A TETRASOMIC MOSAIC IN THE GERM LINE OF *CHORTHIPPUS PARALLELUS*

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1. INTRODUCTION

DURING the study of the population cytology of British grasshoppers a single male *Chorthippus parallelus* was found which proved to be a chromosomal mosaic. About a quarter of the meiotic cells were normal, having eight pairs of autosomes and a single *X* univalent (John, Lewis and Henderson, 1960). The remainder were tetrasomic for one of the large acrocentrics. There have been few detailed analyses of the influence of additional chromosome material on the chiasma forming properties of cells (Mather, 1939). In *Chorthippus* it is possible to identify each bivalent individually at first metaphase, and this, coupled with the fact that the abnormal individual is mosaic, allows a precise analysis of this type of problem, using the normal cells as a control.

2. MATERIAL AND TECHNIQUE

The mosaic individual was one of 22 young male imagines taken near Ashurst Halt, New Forest, England, during the 1962 meiotic season. The testes were removed by vivisection, fixed in acetic-alcohol 1:3, and subsequently squashed in acetic orcein. A number of the follicles were treated individually to determine the pattern of mosaicism; the remainder were squashed in bulk and yielded five preparations (table 1).

3. OBSERVATIONS

The preparations were used to compare the chiasma frequency of disomic (normal) and tetrasomic cells both at diplotene and metaphase I. The cell numbers are summarised in table 1. At diplotene individual bivalents cannot always be distinguished, consequently chiasma frequencies were recorded for the size groups within the complement (3 long:4 medium:1 short). At metaphase I, however, where the positions of the centromeres are apparent, it is possible to assess the chiasma frequency of each bivalent individually.

In all the tetrasomic cells examined the additional chromosomes were present as an extra bivalent. In no case was a multivalent formed. Despite this absence of multivalents there is no reason to believe that the extra pair of chromosomes is not homologous with the M_4 , for in polyploid cells of this genus multivalent formation has only been observed in the L type chromosomes (e.g. fig. 59, John and Henderson, 1962).

The possibility that the extra chromosomes are B-chromosomes has been discounted on the following grounds:

(i) B-chromosomes are usually smaller than normal members of the complement. This is not an invariable rule but it is a useful one.

(ii) No B-chromosomes have ever been reported in a species of *Chorthippus*. Barker (1960) has claimed their occurrence in *Myrmeleotetix maculatus*, though even here they characterise only certain populations and only some individuals in such populations. In such cases, however, they are present in all follicles within the individual.

TABLE 1

A summary of numbers of cells scored in the five slides made from the germ line mosaic. The two samples from slides 3 and 5 are from quite distinct areas

Slide no.	Disomic		Tetrasomic	
	Diplotene	Metaphase I	Diplotene	Metaphase I
1	20	10	...	...
2	...	10	20	...
3 { area a	...	...	...	20
3 { area b	...	...	...	10
4	...	...	...	10
5 { area a	...	...	10	10
5 { area b	...	...	10	10
Totals	20	20	40	60
	40		100	

(iii) The germ line of this anomalous individual of *Ch. parallelus* was atypical not merely in respect of the extra autosomes, but also in the presence of extra X-chromosomes in several of the cells (fig. 5). This applies to both disomic and tetrasomic cell types and clearly indicates non-disjunction in the germ line.

The extra chromosomes were indistinguishable in size from the M_4 . In about 90 per cent. of the diplotene cells, however, one of the M_4 -type bivalents was precocious (figs. 2-4). This is not a normal aspect of M_4 behaviour, though the M_6 bivalent is invariably precocious. Chiasma analysis reveals no difference in either frequency or localisation pattern between the two M_4 -type bivalents, and it is not possible to determine whether the heteropycnotic bivalent consistently represents the same chromosomes. Certainly the heterochromatinisation of one of the two X-chromosomes in female mammals is a random phenomenon: the paternally derived one being heteropycnotic in some cells, the maternally derived one in others (Lyon, 1961). Presumably this also holds where extra X-chromosomes are present in sex aneuploids.

The tetrasomic condition must, in this case, have arisen by a process of double non-disjunction of the M_4 at mitosis. Comparable situations have been reported in single cells of *Schistocerca gregaria* (John and Naylor, 1961) and *Pholidoptera griseoptera* (Henderson, 1961). Single follicle analysis reveals the interesting fact that individual follicles are homogeneous in their chromosome constitutions.

