HORDEUM AND *SECALE* MITOTIC GENOMES LIE APART IN A HYBRID

R. A. FINCH, J. B. SMITH AND M. D. BENNETT
Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, England

SUMMARY

In both unpretreated root tip metaphases and pretreated mitoses of *Hordeum vulgare* L. cv. Sultan × *Secale africanum* Stapf F_1 hybrids, *Hordeum* chromosomes tended to be nearer the centre of the mitosis than *Secale* chromosomes. This was clear in 4 serially sectioned cells examined in the electron microscope. In Feulgen squashes of 38 of 40 cells studied in the light microscope, the mean distances in each cell from the mean centromere position for the cell was less for *Hordeum* centromeres than for *Secale* centromeres.

Such spatial separation of parent genomes might prevent pairing of homoeologues in hybrids.

INTRODUCTION

In crosses of *Hordeum* with *Secale*, all *Secale* chromosomes are clearly larger than all *Hordeum* chromosomes in squash preparations and so the parental origin of each chromosome is identifiable (Finch & Bennett, 1980). Casual observation of pretreated root-tip squashes in *Hordeum vulgare* × *Secale africanum* showed a clear tendency for *Hordeum* chromosomes to be grouped near the centre of a mitosis with *Secale* chromosomes at its periphery (e.g. l.c. Fig. 1). If this separate arrangement of parental genomes were normal *in vivo*, it might be a cause of the non-pairing of homoeologues at meiosis noted in *Hordeum* × *Secale* hybrids (Thomas & Pickering, 1979; Fedak, 1979; Finch & Bennett, 1980). It was decided, therefore, to ascertain *first*, whether the separation was real in squashes; and *second*, if so, whether it is an artefact produced by squashing and/or pretreatment or is a normal arrangement *in vivo*.

MATERIALS AND METHODS

Genotypes and plant culture

F_1 hybrids ($2n = 2x = 14$) were obtained by embryo culture (Finch & Bennett, 1980) from *Hordeum vulgare* L. cv. Sultan ($2n = 2x = 14$) pollinated by *Secale africanum* Stapf ($2n = 2x = 14$) Plant Breeding Institute lines R102 (University of Manitoba line 2D127) or Karoo (Pretoria Department of Agriculture Technical Services line 72077). Nine plants for light microscopic studies of the pretreated roots that gave cells S1–8 and S10–20 in Table 1 were potted in compost after embryo culture and grown in a glasshouse under 400 W high-pressure sodium lamps, operated so that plants were continuously illuminated for about 18 h each day for 1·5–4 months. The compost contained about 3 vol. of moss peat to 1 of silver sand to 2×10^{-2} of Chempak potting base (Chempak Products, Brewhouse Lane, Hertford, Herts, U.K.) containing N, P, K and trace elements. Glasshouse temperatures were usually between 10 and 30 °C. Two weeks before its root was pretreated and fixed the plant that gave cell S9 in Table 1 was taken from embryo culture and grown in hydroponics in a growth room at 20 °C

Table 1. *Mean distances* (GMD:x,y) *of* Hordeum *and* Secale *centromeres from cell mean centromere positions* (MCP:x,y), *ratio of* Hordeum:Secale GMD:x,y *and results of one-tailed* t-*tests for* Hordeum–Secale GMD:x,y *differences in 20 pretreated squashed hybrid cells*

Cell no.	GMD:x,y (μm)		Hordeum GMD:x,y : Secale GMD:x,y	t-test		
	Hordeum	Secale		t	d.f.	P
S1	5·22	13·48	1:2·58	5·167	12	< 0·0005
S2	4·48	11·34	1:2·53	4·915	12	< 0·0005
S3	4·53	11·12	1:2·45	5·276	12	< 0·0005
S4	7·68	16·63	1:2·17	4·500	12	< 0·0005
S5	5·36	12·83	1:2·39	3·664	12	< 0·005
S6	5·67	13·14	1:2·32	3·519	12	< 0·005
S7	5·18	11·61	1:2·24	4·195	12	< 0·005
S8	7·12	15·94	1:2·24	4·260	12	< 0·005
S9	5·13	11·45	1:2·23	4·048	12	< 0·005
S10	5·40	11·06	1:2·05	3·273	12	< 0·005
S11	8·00	13·84	1:1·73	2·839	12	< 0·01
S12	7·11	11·59	1:1·63	2·422	12	< 0·05
S13	9·47	13·79	1:1·46	2·466	12	< 0·05
S14	9·20	13·15	1:1·47	1·184	12	< 0·15
S15	7·06	9·41	1:1·33	1·120	12	< 0·15
S16	8·39	10·67	1:1·27	0·879	12	< 0·20
S17	14·59	15·22	1:1·04	0·148	12	< 0·45
S18	9·30	9·39	1:1·01	0·031	12	> 0·45
S19	9·10	9·01	1:0·99	0·060	12	> 0·45
S20	11·39	10·24	1:0·90	0·276	12	< 0·40
Mean	7·47	12·26	1:1·64	7·24	19	< 0·0005

