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Developmental sequence of chromosome number in a cytologically unstable Rubus hybrid

By G. HASKELL and N. N. TUN

Genetics Department, Scottish Horticultural Research Institute, Dundee

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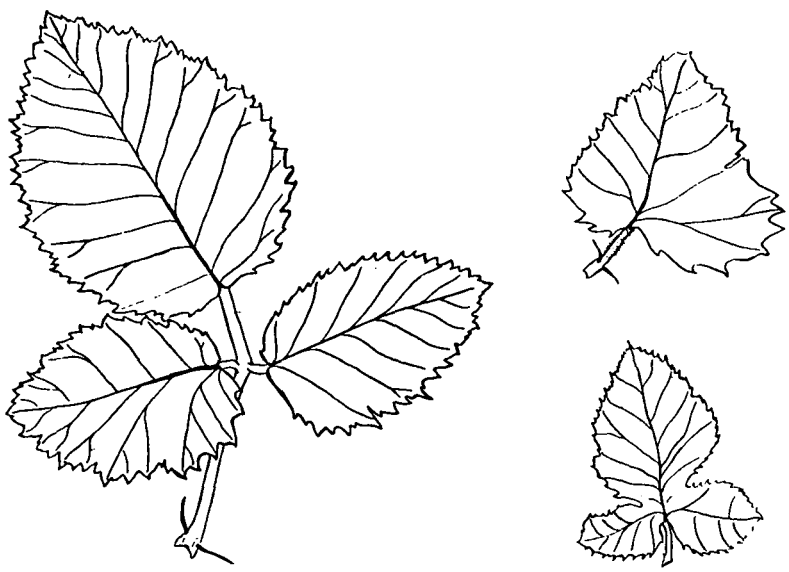
INTRODUCTION

One plant was observed to grow more slowly than the others in a population of thornless segregants in an F_2 from the cross of Merton Thornless (*R. craniensis*, $2n = 28$) with Himalaya Giant (*R. procerus*, $2n = 28$) (Plate I, fig. 1). This seedling was very stunted, with greatly shortened internodes, and with thickened and rugose leaves much smaller than those of normal sibs (Text-fig. 1). The plant was densely



Text-fig. 1. The leaves from (*left*) a normal stable tetraploid ($2n = 28$) thornless seedling ($1^2/59$) taken from the sixth node, and (*right*) leaves from the abnormal, cytologically unstable seedling of the same F_2, taken from the sixth and thirteenth nodes (Plant $1^1/59$).

covered with hairs, and very dark green. Even after twelve months it was approximately only 12 cm. high, compared with an average of 226 cm. for the normal sibs. At first it sent up one shoot only, but eventually it produced a short lateral shoot, although by then the normal sibs had produced three or four long shoots. The root system was also poorly developed, particularly in comparison with the normal vigorous rooting system of the sibs.

Table 1. *Sequential chromosome number variation in the cytologically unstable plant* 1¹/59

Sequential count	No. of cells with chromosome no.																				No. of cells	No. of chromosomes	Mean no. of chromosomes per cell	No. of roots examined
	9	12	19	21	23	24	25	28	29	30	31	32	33	34	35	36	37	38	39	40	46				
1	—	—	—	—	—	—	—	—	—	3	2	2	3	0	6	1	—	—	—	—	—	17	561	33·00	1
2	—	—	—	1	—	—	—	—	—	2	1	1	3	1	5	1	—	—	—	1	—	16	528	33·00	3
3	—	—	1	—	—	1	—	1	1	1	2	3	2	3	9	1	—	—	—	—	—	25	807	32·28	2
4	1	—	—	—	1	—	—	1	—	—	1	1	1	4	8	1	—	—	—	—	—	19	608	32·00	2
5	—	—	—	—	—	—	4	—	1	—	—	4	6	11	29	2	—	1	—	—	—	58	1954	33·69	3
6	—	1	—	—	—	—	—	1	—	—	—	1	3	1	43	—	—	1	—	—	1	52	1794	34·50	5
7	—	—	—	—	—	—	—	—	—	1	0	0	0	0	3	—	—	—	—	—	—	4	135	33·75	2
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	42	1	—	—	—	—	—	43	1506	35·02	1
Total	1	1	1	1	1	1	4	3	2	7	6	12	18	20	145	7	0	2	0	1	1	234	7893	Mean: 33·73	19

The period between the first and eighth count was 168 days.

Analysis of variance

Item	S.S.	N	M.S.	V.R.	P
Between counts	229·86	7	32·837	2·88	<·01
Within counts	2576·18	226	11·399		
Total	2806·04	233			

A detailed cytological analysis within this seedling has revealed great variation in chromosome number which also affects cell size. Britton & Hull (1956, 1957) have reported on mitotic instability in Rubus, but this paper provides the first sustained set of precise cytological observations made at intervals during the growth of a Rubus plant, not previously subjected either to vegetative propagation or to colchicine treatment.

METHODS

Root tips of the abnormal seedling ($1^1/59$) and of six normal sibs ($1^{2-7}/59$) growing in a cool glasshouse were pre-treated in 0·002 M solution of oxy-quinoline for 4 hours at 5 to 10° C. (cf. Tjio & Levan, 1950). The root tips were then fixed overnight in 1:3 acetic alcohol, and were hydrolysed in normal hydrochloric acid for 30 minutes at 58°–60° C. Then they were stained in Feulgen for 2 hours and squashed in carmine; the observations were made at × 1800 magnification. Later examinations were only on the abnormal seedling, but one normal plant was checked for stability at $2n = 28$ chromosomes at intervals. The records of the chromosome numbers are given in Table 1.