Chiasma scores for disomic and tetrasomic cells within the mosaic

at both diplotene and metaphase I are summarised in tables 2 and 3 respectively. As well as the obvious differences between disomic and tetrasomic cells, there is clearly considerable variation between different slides, between different samples from distinct areas on

TABLE 2
Summary of disomic and tetrasomic diplotene scores

Cell condition	Slide no.	Chromosome class			M ₄	Total chiasmata	Total cells	Mean
		Long	Medium	Short				
Disomic	1	178	92	20	30	290	20	14.50
Tetrasomic	2	177	126	20	62	324	20	16.20
	5 { area a	98	61	20	26	169	10	16.90
	5 { area b	94	63	20	29	167	10	16.70

TABLE 3
Summary of disomic and tetrasomic metaphase I scores

Cell condition	Slide no	Chromosome type				Total chiasmata	Total cells	Mean
		L ₁	L ₂	L ₃	M ₄			
Disomic	1	37	29	25	18	151	10	15.10
	2	34	30	24	16	144	10	14.40
Tetrasomic	3 { area a	66	58	45	61	313	20	15.65
	3 { area b	35	31	24	28	158	10	15.80
	4 { area a	36	30	22	26	155	10	15.50
	5 { area a	37	36	28	28	172	10	17.20
	5 { area b	38	32	30	29	171	10	17.10

the same slide, and between stages. To assess the relative importance of these several variables an analysis of variance was performed on a series of samples of ten cells each, chosen as follows:

Slide no.	Disomic		Tetrasomic	
	Diplotene	Metaphase I	Diplotene	Metaphase I
1	10	10	...	...
2	...	10	10	...
5a	...	...	10	10
3 { area a	...	...	...	10
3 { area b	...	...	...	10
4	...	...	...	10
Totals	10	20	20	40
	30		60	

Where more than ten cells had been scored for a particular slide and stage (see table 1) only the first ten were used for this analysis. Since cells are not scored in any particular sequence this may be taken as representing a random sample.

There are three main effects in this analysis:

1. Differences between disomic and tetrasomic.
2. Differences between diplotene and metaphase I.
3. Differences between samples.

The sum of the squares for the first two effects were obtained by comparing the totals of all disomic and tetrasomic cells, and all diplotene and metaphase I cells respectively. Since, however, "samples" is not orthogonal to these other two items, it was obtained by comparing replicate samples within the same stage and within the disomic or the tetrasomic class. This yields five comparisons with five degrees of freedom. The results of this analysis are summarised in table 4.

TABLE 4

Analysis of variance between di- and tetrasomic cells at diplotene and first metaphase based on nine samples each of ten cells from six areas. (See Table on page 507)

Item	dF	SS	MS	VR	P
Disomic <i>v.</i> Tetrasomic . . .	1	33.799	33.799	26.303	<0.1%
Diplotene <i>v.</i> Metaphase . . .	1	6.050	6.050	4.708	≈5%
Samples	5	13.150	2.630	2.047	5%-10%
Remainder	82	105.401	1.285	...	...
Total	89	158.400	...	...	...

The comparison of disomic *versus* tetrasomic is clearly significant, but items 2 and 3 are of borderline significance only. Since item 2 is no more significant than item 3 there is no more difference between the diplotene and metaphase I samples than there is between any two diplotene or any two metaphase samples. We can, therefore, probably disregard the different stages at which these cells were scored.

Examination of the data suggests that the tetrasomic cells differ more between samples than the disomic ones, the range of sample means being 15.50-17.20 in the tetrasomic and 14.40-15.10 in the disomic. The variance of the tetrasomic samples is also higher than that of the disomic samples, 5.32 for 7dF *versus* 1.55 for 2dF respectively. The ratio of the variances is not significant ($P = 20$ per cent.) but this is hardly likely since there are so few degrees of freedom. From our observations in this unit the variance between samples in normal individuals is of the same order as the variance of disomic samples. An analysis of items 2, 3 and 4 in table 4 was therefore carried out on all the tetrasomic cells which had been analysed (see tables 2 and 3);

the results are shown in table 5. This shows that samples differ significantly whereas the stage difference is not significant. Thus while different samples of disomic cells are similar, different samples of the tetrasomic ones are not.

TABLE 5

Analysis of variance of tetrasomic cells from forty diplotene and sixty first metaphase. (See Table 1)

Item	dF	SS	MS	VR	P
Diplotene v. Metaphase	1	2.04	2.940	...	Not significant
Samples	6	34.30	5.717	4.004	0.1%
Remainder	92	131.35	1.428	...	...
Total	99	168.59	...	...	...