with continuous illumination (about 1600 lm/ft² at plant level; i.e. approx. 149 cd sr m⁻²) by cool white fluorescent tubes. About 1 litre of hydroponics solution 2–3 cm deep was used in a shallow enamel pie dish covered by an opaque board with holes through which the shoots protruded. The solution was aerated for 2 h daily and renewed every 2–3 days. Each litre contained about 0·101 g KNO_3, 0·030 g $MgSO_4$, 0·120 g $Ca(NO_3)_2$, 0·027 g NaH_2PO_4, 0·007 g disodium ethylene diamine tetraacetate, 0·005 ml of 60 % (w/v) $FeCl_3$ solution, 25·1 × 10⁻⁵ g $MnSO_4$, 2·6 × 10⁻⁵ g $CuSO_4$, 2·7 × 10⁻⁵ g $ZnSO_4$, 31·0 × 10⁻⁵ g H_3BO_3 and 1·1 × 10⁻⁵ g $(NH_4)_6Mo_7O_{24}$. The pH was brought to about 6·5 with NaOH. An eleventh plant that had been potted in compost after embryo culture and grown in a glasshouse under sodium lamps for 20·5 months, then without artificial light for 3 weeks, was used for electron microscope studies of pretreated roots (cells EM1–3 in Table 3). It was grown for 6 days in hydroponics as described, immediately before one root was pretreated and fixed.

To test for any effect of pretreatment on chromosome arrangement, unpretreated roots were fixed from the plant that had given cell S1 in Table 1. These unpretreated roots were taken when this plant was 2·5 years old; direct from the potted plant for light and electron microscope studies (cells S21–30 in Table 2 and cell EM4 in Table 3, respectively) and from a ramet grown in hydroponics for 6 days for light microscope study (cells S31–40 in Table 2).

Light microscope study

Roots were cut off and fixed within a minute (controls) or, if to be pretreated, put into water at 1 °C for 24 h or fresh saturated aqueous 1-bromonaphthalene at room temperature for 4 h. All roots were fixed for 2 h or more in Carnoy's fixative and stained by the Feulgen method.

Figs. 1–4. Mitotic cells in squashed root tips of *H. vulgare* × *S. africanum* hybrid plants 1 (Figs. 1, 2), 2 (Fig. 3) and 3 (Fig. 4) pretreated in ice-water (Figs. 1, 2, 4) or 1-bromonaphthalene (Fig. 3). Figs. 1, 2, 3 and 4 show cells S2, S6, S15 and S12, respectively. Arrowheads indicate *S. africanum* chromosomes; 6 →, 7 → Sultan SAT chromosomes. Bar, 10 μm.