Some squashes were also made of stipules to see whether the instability was not necessarily confined only to the roots. Owing to the extreme density of the hairs, however, it was generally difficult to see good metaphase plates.

The measurements of cell size were obtained by making camera-lucida drawings at × 1200 on grid paper, and counting the number of squares (unit = 0·01 sq. inch) occupied by the cell.

OBSERVATIONS

Variation in chromosome number

The somatic chromosome complement in a normal sib plant ($1^2/59$) is constant at $2n = 28$ (Plate II, fig. 1). The most frequent number in the abnormal plant $1^1/59$ is $2n = 35$ (Plate II, fig. 2); this plate shows no departure from the usual metaphase plate to be found in Rubus. The complete range of chromosome number within this plant is from 9 to 46. This means that cells lower than the diploid number ($2n = 14$) can occur. A sample of the range of chromosome numbers from 9 to 40 actually observed during the series of observations is given in Text-fig. 2. In Plate II, fig. 3, a metaphase plate with $2n = 12$ is illustrated, to show that there is no difference in the size of the chromosomes as compared with those in the $2n = 35$ cell. This means that reduction in chromosome number has not resulted from terminal fusion, as is known to occur in species like *Cardamine pratensis* (Lawrence, 1931).

The somatic chromosome count from a stipule cell ($2n = 39$) is shown in Plate II, fig. 4. Here the chromosomes look somewhat broader, but these had been pre-treated with colchicine instead of oxy-quinoline so any possible size difference of individual chromosomes might be attributable to the difference in technique.

Fig. 1. Two sibs from the F_2 of *R. craniensis* ($2n = 28$) × *R. procerus* ($2n = 28$). Photographed at 7 months.

Right: abnormal plant ($1^1/59$) with varying chromosome numbers.
Left: normal plant ($1^2/59$) constant with 28 chromosomes.

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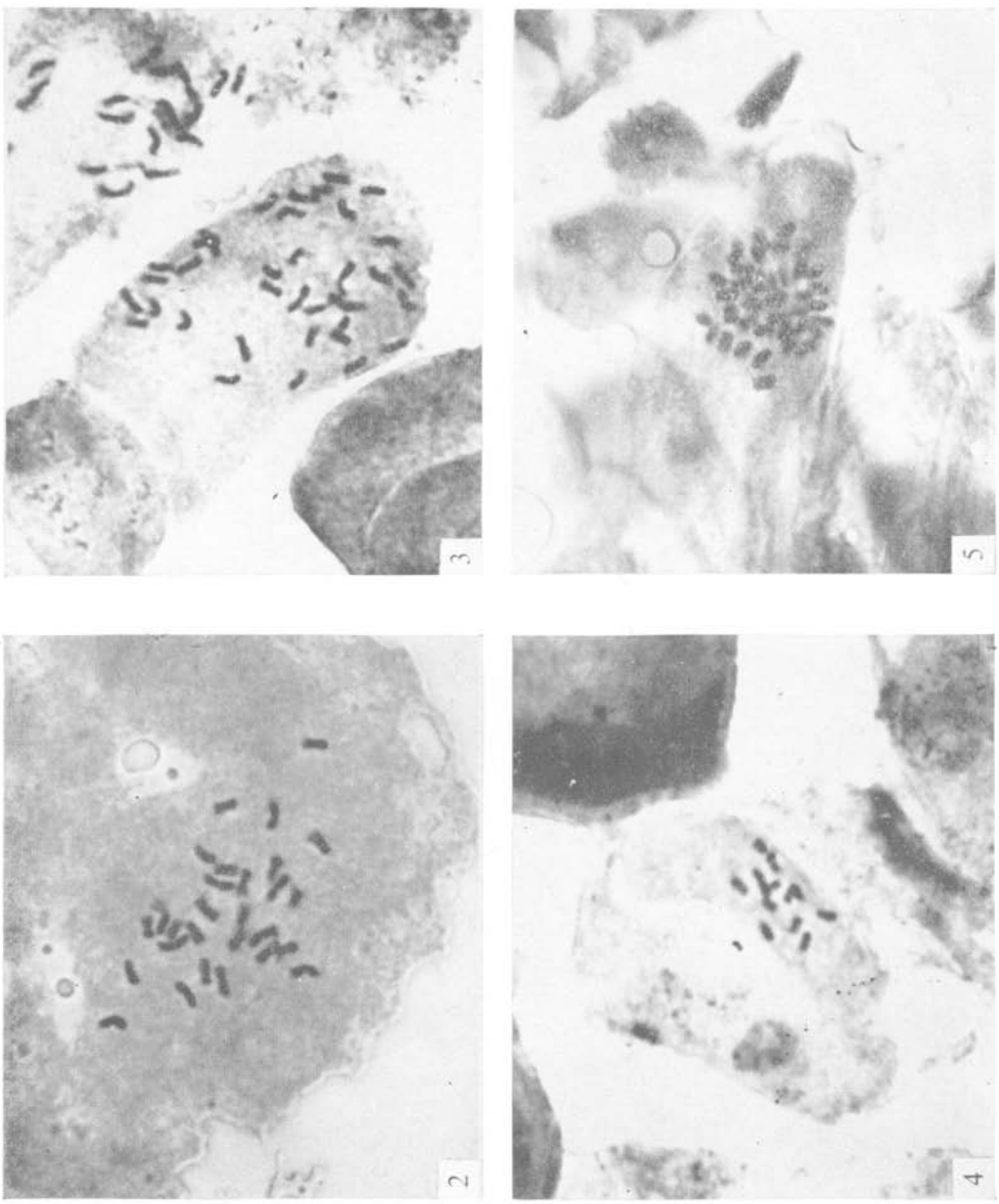

Fig. 2. Somatic chromosome number ($2n = 28$) from a root-tip of a normal sister seedling ($1^2/59$) pretreated with oxy-quinoline.

