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Position and orientation in the metaphase equator of an interchange quadrivalent of *Allium triquetrum*

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SUMMARY

The position and orientation of an interchange quadrivalent in flattened, lateral views of metaphase I were studied in pollen-mother-cells of an interchange heterozygote of *Allium triquetrum*. The quadrivalent is most often located in marginal rather than central positions in the equator. Moreover, when positioned marginally the quadrivalent is more often than expected found in adjacent orientation, whilst when positioned centrally it is more often than expected found in alternate orientation. Consequently, the frequency of alternate (genetically balanced) orientation in the quadrivalent varies sharply according to whether data are obtained from more marginal or more central positions in the metaphase plate.

1. INTRODUCTION

It is well known that chromosomes are sometimes not distributed randomly in the metaphase plate. In some organisms, larger chromosomes tend to lie at the periphery of a roughly circular metaphase plate while smaller chromosomes tend to lie in the centre (Schrader, 1953; Swanson, 1960 for reviews; Juricek, 1975 for recent data). In cultured human cells, certain chromosomes, somewhat regardless of size, centromere location and involvement in nucleolus organization, tend to occupy more central positions in flattened mitotic metaphases, while other chromosomes tend to occupy more peripheral positions. These human data, however, are somewhat contradictory; and in other organisms, too, both random and non-random arrangements have been reported (Comings, 1968, 1980; Avivi & Feldman, 1980 for reviews). In meiotic cells, Kempanna & Riley (1964) noted that certain genetically unrelated bivalents in common wheat are more widely separated along the metaphase equator than expected on the basis of randomness. Kempana & Riley noted this as 'interesting but unresolved', a view equally applicable today to the whole issue of non-random chromosome positioning.

Preferential positioning at metaphase may, on the one hand, be viewed as being *passive* if chromosome positioning at metaphase is determined by preferential positioning during the telophase–interphase period prior to division (Comings, 1968; Costello, 1970; Ford & Wilson, 1972; Ashley & Pocock, 1981). It may also be seen as passive if the various positions in the spindle tend to be filled sequentially

and chromosomes tend to become oriented at particular times. In this way a given chromosome might be 'forced' to take up a particular spindle position. On the other hand, *active* preferential positioning would be indicated if a chromosome in some way takes up a selected position in the spindle regardless of its position prior to metaphase and regardless of whether other positions in the spindle are available.

In the present study, lateral views of metaphase I cells of an interchange heterozygote of *Allium triquetrum* were analysed. The position of the interchange quadrivalent relative to the bivalents was studied, along with the orientation of the quadrivalent. The results show that the quadrivalent is most often found in marginal positions of the equator when it is viewed laterally. But much more surprisingly, adjacent quadrivalents are found more frequently than expected in marginal positions, and alternate quadrivalents found more frequently than expected in central positions. The data on position in the spindle therefore became relevant also to issues concerning orientation of interchange quadrivalents.

2. MATERIAL AND METHODS

Pollen-mother-cells of an interchange heterozygote of *Allium triquetrum* ($2n = 18$; Liliaceae) were studied. The basic characteristics of this interchange have been described previously (Rickards, 1964, 1977). Briefly, a chain-of-four chromosomes (quadrivalent) is formed in nearly all meiotic cells. In all but about 5% of metaphase I cells the quadrivalent is found in alternate or adjacent-1 orientation (Plate 1, *a* and *b*), with about 75% of cases being alternate in the material presently under consideration (Table 1; see also Rickards, 1983).

Meiotic material was obtained from first inflorescences of clonal plants grown together. Inflorescences were fixed in 1:3 acetic-alcohol (two hours) and then stained with alcoholic HCl-carmine (Snow, 1963). After staining, inflorescences were dissected in 45% acetic acid into individual flowers, anthers and pollen sacs. The cells of individual pollen sacs were then extruded and analysed separately (Rickards, 1977). Preparations were scored only when *all* cells of a pollen sac were at metaphase I (Plate 1*a*, *b*).

