

## SHORT PAPER

### Metaphase position and orientation of an interchange quadrivalent of *Allium triquetrum*

#### II. Changes in position with changes in percentage alternate orientation

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#### SUMMARY

The position and orientation of an interchange quadrivalent in flattened lateral views of metaphase I were studied in inflorescences of an interchange heterozygote of *Allium triquetrum* showing high, medium and low alternate orientation frequencies. As the overall frequency of alternate orientation changes in these different inflorescences so the positioning of the alternately oriented quadrivalents changes, in that it becomes more like that of the adjacent quadrivalents. The present data argue for the biological significance of the position/orientation data, and imply the presence of two components of alternately oriented quadrivalents with different positional characteristics.

#### 1. INTRODUCTION

In flattened lateral views of metaphase I, the position in the equator of the 4/6 interchange quadrivalent of *Allium triquetrum* is different for adjacently oriented quadrivalents compared to alternately oriented ones. Adjacent quadrivalents in particular show a strong preference for marginal rather than central placement in the equator (Rickards, 1984). Analysis of well spread versus less-well spread preparations, of the position of large versus small bivalents and theoretical considerations suggest that the positional difference between the two quadrivalents is not an artefact of the scoring or preparation technique, but rather has real biological relevance to preferential positioning of chromosomes in the spindle and to the mechanics of orientation of interchange multiples. However, no adequate explanation of the available data is currently possible. The present data confirm earlier work and show that the degree of marginal placement of the alternately oriented quadrivalent increases systematically as the overall percentage of alternate orientation decreases in different inflorescence material, while the positioning of the adjacently oriented quadrivalent shows no corresponding changes.

#### 2. MATERIAL AND METHODS

The major characteristics of the 4/6 interchange heterozygote of *Allium triquetrum* are described elsewhere (Rickards, 1977, 1983). Very briefly, in pollen mother cells a chain quadrivalent is formed in the place of two of nine bivalents. The quadrivalent at metaphase I is found in alternate or adjacent-1 orientation (Rickards, 1984; plate 1).

Three collections of meiotic material were made, approximately one month apart from

clonal plants grown together. The three collections were respectively of first, second or third inflorescences, which are produced sequentially in each plant at different times of the flowering season. Inflorescences were fixed in acetic alcohol, stained in alcoholic carmine and dissected into individual pollen sacs, the contents of which were then analysed separately (Rickards, 1977, 1983). Pollen mother cells were prepared by heating and squashing, as described previously (Rickards, 1984), the entire analysis being conducted over an essentially continuous period so as to minimise variation in the preparation technique.

The method used to evaluate position of the quadrivalent is described in Rickards (1984, figure 1). In summary, the quadrivalent is assigned two positions out of a total of nine, according to the positions of its two co-oriented pairs of centromeres, relative to the seven bivalents, the total of nine positions in each cell being numbered from the margin nearest the quadrivalent. In this way, cells with varying degrees of overlay of bivalents by the quadrivalent can be evaluated along with those showing marginal through to central location of the quadrivalent.

Table 1. Number of adjacent, alternate and total quadrivalents and percentage of alternate quadrivalents from six pollen sacs from each of two inflorescence collections, results of contingency  $\chi^2$  analyses and summarized data

Pollen sac no.	Collection (inflorescence)			
	Second* quadrivalent type		Third quadrivalent type	
	Adjacent	Alternate (%)	Adjacent	Alternate (%)
1	83	177 (68.1)	36	174 (82.9)
2	58	102 (63.8)	61	249 (80.3)
3	85	159 (65.2)	48	194 (80.2)
4	91	159 (63.6)	62	286 (82.2)
5	66	130 (66.3)	47	175 (78.8)
6	69	117 (62.9)	44	167 (79.5)
Contingency $\chi^2_2$	= 1.92: $P = 0.85$		2.01: $P = 0.85$	
Contingency $\chi^2_{135}$	= 154.30: $P > 0.05$ †		153.60: $P > 0.05$	

#### SUMMARY

Collection (designation)	Quadrivalent type		
	Adjacent	Alternate (%)	Total
Second ('low' alternate)	452	844 (65.1)	1296
First* ('medium' alternate)	388	1191 (75.4)	1579
Third ('high' alternate)	298	1245 (80.7)	1543

Contingency  $\chi^2_2 = 91.0$ :  $P \ll 0.001$ .