Figs. 3 and 4. Two chromosome counts ($2n = 35$ and 12 respectively) from root-tips of the abnormal hybrid $1^1/59$ pretreated with oxy-quinoline.

Fig. 5. Somatic chromosome count, from a stipule of the abnormal plant $1^1/59$ pre-treated with colchicine. ($2n = 39$, but 2 chromosomes are out of focus). All $\times 1920$.

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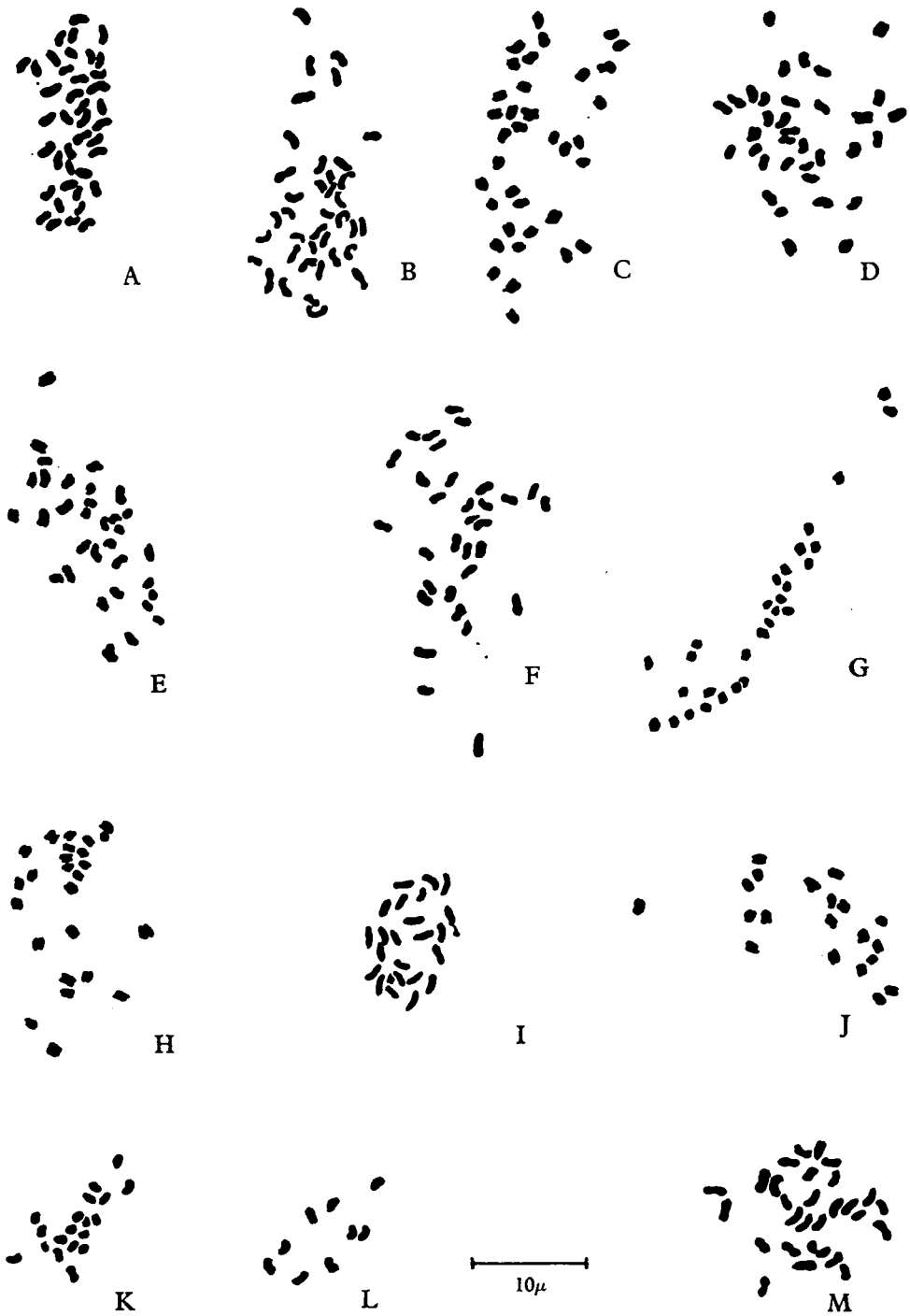

Text-fig. 2. Camera-lucida drawings of some of the varying chromosome numbers found in the root-tips of the unstable Rubus species hybrid ($1^1/59$).

A. $2n = 40$
B. $2n = 38$
C. $2n = 36$
D. $2n = 35$
E. $2n = 33$
F. $2n = 32$
G. $2n = 29$
H. $2n = 25$
I. $2n = 24$
J. $2n = 21$
K. $2n = 19$
L. $2n = 9$
M = Control ($1^2/59$), $2n = 28$