Slides were prepared by gentle heating and then thumb pressure on the coverslip. The heating, in particular, flattens and spreads the chromosomes into a linear array (Plate 1*a*, *b*). Cells subsequently rejected as indecisive comprised (i) cells with atypical orientations of the quadrivalent (about 5% of all cells), (ii) cells flattened from polar or semi-polar aspect, or cells in which the chromosomes failed to spread (4–6%), and (iii) cells in which the quadrivalent atypically was angled widely across the metaphase plate (1%). In all other cells the position and orientation of the seven bivalents and the quadrivalent could be determined.

Ideally, chromosome position should be analysed in polar views of metaphase (Plate 1*c*). However, such cells cannot be analysed easily in *Allium triquetrum* because they are only rarely seen after conventional methods of preparing slides, and because of depth-of-focus difficulties. Hence, quadrivalent position and orientation were analysed in lateral views of metaphase (Plate 1*a*, *b*) – that is, in cells in which the spindle lies in side aspect and, before flattening, the chromosomes had occupied a circular area, but in which now, after flattening, the chromosomes lie in a linear array. Now, if the geometry of the cell is such that it responds to

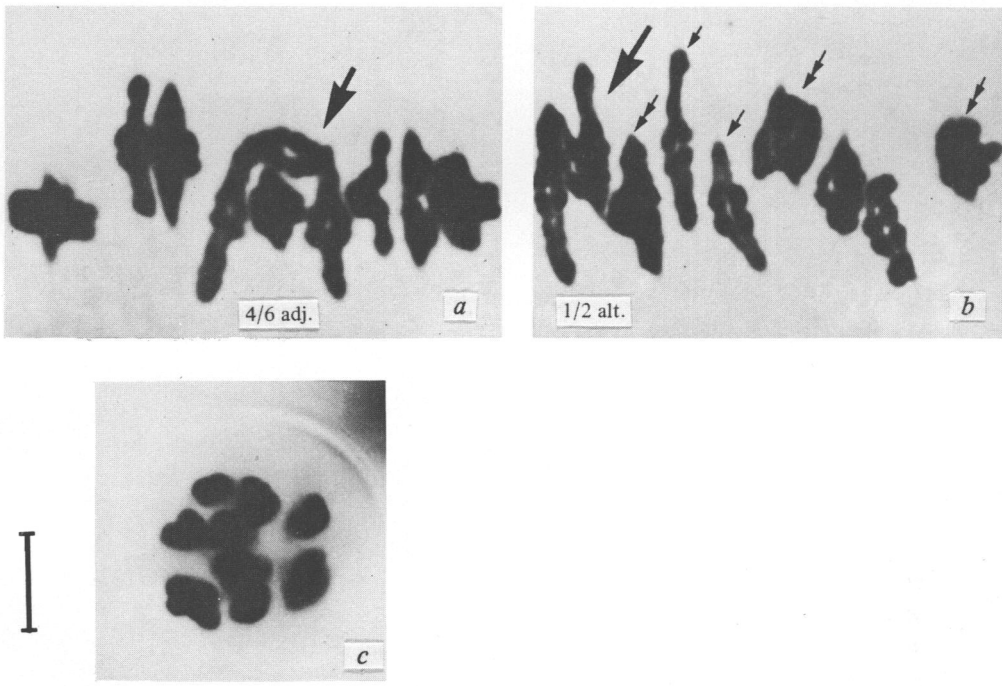

Plate 1. Metaphase I in pollen mother cells of the *Allium triquetrum* interchange heterozygote, in flattened, side view (*a*, *b*) and polar view (*c*). In side view, 7 bivalents and the interchange quadrivalent (large arrows) can be distinguished. In the text, chromosomes at the two edges of the linear array are referred to generally as being located in 'marginal' positions, in contrast to others in 'central' positions. In (*a*), the quadrivalent is in adjacent orientation and occupying specifically position 4/6 (Figure 1 and text); while in (*b*) it is in alternate orientation and occupying position 1/2. In (*b*), the single headed, small arrows indicate two small bivalents, each with subterminal centromeres and a single chiasma, while the double headed arrows indicate three large bivalents, each with median centromeres and two chiasmata. In (*c*), nine chromosome 'bodies' can be distinguished, corresponding to the 7 bivalents and the two cooriented centromere pairs (= halves) of the quadrivalent. In this particular cell, 7 bodies occupy so called 'peripheral' positions (around the outer edge of the spindle), while two bodies occupy 'central' positions. Bar = 10 μm.

