells such that some bivalents have very high chiasma frequencies often four, whereas others remain unpaired. In the second plant, A_1, univalents are very rare and most bivalents form one or two chiasmata, occasionally three.

Finally, there is evidence to indicate that the abnormally high variation in chiasma frequency within *p.m.c.* of A_2 is not achieved by consistently high or low chiasma frequencies in particular bivalents. The indication is that the chiasma frequency of each particular bivalent shows considerable variation between different *p.m.c.* This, along with other evidence described, suggests an instability, a breakdown in developmental control, such as is not uncommon in unbalanced genotypes of hybrid origin.

From the standpoint of gamete fertility this striking difference in the range and frequency of bivalent types in A_1 and A_2 demonstrates the importance of an effective control over the distribution of chiasmata within *p.m.c.* Adaptive adjustments towards efficient chiasma distribution within *p.m.c.* at metaphase 1, and the consequent regular disjunction of homologous chromosomes at anaphase 1 of meiosis, are no doubt achieved through natural selection acting upon variation that is genotypically controlled. Such a mechanism may well account for the regular and complete bivalent formation in species such as *Locusta migratoria* (see Rees, 1957) and *Stenobothrus parallelus* (Darlington and Dark, 1932) where the chromosomes vary widely in length, and where the average chiasma frequency per bivalent is comparatively low. Riley's work on wheat is, of course, another, outstanding, demonstration of the adaptive importance of control over the *distribution* of chiasmata within *p.m.c.*, in this case between particular chromosome pairs in an allopolyploid (Riley, 1960).

It may well be also that control over the distribution of chiasmata between bivalents could be adaptive with regard to the pattern of recombination and hence the type of gametes produced. Evidence on this matter would, however, be very difficult to establish.

5. SUMMARY

1. Within *p.m.c.* of identical chiasma frequencies the distribution of chiasmata between bivalents varies between different inbred and hybrid genotypes.

2. The variation in the distribution of chiasmata between bivalents within *p.m.c.* is to some degree independent of chromosome length.

3. In *p.m.c.* of an F_2 individual derived from a cross between two rye species the chiasmata are distributed between bivalents in an abnormally asymmetric fashion such that univalents are frequent even when the *p.m.c.* chiasma frequency is very high. Other bivalents in these same cells have an unusually high chiasma frequency, four being common. The evidence indicates an instability in developmental control over chiasma distribution in the hybrid genotype.

4. Control exercised by the genotype over the distribution of chiasmata between bivalents within *p.m.c.* clearly plays an important adaptive role in controlling gamete fertility and, as well perhaps, in controlling the pattern of recombination between chromosomes and thereby the nature of the genetic variation released at meiosis.

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