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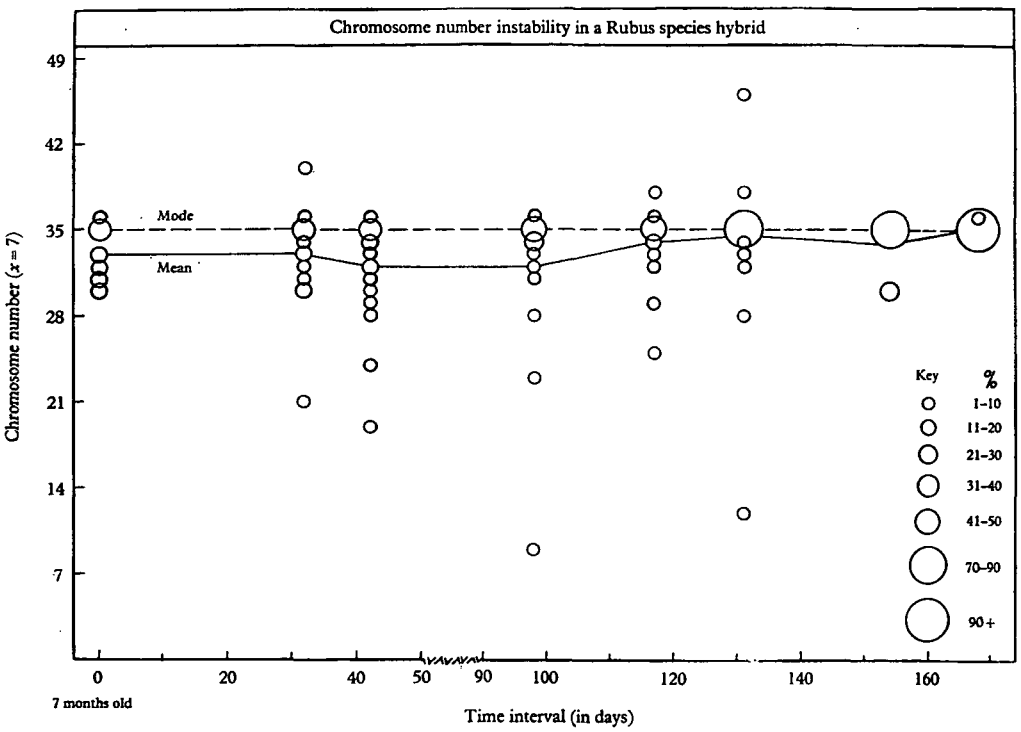
Variation with age of plant

Text-fig. 3 shows the frequency of the various chromosome numbers, determined for a period of 168 days commencing when the plant was 7 months old. This diagram shows the mean chromosome number and also the mode, which is constant at $2n = 35$. During the period under observation the variation in chromosome number, although considerable, clearly is not related to the stage of plant growth. There is a suggestion, however, that there is less likelihood of very low chromosome



Text-fig. 3. The relative frequency of variation in somatic chromosome numbers of root-tip cells in the abnormal seedling. Observations were commenced at 7 months and continued for 168 days.

numbers occurring in later observations, and a greater likelihood of the occurrence of chromosome numbers higher than 35. Throughout the series of observations the mean chromosome number is lower than the mode, i.e. one is more likely to find cells with numbers lower than the pentaploid value of 35. A further interesting feature is that the relative frequency of the $2n = 35$ cells increases with age; this might be a likely consequence of an immediate effect on the cells from actual chromosome number. Throughout the series, the frequency of orthoploid cells with 7 as the basic number does not otherwise increase. Nor is there a balance towards cells with complete chromosome sets of $2n = 28$, which is the number for most species of Rubus; and at no instance during the period of the investigation was the somatic number only $2n = 35$.

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An analysis of variance (Table 1) shows that there is a highly significant difference in chromosome numbers of the various counts. On two occasions counts were made on three roots, and five roots on one occasion. An analysis was carried out to determine whether the five roots differed in their chromosome numbers at any one time. This showed no significant difference between roots. The plant $1^1/59$ is, therefore, unstable with regard to the sequential date when roots are examined, but the roots themselves are generally behaving in a similar fashion.

Satellites

Satellites are a feature of Rubus. A pair of satellited chromosomes were observed in cells of the normal plants with 28 chromosomes; but in the abnormal plant one cell of 24 chromosomes had only one satellite, whereas two cells with 35 chromosomes had two and three satellites respectively (Table 2). This suggests that it is not always the same chromosomes that are being eliminated from one cell to another at mitosis.

Table 2. *Variation of satellite number in Rubus*

Species	Ploidy	Range of satellites per cell
R. tomentosus	$2x$	2 (15)
R. ulmifolius alba	$2x$	1 (5); 2 (25)
R. ulmifolius	$2x$	1 (7); 2 (75)
R. ulmifolius inermis	$2x$	2 (23)
R. tomentosus ×	$3x$	1 (14); 2 (12)
R. ulmifolius ×	$3x$	1 (1); 2 (8)
R. caesius	$4x$	2 (15); 3 (3); 4 (2)
$1^1/59$	$2n=24$	1 (1)
	$2n=33$	2 (1)
	$2n=35$	1 (1); 2 (7); 3 (4)
$1^{2-7}/59$	$2n=28$	2

Numbers in brackets equal cells with the corresponding nos. of satellites.

There are always two satellites per cell in the primary diploid ($2n=14$) species *R. tomentosus*, but in various normal tetraploids so far examined by us four satellites have not always been observed. This difference might result from technical difficulties in observation, e.g. from a satellite folding under a chromosome, or from amphiplasty as suggested by Vaarama (1953) and by Heslop-Harrison (1953). There is generally an increase in satellite number with increase in chromosome number, but the relationship is not absolute.

THE ORIGIN OF CHROMOSOME NUMBER VARIATION

The absence of virus

Tests were made for virus infection, as Caldwell (1952) has suggested that viruses might have wider effects on nuclear division than have been supposed, and as Rubi

generally are susceptible to virus infection. Sub-terminal leaves of the abnormal seedling ($1^1/59$) and a normal tetraploid sib ($1^2/59$) were removed, ground with alumina and rubbed on *Chenopodium alba*. These inoculation tests gave no indication of the presence of any of the five sap transmissible viruses. The cause for the instability must be sought elsewhere.