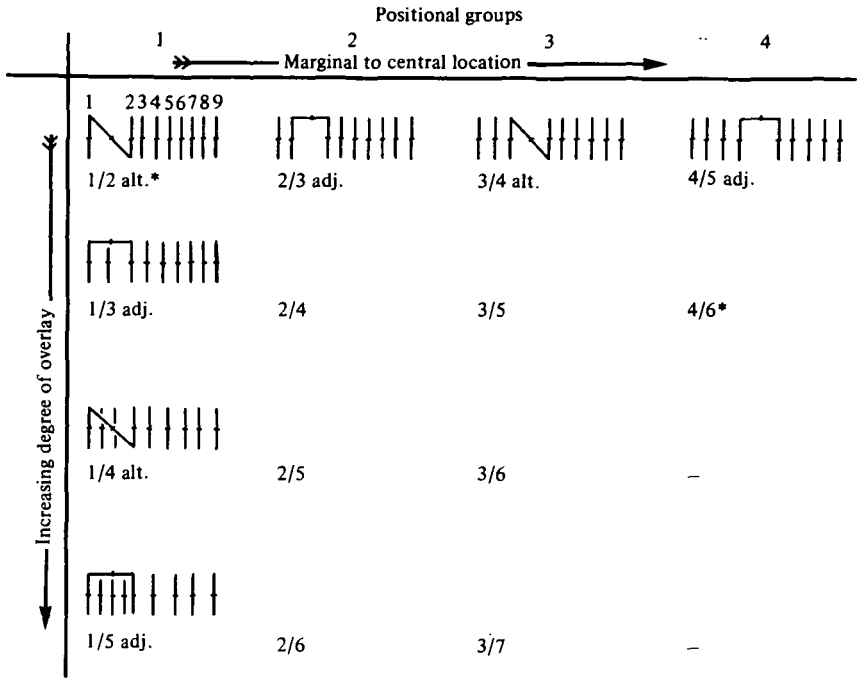

Fig. 1. Illustration of the method used to classify lateral spreads of metaphase I (Plate 1). The quadrivalent is shown as a capital *N* (alternate orientation) or as an inverted *U* (adjacent orientation); and the seven bivalents as vertical lines. The cross bars indicate chiasmata and thus the position of the equator; the poles, therefore, are above and below. The separation of the two cooriented pairs of centromeres (halves) of the quadrivalent has been exaggerated for ease in presentation, particularly in arrangements where the two halves lie in neighbouring positions. In fact, the two halves are usually close together, with the arms linking them somewhat folded.

Only the upper and left hand arrangements of the 14 possible positions are illustrated; the remainder are given positional assignments (2/4, 3/5 etc.) only. The small numbers above the chromosomes in the top/left array indicate the nine positions in the equatorial plate. The *four positional groups* (1–4) represent the most marginal through to the most central locations of the quadrivalent. Positional group 1, for example, includes all cases in which the most marginal half of the quadrivalent lies in position 1. The sequence within each group (1/2–1/5, for example) represents positionally related cases in which the quadrivalent overlies successively more bivalents, and thus represents the *degree of overlay*.

* For actual examples see Plate 1*a*, *b*.

squashing equally from all directions, then chromosomes located in the centre of the spindle (Plate 1*c*) will become located in central or near central positions in the flattened metaphase plate, irrespective of the plane of squashing. But a chromosome initially located in the periphery will, after squashing, be located centrally about as often as it will be located marginally, because of the plane of squashing. Thus for a peripherally located chromosome the position adopted after squashing does not necessarily reflect the original position in the spindle before squashing. Despite this problem the analytical method adopted here can be used to advantage and leads to the accumulation of a wealth of data.