Contingency  $\chi^2_{54} = 185.0$ :  $P \ll 0.001$ †.

\* For details of first inflorescence data see Rickards (1984, table 1).

† There are 14 positional assignments (Rickards, 1984; figure 1) each with the quadrivalent in either alternate or adjacent orientation. Hence for each collection the degrees of freedom (D.F.) are  $(28-1)(6-1) = 135$ . For 135 D.F.,  $\chi^2$  must exceed 168.5 to be significant at the 5% level of probability; and exceed 75.7 for 54  $(27 \times 2)$  D.F.

## 3. RESULTS

The contents of six pollen sacs from each of second and third inflorescence collections were analysed, separate and pooled data of each of which are given in Table 1, along with results of contingency  $\chi^2$  tests. These tests indicate, on one hand, no heterogeneity within each set of inflorescence data (thus justifying the data pooling). On the other hand, the tests indicate very clear differences between inflorescence collections in the overall frequency of alternate versus adjacent quadrivalents (see Rickards, 1983 for discussion on this interesting point). Note that the difference in percentage alternate orientation between the so called 'high' (second inflorescence) and 'medium' (first inflorescence) collections ( $\cong 5\%$ ) is one half of that between the 'medium' and 'low' collections ( $\cong 10\%$ ). The final contingency test of Table 1 indicates very clear differences between inflorescences in their position/orientation data also, as detailed below.

The position/orientation data are presented in Figs. 1 and 2, expressed as percentages of the total alternate or total adjacent quadrivalents, so as to remove the overall differences in frequencies of alternate and adjacent quadrivalents within and between inflorescence collections (Table 1). Plots for first inflorescence data (details in Rickards, 1984) are also given for comparisons.

All three inflorescence types show broadly similar distributions of the quadrivalent. The three main elements of the basic distribution, specified in detail and discussed in Rickards (1984) are (i) a decrease in the percentage of cells in which the quadrivalent overlies successively more bivalents; (ii) a strong preference for the quadrivalent to be located in marginal rather than central positions; and (iii) a stronger marginal preference shown by adjacent quadrivalents compared to alternate ones. While broadly similar, however, the distributions of the adjacent and alternate quadrivalents of Figs. 1 and 2 show differing responses to changes in the overall proportion of alternate orientation over different inflorescence types, as follows.

(a) *Adjacent quadrivalents.* The distributions of the adjacent quadrivalents of high, medium and low inflorescence collections are not significantly different from each other (statistics in caption to Fig. 1). Thus the adjacent quadrivalents behave as a homogeneous group, irrespective of their frequency relative to that of the alternate quadrivalent.

(b) *Alternate quadrivalents.* In contrast to the above, the three alternate distributions differ significantly from each other (caption to Fig. 2). As the overall percentage of alternate quadrivalents decreases over the three inflorescence types, so the proportions of quadrivalents in group 1 positions increase, while those for positions 2–4 decrease (Fig. 2). The changes are systematic (note the single point of intersection), and their magnitudes correspond well to the magnitudes of the differences in overall frequency of alternate orientation in the three inflorescence types (p. 202). Note that the effect on the data of the low collection in particular is to make the alternate distribution more like that of the adjacent quadrivalent (Figs. 1 and 2).

## 4. DISCUSSION

The position/orientation data for second and third inflorescences confirm conclusions made earlier for first inflorescences (Rickards, 1984): the two orientations of the quadrivalent differ in their location in the metaphase equator, with a marginal preference strongest for adjacently oriented quadrivalents. The additional data now also show that the positioning of the alternate quadrivalent (alone) changes systematically, so as to become more like that of the adjacent quadrivalent, as the overall frequency of alternate orientation decreases in different inflorescences. Fig. 2 suggests theoretically that if the percentage of alternate orientation were to be reduced further to, interestingly, 50%, a distribution of alternate quadrivalents essentially identical to that of the adjacent quadrivalent would be produced.