Lagging chromosomes

Most of the observations have been made on pre-treated root-tip cells, which would lessen the chances of seeing the mechanism responsible for maintaining the chromosome number variation. Only in one instance was it possible to make counts on two adjacent cells. One cell had 35 chromosomes and the other had 31 chromosomes; but this need not necessarily mean that both were the products of the same mitosis. Some observations were made later on root-tips without pre-treatment, but by then the chromosome number was beginning to stabilize at 35 and no abnormalities of mitosis, especially regarding spindle formation, could be seen. But one or two dumb-bell shaped nuclei were observed, and these may be comparable to the lobed nuclei observed by Britton & Hull (1957) in their mitotically unstable Rubus plants.

Instability of the egg cell

Plant $1^1/59$ is not like a true pentaploid Rubus plant with $2n = 35$ that has become unstable. Other Rubi of this ploidy are like the Mertonberry, a raspberry-blackberry hybrid (Crane & Thomas, 1949), which is cytologically stable and has large leaves and is vigorous and normal in appearance; it is also highly fertile. Neither is the instability a necessary consequence of species hybridity, for others also were normal and triploid seedlings from *R. rusticanus* ($2x$) × *R. thyrsiger* ($4x$) have remained vigorous and vegetatively stable, although with sterile flowers, for more than 25 years. Hence as the abnormal behaviour of plant $1^1/59$ is not readily attributable to either its being an odd-numbered polyploid or to its being a species hybrid, it must result from an intrinsic chromosome number instability leading to a general state of hormonal unbalance. It would seem that the instability first arose in the egg cell, as the pollen of Rubus is a sensitive reactor to chromosome unbalance (Gustafsson, 1943).

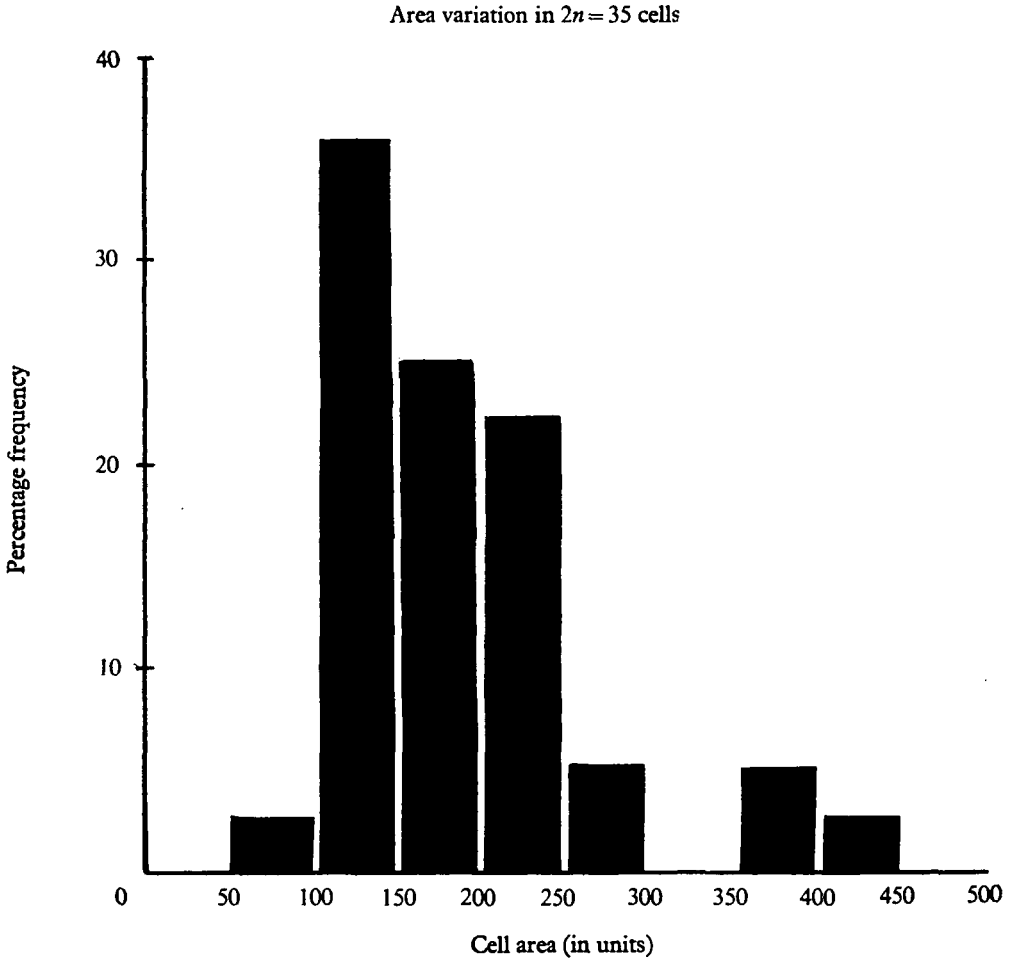
CHROMOSOME NUMBER AND CELL SIZE

Size variation within 2n = 35 cells

Although variation in cell size may be influenced by the technique of squashing not being always at the same pressure in different cells, there is the possibility that a variation in the chromosome karyotype itself may be the basis for the wide range of variation in the distribution for cell area, measured in units (Text-fig. 4). Here the histograms show a skewed distribution, with few cells smaller (viz. 50–100 units) than the most frequent class (101–150 units). Yet there is considerable extension of the skewness, so that there are to be found some cells falling into the

class group (401–450). It is recognized that these are the pooled measurements of cells from various roots, and there might have been some variation between roots. Furthermore, it is known that the volume of cells in the primordia and internodes of *Lupinus albus* is dependent on position (Sunderland, Heyes & Brown, 1956).