Cells were classified by assigning nine positions to the equatorial plate, seven

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occupied by one bivalent each and two by the two co-oriented centromere pairs of the quadrivalent (Figure 1, top left). Assigning two positions to the quadrivalent reflects its structural composition (equivalent to two bivalents); but also, and more importantly, it enables classification of cells in which the quadrivalent overlies one or more bivalents (Figure 1, left hand column). The nine positions were numbered consecutively *from the margin nearest the quadrivalent*, and each quadrivalent was then assigned its two positions. The orientation of the quadrivalent was also recorded. Thus cells were classified as 1/2 alt., 2/4 adj. etc. In Figure 1 note that the two 'missing' positions 4/7 and 4/8 are the same as 3/6 and 2/6 respectively. This duality applies throughout the data: position 1/2, for example, is equivalent to 8/9.

For the position of large versus small bivalents, cells of normal (rather than interchange) material were analysed. The nine bivalents of normal cells include four large bivalents, each with median centromeres and nearly always two chiasmata, one in each arm pair; and 3 small bivalents (approximately one-half the size of the large ones), each with subterminal centromeres and nearly always one chiasma in the long arm pair (see Plate 1*b* for examples; note that the interchange quadrivalent is composed essentially of one each of these two differently sized bivalents; Rickards, 1977). The position of each of the four large and three small bivalents, which can be distinguished easily in nearly all cells, was recorded as 1,2,3,4 or 5, numbering from the margin inwards. The two margins of the metaphase plate were not distinguished, so that each position, except no. 5, is represented twice in each cell. In cell number 1, for example, the large bivalents occupied positions 1--45---1, and the small bivalents occupied positions -23---3--. The remaining two bivalents (small but with sub-median centromeres) were ignored.

3. RESULTS

The contents of 6 pollen sacs (from four flowers from four inflorescences) were analysed. Contingency χ^2 tests indicate that the six pollen sacs do not differ significantly, neither in their frequency of alternately oriented quadrivalents, nor in the relative frequencies of cells classified according to position and orientation (Table 1). Therefore the data were pooled and analysed together.

The position/orientation data are presented in Figure 2, expressed as percentages of the total alternate or total adjacent quadrivalents (thereby effectively removing the overall differences in the frequencies of alternate and adjacent orientations; Table 1). The major points are as follows.

(*i*) *Overlay and position in the equator*

Particularly in positional group 1 (1/2–1/5), there is a sharp decrease in the percentage of cells in which the quadrivalent overlies successively more bivalents (Figure 2*a*). This is explained particularly by the fact that the two 'halves' of the quadrivalent, represented by its two cooriented pairs of centromeres, are linked and thus their independence (wide separation) is restricted.

The decrease is less sharp in positional group 2; and in the adjacents of group

3, and in both the adjacents and alternates of group 4, there are no significant differences between the entries. This implies that the degree of overlay of bivalents by the quadrivalent increases from marginal to central positioning of the quadrivalent; and is explained by the fact that probably most overlay of bivalents by the quadrivalent is induced by lateral flattening of the circular area of chromosomes

Table 1. *Numbers of adjacent, alternate and total quadrivalents and percentage of alternate quadrivalents from six pollen sacs from four flowers from four first inflorescences; and results of contingency* χ^2 *analyses*

Pollen sac No.	Quadrivalent type		
	Adjacent	Alternate (%)	Total*
1	61	183 (75·0)	244
2	75	236 (75·9)	311
3	77	237 (75·5)	314
4	72	235 (76·5)	307
5	59	148 (71·5)	207
6	44	152 (77·6)	196
Total	388	1191 (75·4)	1579

Contingency $\chi^2_5 = 2{\cdot}56$. $P = 0{\cdot}75$.
Contingency $\chi^2_{135} = 150{\cdot}2$, $P > 0{\cdot}05$†.
* The variation has its basis mostly in the number of cells successfully extruded from a pollen sac.
† There are 14 positions for the quadrivalent (Figure 1), each in either alternate or adjacent orientation. Hence the degrees of freedom are (28–1) (6–1) = 135. For 135 degrees of freedom, χ^2 must exceed 168·5 to be significant at the 5% level of probability.