Pretreated root tips were cut off and tapped and squashed in propionic orcein under a coverslip on a slide, one tip per slide. The meristem cells of each unpretreated root tip were gently teased out with a needle into a drop of 45 % (v/v) aqueous acetic acid on a slide and gently squashed under a coverslip, one meristem per slide. Twenty pretreated mitotic cells (1–5 from each hybrid), including 16 cold-treated, and 20 unpretreated metaphase cells were selected solely because each had 14 chromosomes in one focal plane in one field of view under the × 100 objective. They were photographed and printed at × 1875 final magnification. The 2-dimensional mean centromere position (MCP:x,y) for each cell was determined as follows. Two sets of parallel straight lines, each line 2 mm from the next, and the lines in one set intersecting
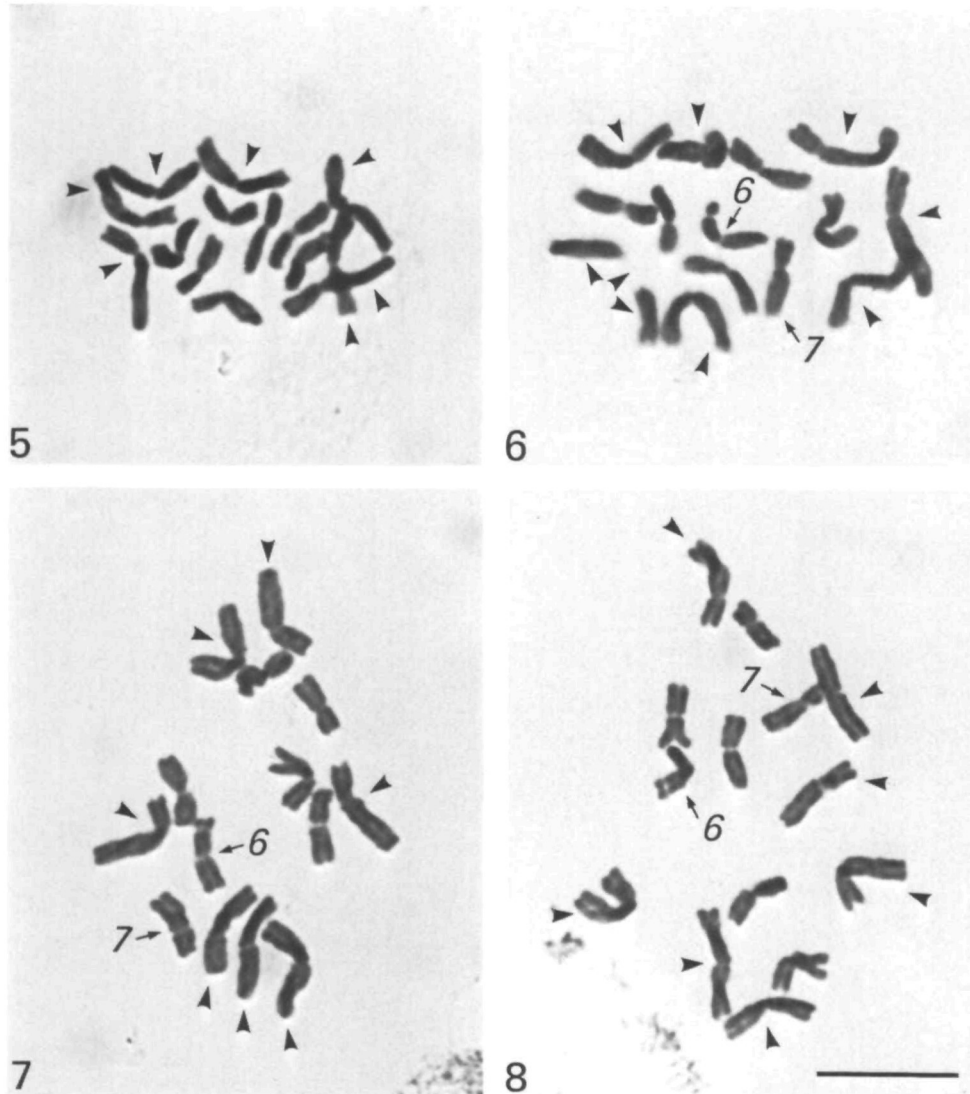
Figs. 5–8. Squashed metaphase cells S21 (Fig. 5), S22 (Fig. 6), S36 (Fig. 7) and S37 (Fig. 8) from unpretreated root tips of H. vulgare × S. africanum hybrid plant 4. Arrowheads indicate S. africanum chromosomes (in Fig. 6, a chromosome with a stretched centromere has each arm and the mid-centromere point marked); 6 →, 7 → Sultan SAT chromosomes. Bar, 10 μm.

those in the other at right angles, were drawn on a transparent sheet to form a grid slightly bigger than the prints. Each print had the grid placed over it with the grid lines parallel to the edges of the print. The coordinates of each centromere were read off the grid with reference to an arbitrarily chosen origin at the bottom left-hand corner of the print. The x and y coordinates of the MCP:x,y were estimated as the means of the x and y coordinates of the 14 centromeres, respectively, and the MCP:x,y was located using the grid, and marked on the print. Using a digitizer (computerized measuring system), the MOP Videoplan image analyser (Kontron Messgeräte GMBH, Munich; British agents: British American Optical Co. Ltd, 820, Yeovil Road, Slough), the distance (CD:x,y) of each centromere from the MCP:x,y was measured.
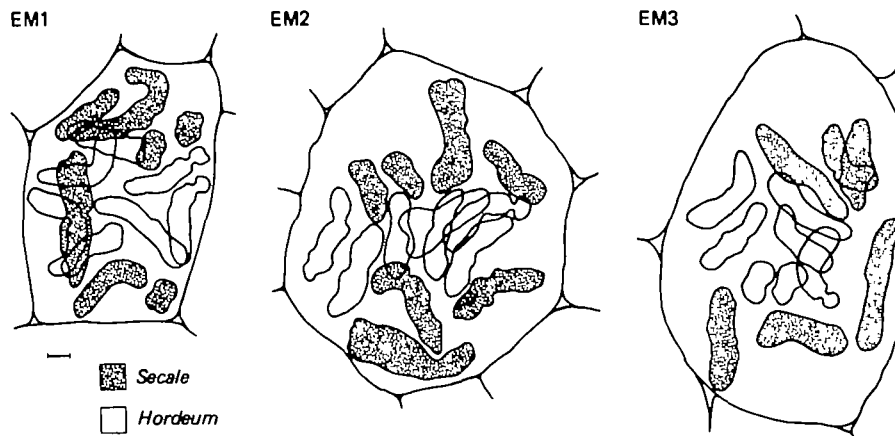
Fig. 9. Diagram of the relative positions of *Secale* and *Hordeum* chromosomes in
the 3 serially sectioned root-tip cells, EM1–3. Bar, 1μm.