The constitution of the chromosomes in the $2n = 35$ cells recorded at the commencement of observations need not necessarily have been the same as the $2n = 35$



Text-fig. 4. Variation in area of a sample of cells in plant $1^1/59$ containing 35 chromosomes.

cells measured later, for the same chromosomes may be increased or decreased numerically through a series of unstable mitoses, and so the value '$2n = 35$' itself becomes a misleading guide to chromosome constitution. This is even more of a possibility not only when taken in conjunction with the reports of Sharma & Sharma (1957) on mitotic changes in chromosomal karyotypes of various species, but also because the genes so far tested (7 out of the 17 known) in *Rubus idaeus* have been assigned to two linkage groups only. This might indicate that of the

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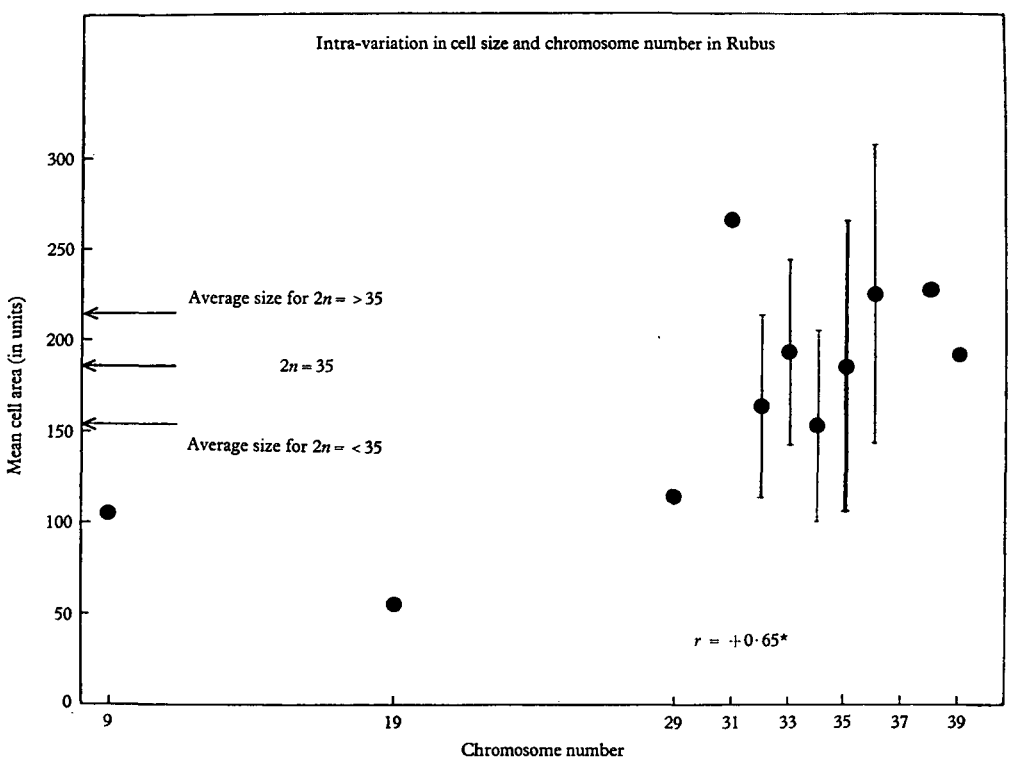
basic 7 chromosomes, some are almost genetically inert, at least for oligogenes. Hence, if this were so, any of these chromosomes could be interchanged during successive somatic mitoses, without particular detriment to the plants, other than for biometrical effects which ensue from changes in the polygenic system. Their interplay may also effect vegetative segregation, which is a widely recognized feature of Rubus, e.g. having been observed several times in the *S/s* and the *H/h* genes, and it may also partly explain the variation in the vegetative parts of the plant $1^1/59$.

The occurrence of Rubus chromosomes as a cohort of 7 is essential to give balance to the polyploid series, and to the production of fertile pollen, necessary for the maintenance of the species, whether they are sexual or apomictic (Haskell, 1960*a*). Thus '$2n = 35$' cells in this unstable plant may or may not all have the same genomic constitution.

Size variation in cells of a chromosome number series

There is a correlation between cell area and actual chromosome number within the root-tips of $r = +0{\cdot}65$, which is significant at the 5% level. The average size for the $2n = >35$ cells is 214 units, and for $2n = <35$ cells it is 154 units, and the



Text-fig. 5. Mean areas of cells from plant $1^1/59$ plotted against the number of chromosomes within those cells. Twice the standard deviation is shown in the larger samples.

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mean of the $2n = 35$ cells is 186 units. Thus clearly the size of the cells is associated with chromosome number. It would appear, too, that euploid cells with the strict multiple of the basic number are not necessarily intrinsically larger in size than aneuploid cells, for the mean of the $2n = 35$ cells fits in with the general regression (Text-fig. 5). Thus cell size, at least in Rubus, appears to be more influenced by actual chromosome number than by a euploid constitution.

The steady increase in cell area with increase in chromosome number also suggests that variation in pressure during squashing does not have the influence that might have been expected. Hence it might not have so greatly affected the variation in size of the $2n = 35$ cells (Text-fig. 4), making this due more likely to the other suggested causes. The changes in variation within the cells carrying a particular chromosome number are seen from Table 3. The variation is found to

Table 3. *Internal variation in cell area for cells of different chromosome number*

Chromosome number	Departure from $2n = 35$	Mean area (in units)	Coefficient of variation
36	+1	$226{\cdot}1 \pm 81{\cdot}8$	36·18
35	0	$186{\cdot}5 \pm 78{\cdot}4$	42·04
34	−1	$152{\cdot}6 \pm 52{\cdot}2$	34·21
33	−2	$193{\cdot}1 \pm 50{\cdot}8$	26·31
32	−3	$163{\cdot}8 \pm 49{\cdot}5$	30·22

be greatest in the $2n = 35$ cells, and there is some decrease in the coefficient of variation (C) with the addition or subtraction of 1, 2 or 3 chromosomes. Variation in cell area is not, therefore, most stable at the euploid level.