into a linear array. In general, quadrivalents lying in a peripheral and 'side' (left or right) position in the circular area before squashing (Plate 1*c*) will be less vulnerable to having bivalents positioned between the two halves of the quadrivalent during flattening than will those positioned further around, or toward the centre of, the circular area. The important implication of these overlay data is that numerical values for position 1/2, for example, should be compared not solely with those of 2/3, but with 2/3 plus a proportion of 2/4. In general, to make valid comparisons between successively more central positions of the quadrivalent the data within each positional group must be pooled. In effect this is classifying the position of the quadrivalent according to the position of its most marginal half. The pooled data given in Figure 2*b* show an obvious trend. There is a systematic decrease in the proportions of assignments from the most marginal to the most central position. (The group 4 totals cannot be compared directly with the others because they contain only two, rather than four, entries; see Fig. 1).

(ii) *Position and orientation*

While both quadrivalent types show a preference toward marginal rather than central location, this preference is much stronger for adjacent quadrivalents than for alternate ones. Conversely, alternate quadrivalents are over-represented in

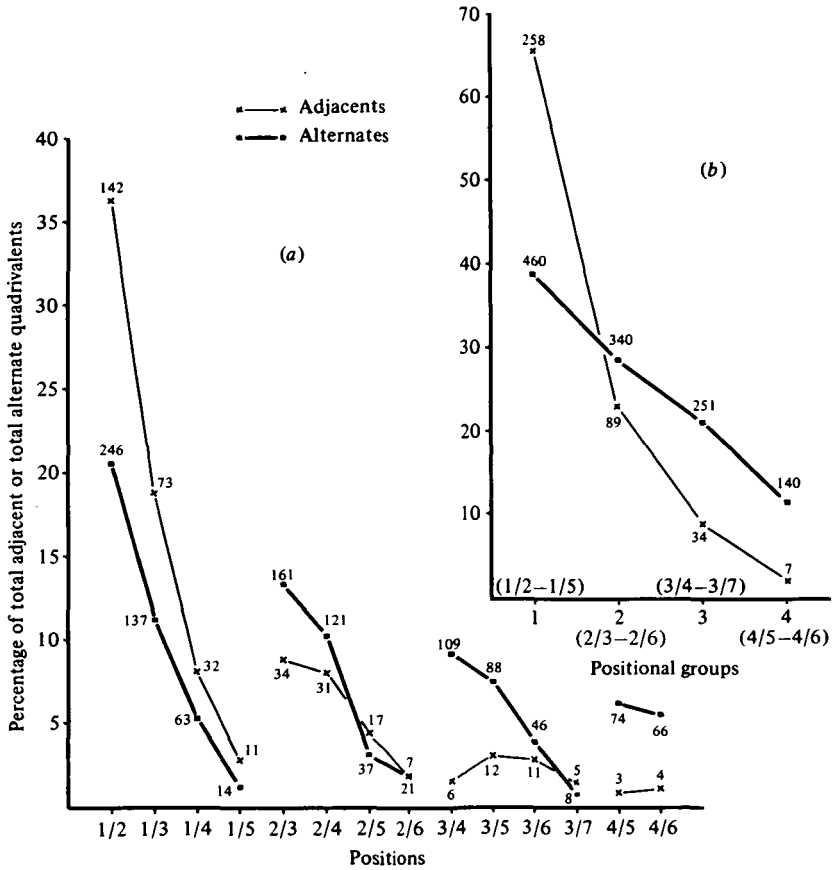

Fig. 2. Numerical and graphical presentation of data on position and orientation of the quadrivalent in lateral views of metaphase, classified according to the scheme illustrated in Figure 1, data from six pollen sacs from four first inflorescences pooled. Numbers indicate cells scored. For total numbers of alternate and adjacent quadrivalents, see Table 1. (*a*) data for individual positions; (*b*) data for positional groups.

central locations (groups 3 and 4). The rarity of adjacent orientations in group 4 (7 out of 147 cells) contrasts sharply with their relative abundance in group 1 (258 out of 718). Overall, the differences in the distributions of the two quadrivalents are statistically highly significant ($\chi^2_3 = 214{\cdot}4$, $P \ll 0{\cdot}001$).