Thus each of the alternate distributions of Fig. 2 are composites of two different

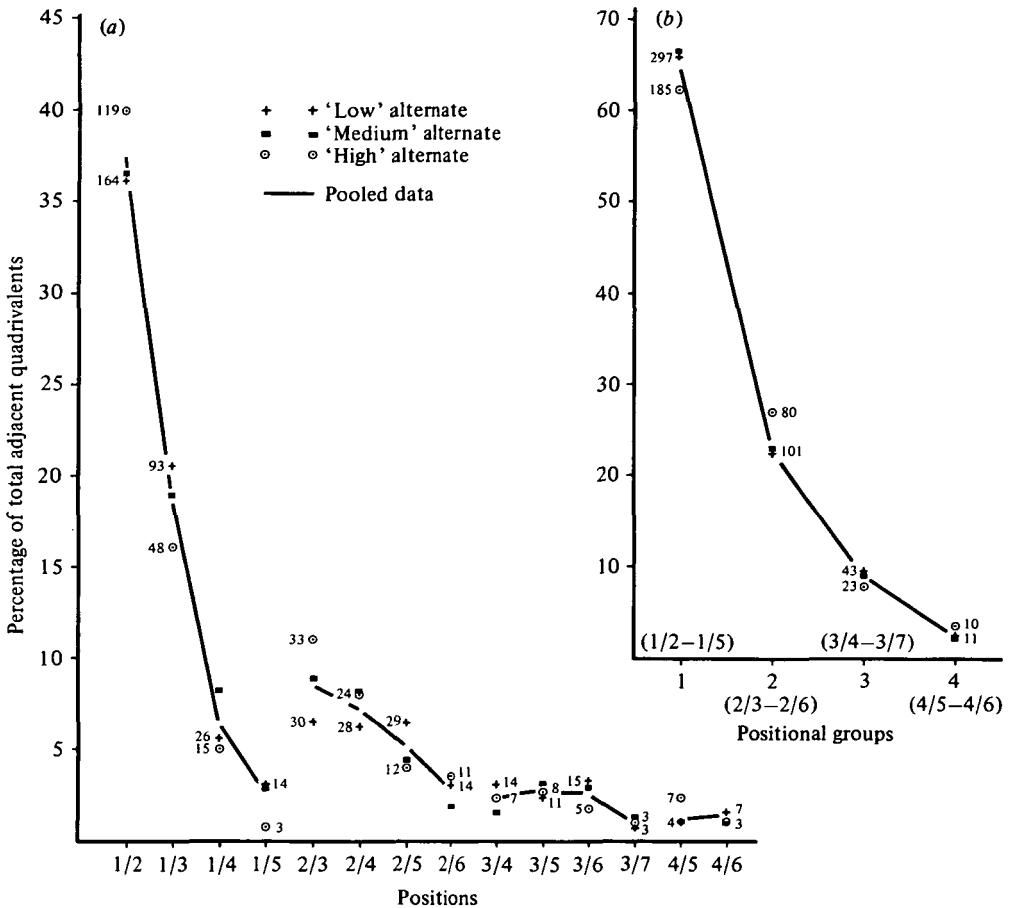


Fig. 1. Numerical and graphical presentation of data on the position of *adjacent* quadrivalents in lateral views of metaphase I from first, second and third inflorescence collections; data of each collection from six pollen sacs pooled. Numbers indicate cells scored. For total numbers of adjacent quadrivalents, see Table 1. For numerical data for first ('medium' alternate) inflorescences, see Rickards, 1984 figure 1. (a) data for individual positions; contingency  $\chi^2 = 29.5$ , D.F. =  $(14-1)(3-1) = 26$ ,  $P = 0.3$ ; (b) data for positional groups; contingency  $\chi^2_6 = 4.6$ ,  $P = 0.6$ .

distributions, conceivably representing early versus late orienting quadrivalents (Rickards, 1977, 1983) or alternate-1 versus alternate-2 quadrivalents (Rickards, 1983). The data are then explained if (a) positioning of most of the quadrivalents, in either alternate or adjacent orientation, is as shown for adjacent quadrivalents (Fig. 1) and (b) elevation of the overall percentage of alternate orientation above 50% involves a component of quadrivalents that, in becoming alternately rather than adjacently oriented, are positioned atypically toward the centre of the metaphase plate.