To obtain separate data for each parental genome, the size difference between Sultan and
S. africanum chromosomes was used to identify the species of each chromosome, and the 7
smallest chromosomes in each hybrid nucleus were identified as Sultan chromosomes and the
7 largest as *S. africanum* chromosomes. Identification by size was often confirmed by features
such as characteristic arm ratios, heterochromatin and satellites (Figs. 1–8). The mean of the
7 *Hordeum* CD:*x,y* values was taken as the genome mean distance (GMD:*x,y*) of *Hordeum*
and the mean of the 7 *Secale* CD:*x,y* values as the *Secale* GMD:*x,y*. As the print magnification
was known, the absolute distances on the microscope slide were calculated.

EM preparations

Roots were cut off and fixed either after 24 h pretreatment in water at 1 °C (cells EM1–3 in
Table 3) or within a minute (cell EM4 in Table 3). Fixed roots were prepared for electron
microscopic (EM) study as described by Bennett, Smith, Simpson & Wells (1979).

Serial sections (0·1 μm thick) of cells EM1–4 were photographed and printed at a final
magnification of × 11800. For each cell, the prints were put in a separate binder. Each print
was arranged and numbered according to the order in which the section depicted was cut by
the microtome; print and section having the same serial number.

The volume of each chromosome was calculated by tracing its outlines from all prints showing
it, using the digitizer. In cell EM1, one section was obscured and outline-area values of the
chromosomes on it were calculated as the means of the corresponding values from the 2
adjacent sections and included in cell totals. Observations on the other 3 cells were complete.
For the reasons outlined in the section above, the 7 chromosomes with the smallest volumes in
each cell were identified as Sultan and the rest as *S. africanum* chromosomes.

The spatial arrangement of the genomes within each cell was studied as follows. Individual
chromosomes were identified and labelled on the prints. The centromere was identified as
described by Bennett, Smith, Ward & Jenkins (1981) on each chromosome and its centre
(judged by eye) marked as a point on a single print. For simplicity, the position of each chromo-
some was deemed to be at its mid-centromere point and analysis of the spatial distribution of
chromosomes used the coordinates of these points alone.

Cell walls, some cell inclusions, all chromosome outlines and mid-centromere points were
traced from each print on to transparent sheets. These sheets were stacked in order of print
number and fixed together so that all cell walls and other markers were aligned as *in vivo*, when
viewed at right angles to the plane of sectioning. The stack was placed on a light box and over-
laid with a further transparent sheet. The widest possible outline of the cell wall and of each
chromosome and the position of each centromere and its mid point were traced or marked on the

top sheet to form a composite tracing. Such composite tracings when redrawn formed the basis of Fig. 9.

For each cell, a composite tracing was laid on a transparent grid (see preceding section) and x and y axes were drawn on the tracing at right angles to each other, meeting at an arbitrarily chosen origin outside the cell wall at the bottom left corner of the tracing. Next, the coordinates of each mid-centromere point on the 3 axes (x, y and z) at 90° to one another and with the same origin were obtained. The x and y coordinates were obtained directly from the composite tracing and expressed in units corresponding to μm *in vivo*. The z coordinate was obtained in the same units by multiplying by 0·1 the serial number of the section with the relevant mid-centromere point, as each section was 0·1 μm thick. The coordinates of the 3-dimensional mean centromere position (MCP:x,y,z) were calculated as $\bar{x}$, $\bar{y}$ and $\bar{z}$ and the MCP:x,y was marked on the composite tracing. The CD:x,y was found for each centromere on the composite tracing using the digitizer and expressed in μm. Next, for each centromere, the minimum distance (d) between that centromere and the section with the MCP:x,y,z was found and expressed in μm. To do this, the difference between the serial numbers of the print with the relevant mid-centromere point and the section with the MCP:x,y,z was multiplied by 0·1. The distance (CD:x,y,z) of each centromere from the MCP:x,y,z was calculated using the formula, CD:$x,y,z = \sqrt{((CD:x,y)^2 + d^2)}$. The mean of the 7 *Hordeum* CD:x,y,z values was taken as the 3-dimensional genome mean distance (GMD:x,y,z) of *Hordeum* and the mean of the 7 *Secale* CD:x,y,z values (6 in nucleus EM3) as the *Secale* GMD:x,y,z.