Since it is possible to distinguish each chromosome type at metaphase I, the correlation of any bivalent with any other bivalent for chiasma frequency can be calculated. The results of such an analysis are given in table 6 for both disomic and tetrasomic metaphase I samples. The correlation coefficients for the remaining chromosomes

TABLE 6

Correlation coefficients of the L and M₄ bivalents in disomic and tetrasomic metaphase I samples

Class	Sample	dF	Bivalent on bivalent correlation					
			L ₁ L ₂	L ₁ L ₃	L ₁ M ₄	L ₂ L ₃	L ₂ M ₄	L ₃ M ₄
Disomic	1	18	+0.406	+0.212	+0.066	+0.379	+0.199	-0.066
Tetrasomic	3a	18	+0.155	+0.378	+0.142	+0.132	-0.326	-0.043
	3b	8	+0.271	-0.452	+0.494	-0.220	+0.126	-0.100
	4	8	0.000	-0.600	+0.639*	0.000	0.000	-0.600
	5a	8	-0.566	+0.168	+0.168	+0.082	+0.490	+0.224
	5b	8	+0.149	0.000	-0.617	-0.423	+0.069	0.000

* Significant at the 5 per cent. level.

(M₅-M₇ and S₈) are very small, because these almost invariably form only one chiasma, and for this reason are not included in the table.

Only one correlation coefficient is significant and then only at the 5 per cent. level of probability, and since there are 36 calculations this is to be expected. There also appears to be no pattern in these correlation coefficients. No sample or particular bivalent is consistently positively or negatively correlated; even the L₁M₄ positive correlation is offset by a high negative one in the 5b sample.

4. DISCUSSION

The existence of, and the conditions within, this chromosomal mosaic has a bearing on two points of interest. First, the mechanism of mitotic non-disjunction and secondly the conditions governing chiasma formation within nuclei.

(i) *Non-disjunction*

Mitotic non-disjunction is theoretically possible by one of two methods. On the one hand both sister half centromeres might orientate to the same mitotic pole. Alternatively, if sister half centromeres auto-orientate, the regions of special cycle of division may persist undivided through anaphase. These possibilities have never been visibly distinguished: a modified form of the second has been seen in the B-chromosome of rye (Müntzing, 1946), where at the first pollen grain mitosis sister chromatids fail to separate despite the division of both centromeres and regions with special cycle of division. This is because of the existence of two adhesion loci situated equidistant from the centromere.

Whilst the cytological evidence is unsatisfactory, there are some suggestive genetical observations on the behaviour of the centromere at meiosis. Wallace (1961) has shown that if two distinct inbred lines of mice are crossed, then in the heterozygote the centromeres of similar ancestry tend to move to the same pole. This has been interpreted as implying that genetically similar centromeres, and hence the loci linked closely to them, show an affinity in movement.

This affinity is, of course, a meiotic phenomenon and leads to non-random segregation of non-homologous centromeres. Affinity is presumably dependent upon inter-centromeric interaction, and for this reason might operate at mitosis as well as meiosis, though here, of course, it would not be detectable under normal circumstances.

In a normal mitotic cycle sister half-centromeres auto-orientate and move to opposite poles; non-sister half-centromeres of homologous chromosomes move to the same pole. Where mitotic non-disjunction occurs sister half-centromeres behave in an essentially meiotic manner and this may facilitate the detection of intra-centromeric interaction. Whereas meiotic affinity is necessarily between non-homologues the opportunity for interaction between centromeres at mitosis is not so limited. Indeed, since the possibility of close genetic relationship between the homologous centromeres should exceed that between non-homologous ones, affinity relations at mitosis might be expected to involve homologues preferentially. The absence of trisomic cells in the mosaic indicates that the two non-disjunctional events cannot be separated by more than one mitotic cycle. This means that, either both homologues undergo non-disjunction in the same mitotic sequence, or that one of the three homologous chromosomes produced by single non-disjunction undergoes further non-disjunction at the very next mitosis.

Plate

All figs. are from acetic-orcein squash preparations and are reproduced at $ca \times 1500$.

FIG. 1.—The normal diplotene complement of *Chorthippus parallelus*.

FIGS. 2-4.—Tetrasomic cells at diplotene from the tetra-disomic mosaic. In fig. 4 note the marked heteropycnosity of one of the M_4 -type bivalents which is associated by stickiness with the precocious M_6 .

FIG. 5.—A tetrasomic cell with an extra X-chromosome at diakinesis.

The simplest and most probable interpretation is simultaneous non-disjunction of both homologues. And in this we see, perhaps, the expression of something comparable to meiotic affinity, but acting between homologous rather than non-homologous centromeres and conditioned in this instance by some unique nuclear-cytoplasmic interaction. This suggests also that the centromere of M_4 at least is in some way different from the other centromeres.