DISCUSSION

The basic chromosome number in the genus Rubus is $x = 7$, and polyploidy, especially tetraploidy, predominates in most true blackberries (Eubatae). Exceptions from the euploid series have been recorded: Crane (1940) had a dwarf monosomic ($2n = 27$) seedling following the selfing of tetraploid *R. thyrsiger*. Gustafsson (1943) found aneuploidy in *R. pyramidalis* Kalt. with $2n = 26$–28.

Chromosome number is more flexible in the Corylifolii (i.e. *R. caesius* and its hybrids), both in degree of ploidy and in occurrence of aneuploid numbers. Heslop-Harrison (1953) considered aneuploidy as rare in the subgenus Eubatus wild in Britain, and in several other Rubus subgenera. Among seedlings of North American polyploid Rubi, Einset (1951) found several aneuploids, but none were unstable. One small rosette plant, a $2n/4n$ mixoploid chimera, was found by Haskell (1960*b*) among diploid progenies of raspberry (Idaeobatus) from seeds harvested wild in Britain; it produced multiple growing points that failed to elongate and flower.

Therefore instability, whether cytological and/or morphological, is not necessarily an adjunct of either aneuploidy or of tetraploidy within diploid tissue.

However, in some vegetatively reproducing plants Sharma & Sharma (1957) have reported abundant varying chromosome complements in root tips, contrasting with sexually reproducing dictyledons.

Hegwood & Hough (1958) found a mosaic of chromosome numbers in apples with modes either at the diploid or triploid values, apparently due to abnormalities in mitosis. The tendency seemed to be heritable. Cells with double chromosome numbers scattered in normal root-tips occur in *Soldanella* spp.; Satczek (1951) attributed this to low temperatures following snowfall. The earlier observations, providing more of the lower chromosome numbers in plant $1^1/59$, were from roots growing at lower temperatures, very short days, and weak light intensity; but this applied also to the tetraploids. This conflicts with the hypothesis that tetraploid Rubi of Europe may have spread and replaced primary diploids following retreat of the ice front, i.e. following subjection of the diploids to low temperatures (cf. Gustafsson, 1943). Hull & Britton (1958) found in cuttings of a Boysen × Eldorado plant some cells with both high (e.g. 35–60) and low chromosome numbers. Re-examination in the following spring gave only low chromosome numbers (e.g. 20–35). This may represent differential growth rates of cells with varying chromosome numbers at different seasons of the year, or that lower temperatures during growth of a Rubus plant may reduce chromosome numbers, particularly in mitotically unstable individuals.

Various aneuploid complements occur in isolated cells of the orchid *Paphiopedilum wardii* root tips, although only three types might be multiplied of the twenty kinds of chromosomes (Duncan, 1945). They occur through a process resembling that for polysomaty, but not all chromosomes are replicated. Aneusomaty may be acting in seedling $1^1/59$, but owing to the difficulty in identifying individual Rubus chromosomes this has not been verified. The phenomenon is different from true endomitosis, not uncommon in Rubus, as illustrated by diploid *R. idaeus* which produces tetraploid sports from diploid shoots.

Spindle abnormalities were responsible for the instability in root tip cells and P.M.C.s of *Hymenocallis calathinum* (Amaryllidaceae) (Snoad, 1955). Chromosome number mosaicism also occurs in plants with stable root tips, e.g. Sachs (1952) found mosaicism in P.M.C.s of amphiploids involving Triticum, Aegilops and Agropyron, and spindle abnormalities might have occurred late in development, i.e. during premeiotic mitosis. Britton & Hull (1956) studied mitotic instabilities of six plants from Boysen × Eldorado and Young × Eldorado in Maryland (latitude 38° 55′), but their chromosome counts could only be ascertained with an error of ± 2 chromosomes. Accurate records have been made from clear plates only in the present observations. Although taken at latitude 56° 28′, i.e. almost 18 degrees further north of Maryland, which involves wide differences in daylength and light intensity, the same general pattern of instability occurs, providing that the plant under examination is itself unstable.

Besides the suggestion that the egg is a primary cause of instability, there is also some evidence ruling out an apomictic mechanism as responsible. Thus the F_1 plant that produced $1^1/59$ was vigorous and thorned, and might have produced

some progeny apomictically, for the parental *R. thrysiger* is a tetraploid apomict. Yet there is no indication that the instability arose from an apomictic egg, for the F_1 parent was heterozygous for the thorned (*S*) *v.* thornless (*s*) gene, and as plant $1^1/59$ is thornless it must have arisen sexually to be homozygous recessive, even allowing for the diplorous mechanism possibly involving a modified meiosis and recombination.

Morphological mosaicism occurs in polygenomic Gossypium hybrids, some characteristics being more frequent among hexaploid ($2n = 78$) combinations than corresponding tetraploids (Menzel & Brown, 1952). The mosaicism was absent in aneuploid species with one or two extra chromosomes, and does not appear to result from interaction of specific incompatible genomes, but occurs in all hexaploid hybrids. Thus one hexaploid hybrid combining three genomes had a sector with altered chromosome number, and also some buds with $2n = 65 \pm$; and, less frequently, 2–6 extra chromosomes. This parallels the situation in plant $1^1/59$, where eighty-eight counts of cells gave the somatic number less than 35, and eleven counts gave more than 35 chromosomes (Table 2). Yet the polygenomic history of this plant, involving *R. ulmifolius*, *R. thyrsiger* and *R. procerus*, cannot itself be the prime cause of the instability, as its sibs are vigorous, constant tetraploids.