RESULTS

In the Feulgen-stained squashes, the chromosomes in cells S1–20 from pre-treated roots (see examples in Figs. 1–4) were similar in appearance to metaphase chromosomes in cells S21–40 from unpretreated roots (see examples in Figs. 5–8), except that chromosomes seemed larger in cells S1–20 than in cells S21–40. The GMD:x,y of the *Secale* centromeres from the MCP:x,y exceeded the GMD:x,y of the *Hordeum* centromeres in 18 of cells S1–20 (Table 1). The difference was significant to very highly significant by Student's t-test in cells S1–13 and the grand means of the GMD:x,y values were also very highly significantly different in a paired t-test (Table 1). The *Secale* GMD:x,y varied from 0·90 to 2·58 (mean 1·64) times the size of the *Hordeum* GMD:x,y. Pooled data from the 4 cells from roots pretreated with 1-bromonaphthalene (cells S13, 15, 16 and 19) gave a *Secale*:*Hordeum* GMD:x,y ratio of 1:1·26. This ratio agreed with the *Secale*:*Hordeum* GMD:x,y ratio of 1:1·75 given by pooled data from the 16 squashed cells from roots pretreated with ice-water (cells S1–12, 14, 17, 18 and 20) in that the *Hordeum* GMD:x,y exceeded the *Secale* GMD:x,y, but the *Secale*:*Hordeum* GMD:x,y ratio was significantly lower ($P = 0·02$–$0·05$) after the 1-bromonaphthalene pretreatment than after the ice-water pretreatment.

Results from unpretreated squashed cells agreed closely with those from pretreated ones. Thus in all 20 cells, the GMD:x,y of the *Secale* centromeres from the MCP:x,y exceeded the GMD:x,y of the *Hordeum* centromeres by 1·004–2·269 (mean 1·485)-fold and the difference was significant in 12 cells (Table 2). In t-tests of *Secale*:*Hordeum* GMD:x,y ratios in squashed cells, there was no significent difference between pot-grown and hydroponically grown unpretreated roots or between pretreated and unpretreated roots.

The 3-dimensional EM study of the 4 unsquashed serially sectioned cells, EM1–4,

Table 2. *Mean distances* (*GMD*:x,y) *of* Hordeum *and* Secale *centromeres from cell mean centromere positions* (*MCP*:x,y), *ratio of* Hordeum:Secale *GMD*:x,y *and results of one-tailed* t-*tests for* Hordeum–Secale *GMD*:x,y *differences in 20 unpretreated squashed cells from one hybrid plant. Cells S21–30 came from the potted plant and cells S31–40 from a ramet grown in hydroponics*

| | GMD:x,y (μm) | | *Hordeum* GMD:x,y: | t-test | | |
Cell no.	*Hordeum*	*Secale*	*Secale* GMD:x,y	t	d.f.	P
S21	4·61	10·46	1:2·27	4·706	12	< 0·0005
S22	3·40	6·72	1:1·97	5·245	12	< 0·0005
S23	2·87	5·44	1:1·90	4·016	12	< 0·005
S24	3·45	6·14	1:1·78	2·817	12	< 0·025
S25	4·60	8·08	1:1·76	3·378	12	< 0·005
S26	4·35	7·02	1:1·62	2·361	12	<0·025
S27	4·86	6·83	1:1·40	2·222	12	< 0·025
S28	5·40	7·14	1:1·32	1·217	12	< 0·15
S29	7·95	10·41	1:1·31	1·453	12	< 0·10
S30	6·97	7·64	1:1·10	0·479	12	< 0·35
S31	3·28	7·23	1:2·21	4·576	12	< 0·0005
S32	4·47	8·26	1:1·85	3·704	12	< 0·005
S33	4·43	7·24	1:1·63	2·166	12	< 0·05
S34	5·32	8·02	1:1·51	1·682	12	< 0·10
S35	5·19	7·70	1:1·48	2·013	12	< 0·05
S36	6·65	9·54	1:1·47	2·262	12	< 0·025
S37	7·88	10·65	1:1·35	1·574	12	< 0·10
S38	8·56	10·90	1:1·27	1·448	12	< 0·10
S39	8·21	10·14	1:1·23	1·485	12	< 0·10
S40	6·68	6·71	1:1·00	0·016	12	> 0·45
Mean	5·46	8·11	1:1·49	9·939	19	< 0·0005