(ii) *Chiasmata conditions within nuclei*

Mather (1936, 1939) showed that in some species bivalents within a nucleus compete for chiasmata. This demonstration depended upon the finding of a negative correlation between chiasma frequencies of different chromosome classes within nuclei. More recently similar demonstrations have been made in *Delphinium* under normal conditions of chiasma formation (Basak and Jain, 1962), abnormal conditions of chiasma formation leading to the production of univalents (Jain and Basak, 1963) and in interchange heterozygotes (Basak and Jain, in press).

Mather explained this competition in terms of an upper limit in cell chiasma frequency; in those cases where no competition exists this upper limit is not attained. By comparing disomic and trisomic maize Mather was able to show that the addition of an extra chromosome led to an elevation of the upper limit.

In the present case the presence of extra chromosomes does not affect the correlation between bivalents for chiasma frequency. Since, however, the upper limit is not operating even in the disomic cells, then competition is not expected in the tetrasomic ones.

Whilst there is no competition in tetrasomic cells there is an interesting and significant difference between the total cell chiasmata in different samples of the mosaic. This probably reflects a follicle difference in the pattern of mosaicism. Certainly single follicle analysis shows that the chromosome condition within a follicle is constant. Unfortunately it was impossible to obtain sufficient scorable cells from single follicles to investigate the chiasma conditions within follicles. But if this interpretation is correct, it implies that the tetrasomic cells may be more sensitive to differences in developmental background than are disomic cells.

This has an interesting corollary: clearly where multivalent formation does not occur there will be no mechanical obstacle to the production of polysomics in species where, as in *Chorthippus*, no competition occurs, or where, as in maize, the competitive limit rises on the addition of extra chromosomes. Indeed, the only situation under which an aneuploid would face a chiasma problem is where all the chromosomes in the complement are similar in size and chiasma potential, and where competition already exists at a level which is not increased by polysomy.

Mechanical difficulties of chromosomal behaviour, therefore, are not likely to be serious, and the fitness of a tetrasomic aneuploid will depend essentially on the physiological effect in development that results from the new and unbalanced condition of the genes.

5. SUMMARY

1. In a tetrasomic germ line mosaic of *Chorthippus parallelus* 75 per cent. of the meiotic cells observed were found to contain an extra autosomal bivalent.

2. With the possible exception of increased pycnosity, the additional chromosomes were indistinguishable in morphology and behaviour from the normal M_4 member of the complement. The absence of multivalent formation in tetrasomic cells is not surprising since tetraploid cells of this species rarely form multivalents in the M_4 category.

3. The tetrasomic cells are the products of double mitotic non-disjunction at an early stage in the development of the germ line.

4. No competition for chiasmata occurs between bivalents in this species, and the addition of the two extra chromosomes leads to a simple increase in the chiasma potential of the cell.

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6. REFERENCES

- BARKER, J. F. 1960. Variation in chiasma frequency in and between natural populations of *Acridide*. *Heredity*, **14**, 211-214.
- BASAK, S. L., AND JAIN, H. K. 1962. Autonomous and interrelated formation of chiasmata in *Delphinium* chromosomes. *Chromosoma (Berl.)*, **13**, 577-587.
- BASAK, S. L., AND JAIN, H. K. The interchange distribution of chiasmata in interchange heterozygotes of *Delphinium*. *Heredity*, **19**.
- HENDERSON, S. A. 1961. Chromosome number and behaviour in the grasshopper *Pholidoptera*. *Heredity*, **16**, 181-186.
- JAIN, H. K., AND BASAK, S. L. 1963. Interchromosome effects of chiasmata and crossing-over. *Nature*, **197**, 725-726.
- JOHN, B., LEWIS, K. R., AND HENDERSON, S. A. 1960. Chromosome abnormalities in a wild population of *Chorthippus brunneus*. *Chromosoma (Berl.)*, **11**, 1-20.
- JOHN, B., AND HENDERSON, S. A. 1962. Asynapsis and polyploidy in *Schistocerca paranensis*. *Chromosoma (Berl.)*, **13**, 111-147.
- JOHN, B., AND NAYLOR, B. 1961. Anomalous chromosome behaviour in the germ line of *Schistocerca gregaria*. *Heredity*, **16**, 187-198.
- LYON, M. F. 1961. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature*, **190**, 372-373.
- MATHER, K. 1936. Competition between bivalents during chiasma formation. *Proc. Roy. Soc. B*, **120**, 208-227.
- MATHER, K. 1939. Competition for chiasmata in diploid and trisomic maize. *Chromosoma*, **1**, 119-129.
- MÜNTZING, A. 1946. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at pollen mitosis. *Hereditas*, **32**, 97-119.
- WALLACE, M. E. 1961. Affinity: evidence from crossing inbred lines of mice. *Heredity*, **16**, 1-23.