The present data all but rule out the position/orientation data being an artefact brought about by, for example, differential lateral movement of the quadrivalent during slide preparation (Rickards, 1984). This is because overall differences in the percentage of alternate quadrivalents effectively have been removed by expressing the data as

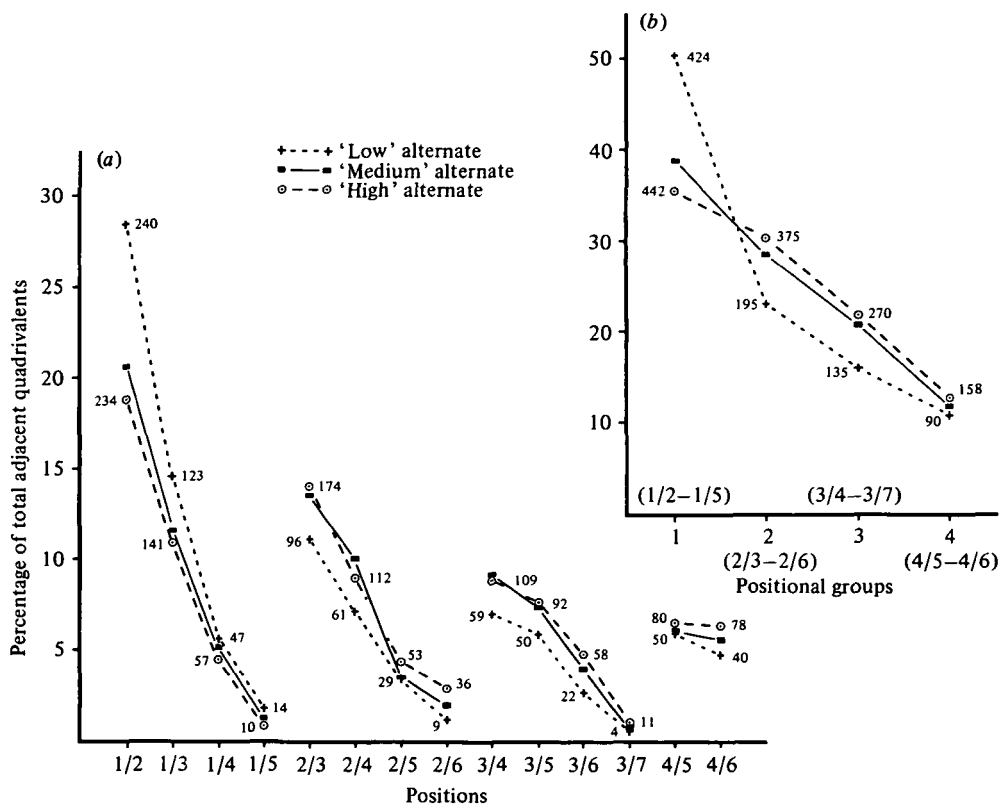


Fig. 2. Numerical and graphical presentation of data on the position of *alternate* quadrivalents. Further explanation as for Fig. 1. For data in (a), contingency  $\chi^2_{26} = 67.3$ ,  $P < 0.001$ ; for data in (b), contingency  $\chi^2_3 = 48.9$ ,  $P < 0.001$ .

percentages of total alternate or total adjacent quadrivalents. For differential movement, the changes in Fig. 2 would imply a degree of differential movement varying as the overall percentage of alternate orientation in the pollen sac changes, affecting alternate but not adjacent quadrivalents. This is improbable. The biological relevance of the position/orientation data are thus further emphasized (see also Rickards, 1984).

#### REFERENCES

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