clearly confirmed that *Hordeum* centromeres were grouped closer to the mean centromere position (MCP:*x,y,z*) than were *Secale* centromeres (Fig. 9). The chromosomes in cells EM1–3 from a cold-treated root were similar in appearance to those in the metaphase cell EM4 from an unpretreated root. There were 14 chromosomes in cells EM1, EM2 and EM4 but only 13 in cell EM3. Aneuploid cells with 13 or 15 chromosomes are more common in *Hordeum* × *Secale* hybrids, including the present hybrid (Finch & Bennett, 1980) than in the parental species. The ranked volumes and the distance (CD:*x,y,z*) of each centromere from the mean centromere position (MCP:*x,y,z*) in cells EM1–4 are given in Table 3. Using morphological criteria, the 7 largest chromosomes in cells EM1, EM2 and EM4, and the 6 largest in cell EM3 were identified as *Secale* chromosomes. The chromosomes ranked seventh in cells EM1 and EM2 and sixth in cell EM3 had satellites.

The mean distance (GMD:*x,y,z*) from the mean centromere position of the cell (MCP:*x,y,z*) was significantly less for *Hordeum* than for *Secale* centromeres in all 4 cells and the grand mean (2·66) of the *Hordeum* GMD:*x,y,z* values was highly significantly less than that (4·64) of the *Secale* GMD:*x,y,z* values (Table 4). *Hordeum*

Table 3. *Chromosome volume and distance (CD:x,y,z) of centromere from cell mean centromere position (MCP:x,y,z) for each chromosome in 4 serially sectioned hybrid cells studied by EM. The 7 smallest chromosomes in each cell are Hordeum and the rest Secale. Cells EM1–3 came from a pretreated root and cell EM4 from an untreated root*

| Cell EM1 | | Cell EM2 | | Cell EM3 | | Cell EM4 | |
Chromosome volume (μm^3)	CD:x,y,z (μm)	Chromosome volume (μm^3)	CD:x,y,z (μm)	Chromosome volume (μm^3)	CD:x,y,z (μm)	Chromosome volume (μm^3)	CD:x,y,z (μm)
10·89	4·15	12·61	6·04	14·32	4·75	12·77	3·46
10·60	4·60	12·34	5·47	13·29	5·89	12·07	3·69
10·45	3·64	12·32	4·88	11·36	5·40	12·02	5·05
9·98	5·56	12·06	4·70	11·30	3·98	11·68	3·78
9·44	5·74	11·34	2·26	11·02	4·77	10·82	3·84
8·83	3·67	10·90	5·17	10·72	4·72	10·35	5·40
8·45	5·25	10·13	4·93	—	—	10·19	4·58
7·23	2·34	8·75	4·11	8·81	2·46	8·54	4·02
7·06	1·95	8·32	1·94	8·53	2·01	8·15	1·48
7·06	1·72	7·86	2·97	8·46	4·56	7·99	2·39
6·99	1·73	7·71	2·33	8·27	0·58	7·94	4·08
6·96	3·45	7·69	2·99	7·94	1·62	7·87	1·44
6·31	3·03	7·52	3·76	7·62	2·58	7·42	3·61
5·89	3·06	6·31	1·43	6·96	2·89	6·67	4·06

Table 4. *Mean distances* (GMD:x,y,z) *of* Hordeum *and* Secale *centromeres from cell mean centromere positions* (MCP:x,y,z), *ratio of* Hordeum:Secale *GMD*:x,y,z *and results of one-tailed* t-*tests for* Hordeum–Secale *GMD*:x,y,z *differences in serially sectioned hybrid cells studied by EM. Cells EM1–3 were pretreated and cell EM4 was not*

| | GMD:*x,y,z* | | Hordeum GMD:*x,y,z*: | t-test | | |
| | Hordeum | Secale | Secale GMD:*x,y,z* | t | d.f. | P < |
Cell no.						
EM1	2·79	4·78	1:1·71	3·431	12	0·005
EM2	2·47	4·66	1:1·89	5·129	12	0·0005
EM3	2·39	4·92	1:2·06	4·518	11	0·0005
EM4	3·01	4·26	1:1·42	2·310	12	0·025
Mean	2·66	4·64	1:1·74	7·667	53	0·0005