The relationship between position and orientation of the quadrivalent means that the percentage of alternate (genetically balanced) orientation varies according to whether data are obtained from more marginal or more central positions. Thus, while less than 65 % of quadrivalents in group 1 are alternates, in group 4 the figure is 95 % (see also Rickards, 1983 p. 471).

(iii) *Effects of spreading on position/orientation relations*

Cells from a further three pollen sacs were prepared solely by flattening with pressure on the coverslip, without initial heating. The chromosomes are distinctly

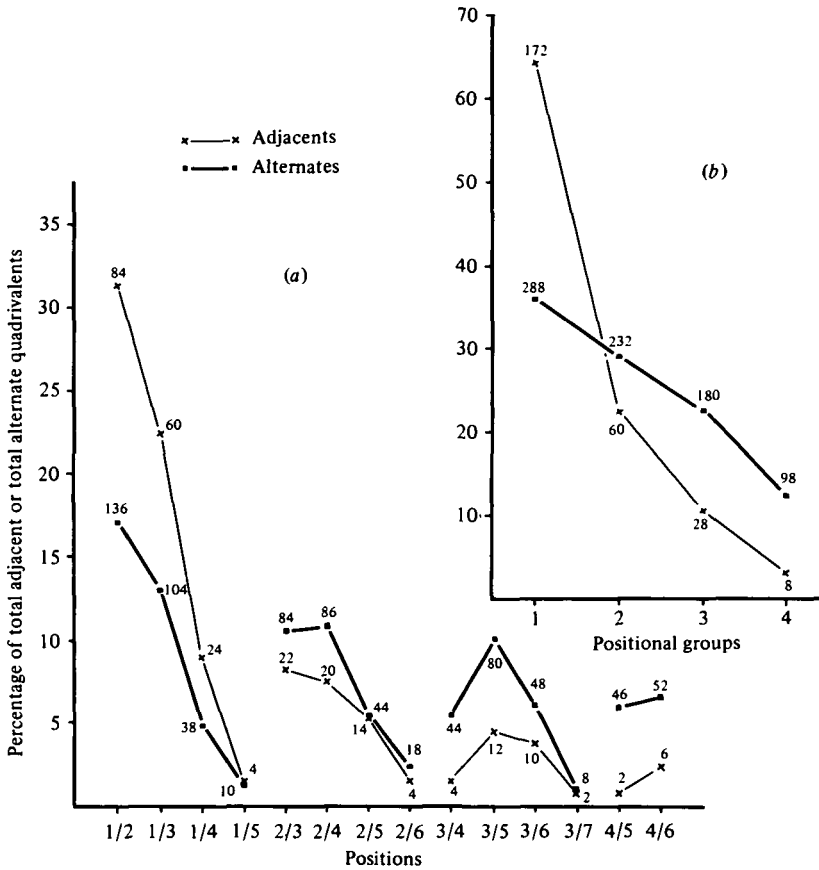

Fig. 3. Numerical and graphical presentation of data on position and orientation of the quadrivalent in lateral metaphases from 3 pollen sacs in which the average degree of chromosome spread was less than that for the cells represented in Figure 2. A total of 798 alternate quadrivalents and a total of 268 adjacent quadrivalents were scored.

less well spread by this procedure. The obvious visual effect is quantified by the facts that (*a*) 35% rather than 10% of cells could not be classified as to position/orientation, because of inferior spreading and (*b*) cells in which the quadrivalent overlay one bivalent (in particular) were much more frequent than for cells prepared with heat plus flattening (Figure 3*a*; cf. Figure 2*a*). Despite this, the distributions of cells over the four positional groups for less-well-spread preparations are essentially the same as for well-spread ones (compare Figures 3*b* and 2*b*). Thus flattening and spreading apparently affects the quadrivalent and bivalents equally, and alternate and adjacent quadrivalents are also equally affected.