If the plant consisted of $2x$, $3x$, $4x$, etc., cells, it might merely be a polyploid mixo-chimera; but the behaviour is more complex, as the observed numbers do not correspond to a simple polyploid series. As the most frequent chromosome number is 35, one might expect cells with 34 or 36 chromosomes due to occasional lagging of a chromosome at anaphase; but these numbers do not preponderate. Furthermore, there is no corresponding chromosome number to balance with the same frequency the particular chromosome number to make up to 35 ($= 5x$). Nor do we know whether mitosis takes place at the same rate in the cells with different chromosome numbers. There is no extra frequency of cells other than those with 35 chromosomes carrying the orthoploid series of the basic number $x = 7$, viz. 14, 21, 28 and 42.

Although attempts were made to obtain chromosome counts from leaves and buds, the rugose nature and dense hairiness prevented this, but one plate was clearly countable from a stipule. This cell had 39 chromosomes (Plate II, fig. 4). In this respect Berger & Witkus (1954) have shown in *Xanthisma texanum* ($2n = 8$) that only plants with shoots having supernumerary chromosomes produced occasional root cells with 9 or 10 chromosomes, but they never constituted more than 2% of the dividing cells. Elimination of the supernumeraries in the cells of Xanthisma root tissue during early differentiation results from lagging of the supernumeraries at mitosis (Berger *et al.*, 1955).

In a literature survey on cell size and polyploidy, Noggle (1946) suggested that 'chromatin : cytoplasm' ratios are different between diploid and tetraploid cells within various species; but they depended on the stage of development of the organs carrying the cells. The ratios for root tip cells in plant $1^1/59$ carrying the series of aneuploid chromosome numbers are given in Table 4. Here each ratio is

Table 4. *The* $\frac{\text{'Chromatin'}}{\text{cytoplasm}}$ *ratio in cells with increasing chromosome numbers*

Chromosome no.	Ratio
(9	11·78)
(19	3·42)
29	3·91
(31	8·97)
32	5·12
33	5·85
34	4·49
35	5·32
36	6·28
(38	6·02)
(39	4·95)
Mean	6·01

Figures in brackets are for single counts only.

assessed from the value of $\frac{\text{mean cell area}}{\text{chromosome number}}$. There is no trend in the series between chromosome number and the chromatin : cytoplasm ratio, so this constancy within similar tissue indicates evidence of a balance between cell size and chromosome number, and this balance leading to stability between the chromatin and the cytoplasm is predetermined.

On the other hand, there is correlation between cell area and chromosome number (Text-fig. 5). In liver and kidney cells of rats (Harrison, 1951) there is a direct relationship between the numbers of chromosomes within the cells and the amount of DNA per nucleus. It is not, therefore, unfeasible to suggest that in the chromosome mosaic root tissue of plant $1^1/59$, cell size and the amount of DNA are correlated, and that the quantity of DNA decides the ultimate size of the particular cell after cell-wall formation. Because the chromosomes of Rubus are alike in size, this produces a significant correlation in the aneuploid series, for the addition of single chromosomes is tantamount to adding a proportional quantity of DNA within cells of the same tissue and growing under similar environments.

The discovery by several workers that mitotic instability occurs in Rubus plants of widely different sources, e.g. from British and North American origin, indicates that this is more widely spread than previously supposed. Interesting material is now available to study how important is this form of somatic variation in the life-cycle in Rubus, where vegetative reproduction is prominent. That there is a mechanism affording somatic variation in Rubus is in line with earlier knowledge of the versatile breeding system of the genus. It may also help to explain why some varieties of Rubus in cultivation have changed ploidy since chromosome counts were first made. Thus Crane & Darlington (1927) originally gave the chromosome number of Himalaya Giant (*R. procerus*) as $2n = 49$, but all recent counts have been consistent at $2n = 28$; the Mahdiberry, previously a semi-sterile triploid with $2n = 21$, is now a fertile pentaploid with $2n = 35$ (Crane & Thomas, 1949); and the tetraploid cultivated raspberry variety of Hailshamberry ($2n = 28$) is now only available

as a triploid ($2n = 21$). We need not look only, as previously supposed, to replacement of the stock by self-sown seedlings, but can consider also the probability that somatic changes in chromosome numbers occur in the vegetative system, and with greater frequency than hitherto supposed.

SUMMARY

1. Serial cytological observations were made on one abnormal seedling from the thornless segregants of the F_2 progeny from crossing two tetraploid ($2n = 28$) Rubus species. The number of root-tip chromosomes was found to vary between 9 and 46 per cell, with the mode always at 35. The wide variation in chromosome number decreased with time, until there was almost stability at $2n = 35$. A vegetative (stipule) cell was $2n = 39$. The numbers lower than 35 were not due to terminal fusion.

2. Evidence from the number of satellites per cell suggests that not always were the same chromosomes being eliminated, although there is a general increase in satellite number with increased numbers of chromosomes per cell.

3. There was a correlation between the chromosome numbers and corresponding cell sizes within the aneuploid series. This may be associated with the incremental change of DNA following the addition or subtraction of individual chromosomes, which are highly similar in Rubus.

4. The cause of the instability was not definitely established. Its initiation is probably attributable to the egg mother cell, as Rubus pollen reacts sensitively to chromosome unbalance. No abnormalities were observed in the mitoses, and virus infection was not responsible for the instability.

5. Chromosome number instability of this plant is discussed in relation to other examples from the same and different genera: it is suggested that the changes in chromosome number known in various Rubi may have arisen somatically.

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