Table 5. *Correlation coefficients and their probabilities in linear regression analysis of centromere distance* (CD:x,y,z) *from cell mean centromere position* (MCP:x,y,z) *and chromosome volume in unsquashed hybrid cells with* (EM1–3) *and without* (EM4) *pretreatment*

Cell	Chromosomes	Correlation coefficient	P
	All 14	+0·67	0·008
EM1	*Hordeum*	−0·57	0·185
	Secale	−0·27	0·555
	All 14	+0·76	0·001
EM2	*Hordeum*	+0·59	0·162
	Secale	+0·30	0·512
	All 13	+0·75	0·003
EM3	*Hordeum*	−0·04	0·936
	Secale	+0·34	0·511
	All 14	+0·39	0·167
EM4	*Hordeum*	−0·29	0·522
	Secale	−0·56	0·191
	All 14	+0·63	<0·001
EM1–4 aggregate	*Hordeum*	+0·03	0·863
	Secale	+0·03	0·892

and *Secale* chromosomes showed no overlap in the value of CD:*x,y,z* in cell EM1, and in cells EM2 and EM3, only one *Secale* chromosome had a lower value than the highest *Hordeum* value for its cell. In cell EM4, 4 *Secale* chromosomes had lower CD:*x,y,z* values than the highest *Hordeum* value. The *Secale* GMD:*x,y,z* was 1·71 1·89, 2·06 and 1·42 times that of *Hordeum* GMD:*x,y,z* in cells EM1, 2, 3 and 4, respectively. These values are within the range of, and together are not significantly different from, the corresponding GMD:*x,y* ratios in cells S1–40 examined by light microscopy.

Comparisons of the ratio of the *Hordeum* to the *Secale* GMD:x,y,z between the 30 cells fixed after pot culture and the 14 cells fixed from hydroponics, and between the 23 pretreated cells and the 21 unpretreated cells showed no significant differences in 2-tailed t-tests.

Linear regression analysis showed in each of cells EM1-4 a positive correlation, highly significant in cells EM1-3, between chromosome size and CD:x,y,z within the total complement, but no consistent or significant correlation within either parent genome separately (Table 5). Pooled data from all 4 cells showed a very highly significant positive correlation for the whole genome, but the positive correlations within each separate genome were not significant.

DISCUSSION

Three-dimensional EM estimates of centromere positions in unsquashed cells are more true to life than are estimates from squash preparations because of both the extra dimension and the absence of distortion by squashing in EM techniques. The mean CD:x,y of all centromeres in cells S1-40 was 8·33 and the mean CD:x,y,z of all centromeres in cells EM1-4 was 3·64, but the mean *Hordeum*:*Secale* CD:x,y and CD:x,y,z ratios were 1:1·57 and 1·74, respectively, and not significantly different. Obviously, however, squashing forced chromosomes an average of 2-3 times further from the centre of the mitosis than they had been *in vivo*. The close agreement between these *Hordeum*:*Secale* ratios in EM and light microscopic studies in our hybrid and the constancy and clearness of the EM results proves that the subjective impression obtained from squash preparations, that *Hordeum* chromosomes were grouped near the centre of the mitosis with *Secale* chromosomes nearer the periphery, was real and not an artefact produced by squashing. Thus, squash preparations viewed by light microscopy can give valuable information about the spatial arrangement of metaphase chromosomes *in vivo*. As results from pretreated roots were so similar to those from unpretreated roots, it is clear that pretreatment neither causes nor destroys the separation of the 2 parental genomes observed in our material.

In several species, chromosome position at mitosis is correlated with chromosome size. For example, small chromosomes tend to lie nearer the cell centre than do large chromosomes in a grasshopper (Nur, 1973), the Chinese hamster (Juricek, 1975), and man (Rohlf, Rodman & Flehinger, 1980). The grouping of *Hordeum* chromosomes near the centre of the mitosis in the present hybrid is probably not due to a general effect of their smaller size. If it were, the positive correlation between chromosome volume and CD:x,y,z found in cells EM1-4 for the total complement would also apply for each parental genome separately. However, regression analysis of cells *EM*1-4 showed that within each parental genome, chromosome volume was not correlated with CD:x,y,z even with pooled data (Table 4). Indeed, the correlation coefficient was negative in 5 of the 8 genomes considered.