As a further check on possible differential effects of spreading, the relative positions of large and small bivalents (p. 134) were examined. The number of cases in which large and small bivalents occupied each of positions 1–5 were summed over 100 cells. The two distributions obtained do not differ significantly from each other (Table 2).

Table 2. *Positions of* 400 *large and* 300 *small bivalents in lateral views of metaphase I, from* 100 *normal cells. See Plate* 1 *b for examples of large and small bivalents; positions* 1–5 *are described in the text*

	Position					
	1	2	3	4	5	Total
Large bivalents	109	94	75	78	44	400
Small bivalents	62	80	74	56	28	300

Contingency $\chi^2_4 = 7{\cdot}2$, $0{\cdot}1 < P < 0{\cdot}2$.

4. DISCUSSION

The two principal issues of the above data requiring further consideration are (i) the strong tendency for the quadrivalent to occupy marginal rather than central locations in the linear array of chromosomes and (ii) the much stronger marginal preference shown by adjacently oriented quadrivalents (Figure 2).

Theoretically, one might expect on the basis of randomness approximately equal proportions of cells in which the quadrivalent occupies positional groups 1–3 and half this proportion for group 4, analogous to the 2:2:2:2:1 distributions for large and small bivalents (Table 2). This expectation clearly is not met. However, full understanding of expectation based on randomness requires critical analysis of models based on actual cells (work in progress); and must consider the facts that (*a*) the quadrivalent is assigned not one but *two* positions, which in the circular area of chromosomes must necessarily be neighbours or near neighbours (since the quadrivalent halves are linked), and (*b*) in the linear array the quadrivalent has, in effect, been classified according to the position of its most *marginal* half (p. 135).

Irrespective of expectation based on randomness, the stronger marginal preference shown by adjacently oriented quadrivalents requires special consideration. Conceivably the different distributions of adjacent and alternate quadrivalents reflect the same tendency seen in some organisms for large chromosomes to be positioned peripherally and small chromosomes centrally in polar metaphases (see Introduction); or may be technical artefacts caused by differential lateral movement of the two quadrivalents during slide preparation. Such explanations imply actual or effective size differences in the two quadrivalents, which, however, are identical in chromosome make up, and have the same chiasma frequency and distribution (latter data not shown). Diagonal versus horizontal placement of the arms linking the two halves of the quadrivalent might conceivably produce an effective size difference in the two quadrivalents, but a conclusion as to which quadrivalent is therefore effectively larger or smaller is debatable either way. Moreover, adjacent quadrivalents do not slant systematically across the equator as expected if the arms linking the quadrivalent halves cause differential movements; the added preference toward marginal location of adjacent quadrivalents is as strong in less-well-spread preparations as well-spread ones (Figures 2, 3); and small and large bivalents are neither found preferentially in marginal nor central positions in flattened metaphases (Table 2). Note in all this regard that the relative physical

size differences between small bivalents, large bivalents and the quadrivalent (in either orientation) are 0·5, 1·0 and 1·5, respectively.

Alternatively, position and orientation of the quadrivalent may in some way be correlated with varying geometries of the cell and thus the way the cell will lie before flattening, so that adjacent orientations in marginal locations are preferentially revealed. This seems unlikely.

Finally, the patterns seen in the linear squashes may indicate genuine differences in location of the two quadrivalents in the living spindle. Perhaps quadrivalents destined to be oriented in an adjacent manner are located, in an active or passive manner (see Introduction), preferentially in peripheral rather than central positions of the living spindle, which would increase their probability of being located in marginal positions after flattening; and conversely for alternately oriented quadrivalents.

Clearly, we are not yet in a position to decide for or against these possibilities. The date available at present appear to argue most clearly for there being real biological significance to the distribution patterns shown, with implications to possible ordering of chromosomes in the nucleus (Ashley & Pocock, 1981; Bennett, 1982) and to the mechanics of orientation of interchange quadrivalents (Rickards, 1983 for review). Further data are bound to be informative, especially if derived from material with different overall percentages of alternate versus adjacent orientation. Also, three dimension reconstructions of sectioned material (Maguire, 1983) should contribute important information.

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