In the present work, separation of parental genomes was seen in roots of various sizes from plants of various ages grown in different environments, and even after 24 h cold treatment. Observation of cells at all stages of the cell cycle in many root-tip

squashes showed that all chromosomes were integrated and behaved (except for the production of occasional aneuploid cells) regularly, alike and synchronously. This indicates that the separation persists through many cell divisions and is a normal condition not restricted in roots to one developmental phase or environmental situation. *Hordeum* × *Secale* hybrids are particularly favourable material for studying genome separation since the very precise estimates of chromosome volume obtainable by electron microscopy constitute a simple means by which each chromosome can be identified as to species, in cells where undisturbed chromosomal positions are also accurately known. This provides a valuable check on the interpretation of chromosome positions in squash preparations where disturbance of chromosome positions is inevitable. However, in many genotypes, genome separation would be hard to detect since maternal and paternal sets look identical. Perhaps, therefore, genome separation in somatic tissues is a general phenomenon that has been overlooked because paternal and maternal genomes are usually indistinguishable in appearane. Thus in a *Rubus* hybrid, 2 parental genomes commonly appeared separated in the same meiocyte at MI (Bammi, 1965). However other abnormalities were common, in contrast to the case of our hybrid.

Non-random positioning and orientation, and behavioural differences between paternal and maternal chromosomes occur in many animals. For instance, gonomery, the condition in which paternal and maternal chromosomes remain in 2 separate groups in the zygote and early cleavage stages, is known in several species (see Costello, 1970), but the separation was not reported to have persisted further in these examples. Indeed, in gonomeric *Tigriopus* zygotes, 'mixing of paternal and maternal chromosomes was effected in late anaphase of the first cleavage division' (Ar-Rushdi, 1963). White (1973) and Brown & Chandra (1977) have reviewed many cases in animals where paternal and maternal genomes are distinct in position and/or behaviour. Recognizable separation of parental genomes, such as is conspicuous at somatic interphase in scale insects, usually involves some abnormality such as complete heterochromatization of one genome (e.g. Kitchin, 1970), which does not occur in the present hybrid, or is reported from the germ line. In *Sciara*, maternal and regular paternal genomes are separated during male meiosis while not heterochromatized, and the paternal set is eliminated (Crouse, Brown & Mumford, 1971). The present authors know of no other published case than the present hybrid where separation of parental genomes is maintained during development of nuclei or cells during a large indeterminate number of regular mitoses in the soma. Interestingly, however, genome separation was found in cultured man–mouse hybrid cells and was thought to persist 'through several mitoses' (Rechsteiner & Parsons, 1976).

In the cereals, non-random arrangement of chromosomes has been noted at meiosis (Kempanna & Riley, 1964) and mitosis (Fedak & Helgason, 1970; Singh, Röbbelen & Okamoto, 1976; Thomas, 1973), but associations are usually found or assumed to be homologous or homoeologous, not genomic (e.g. Feldman, Mello-Sampayo & Avivi, 1972). In *Hordeum* × *Secale* hybrids that reach meiosis, there is almost complete failure of effective pairing in so far as most pollen mother cells contain 14 univalents at first metaphase (Wagenaar, 1960; Thomas & Pickering, 1979; Finch & Bennett,

1980). If premeiotic association of homologues is essential for normal meiotic pairing, as suggested for wheat (Feldman, 1966), genome separation like that noted in the roots of the present hybrid might be an important cause of pairing failure if it occurred in shoots. Such genome separation might prevent normal meiotic pairing, either because the separation persists into meiotic prophase or because it breaks down before meiosis, but so late in development that normal meiotic pairing is still prevented. To prevent recombination, genome separation in hybrids would have to separate homoeologues or their pairing initiation sites, at least at the stage or stages when their coincidence is essential for normal pairing.

Unfortunately, plants of the present hybrid have not produced spikes and so it has not been possible to investigate the spatial arrangement of the chromosomes in archesporial cells or meiocytes. However, Tozu (1976) obtained mature spikes in his *H. vulgare* × *S. africanum* hybrid; and so, using serially sectioned cells of suitable hybrids with or without normal pairing and/or chiasma formation, it will be feasible to test whether genome separation occurs, how late it persists in the germ line and whether or not it is correlated with failure of pairing and/or chiasma formation in hybrids. However, if genome separation should prove to be a general phenomenon, as suggested earlier in this discussion, then it obviously cannot explain on its own the failure of pairing and chiasma formation in *Hordeum* × *Secale* hybrids.

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