Karyotype Variation in *Rubus* with Particular Reference to *R. idaeus* L. and *R. coreanus* Miquel

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Received May 9, 1979

It is generally believed that little chromosome differentiation has occurred in the genus *Rubus*. But this belief is based largely upon studies in the *Eubatus* sub-genus which contains the blackberries: Heslop-Harrison (1953), for example, reported that the chromosomes of a large number of British blackberry species ranged in size from 1.5 to 2.0 μm and were too small for morphological study. Similarly, Einset (1947) reported that in American blackberries the chromosomes showed little variation, ranging in length from 1.0 to 4.0 μm . These conclusions are supported by meiotic studies, which have led to the idea that chromosome differentiation between *Rubus* genomes has occurred through minor alterations caused by gene mutations, and by minute structural changes where the rearrangements are so small that the ability of the chromosomes to conjugate has not changed; hence most chromosome combinations are capable of pairing and permitting crossing over, though in allopolyploid hybrids the chromosome differences are frequently sufficient to compel preferential pairing (Gustafsson 1943, Thomas 1940). More recently, Jinno (1958) reported that individual chromosomes of a range of Japanese *Rubus* species differed in the position of their constrictions, and that species differed in the frequency of chromosomes with median, submedian and sub-terminal constrictions. A greater degree of differentiation was detected by Bammi (1965) for *Rubus parvifolius*, a Japanese species of the *Idaeobatus* sub-genus, which contains the raspberries. Bammi used pachytene analyses to identify each of the seven chromosomes, recording their absolute lengths, the ratio of the lengths of the long and short arms and the pattern of their chromatic regions. He considered that the chromosomes of this species were unusually easy to spread and that meiotic studies of other *Rubus* species would be less productive. This paper describes an attempt to detect differences among somatic chromosomes of the Korean species *R. coreanus* and the raspberry 'Glen Clova', a cultivar of *R. idaeus*. Both are from the *Idaeobatus* sub-genus and *R. coreanus* is used extensively in raspberry breeding as a source of genes for disease resistance (Williams 1950, Keep *et al.* 1977, Jennings 1978). Four F_1 hybrids of the two species were made available from the breeding programme at the Scottish Horticultural Research Institute.

Materials and methods

For staining mitotic chromosomes, root tips were placed for seven minutes in a watch glass containing one part of N. HCl and five parts of lacto-propionic-orcein(lpo) at a temperature of 60°C. They were then mounted in lpo and squashed hard. Slides were examined under phase contrast and photographs taken with a magnification of $\times 1000$ and subsequently enlarged to $\times 3800$. Twenty cells were suitable for measurement on the photographs of both *R. coreanus* and 'Glen Clova' and 14, 12, 13 and 12 cells respectively on those of the F_1 hybrids referred to as hybrids A, D, E and M. The total and short-arm lengths were determined from the photographs for each chromosome of each cell, the lengths of satellites when present being included within the measurements. The chromosomes of the two species were numbered one to seven in order of decreasing size, chromosomes of homologous pairs receiving the same number, while those of the hybrids were numbered from one to 14, because the chromosomes from the two species were found to be sufficiently differentiated to be distinguished.

For meiotic studies, flower buds were collected in January 1978 from plants which had been stimulated to flower out-of-season (Jennings 1964). Anthers were fixed in a 6-3-2 mixture of absolute alcohol, chloroform and propionic acid, and simultaneously stained and hydrolysed at 60°C for five minutes in a mixture of 5 parts 2% lpo to 1 part N. HCl prior to being mounted in 2% lpo and squashed.

For pollen studies, single mature anthers from fresh flower buds were placed on a slide in a drop of cotton blue and then tapped and squashed to release their pollen grains.

Results

The preparations obtained showed clear differences between each of the seven chromosomes of the two species (Fig. 1), providing data which were first subject to statistical evaluation.

i) Statistical analyses

The objectives of statistical analyses were, first, to assess the accuracy with which chromosomes could be recognised as individuals, and second, to determine whether the chromosome lengths and arm ratios were influenced by the cellular environment of the parent or hybrid where the chromosomes occurred.

Chromosome 4 of *R. coreanus*, which was numbered 10 in the hybrids, was a suitable test subject for the first objective, because its satellite ensured its recognition in all the material. Analyses showed that its length differed slightly but significantly in the different hybrids (Tables 3 and 4), though there were no significant differences for its arm ratios. The standard errors of the means for its length in *R. coreanus* and hybrids A, D, E and M were respectively 0.0146, 0.0174, 0.0188, 0.0181 and 0.0188 and those for its arm ratios were 0.0264, 0.0315, 0.0341, 0.0327 and 0.0341. Hence the chromosome was reliably separated from those most similar to it. Thus, if this variation was characteristic of all the chromosomes, it is clear that identification was reliable, though in some instances, for example chromosomes 7 and 9 in hybrid D, a consideration of both length and arm ratio was necessary.

Figs. 1-2. 1, somatic chromosomes of hybrid A. 2, late metaphase 1 in hybrid D.

Additionally, an analysis of variance was done on chromosome numbers 1, 2, 5, 7, 10 and 12 in the two parents and four hybrids, the chromosomes again being chosen because they were relatively distinct. The results (Table 2) clearly show large differences due to chromosomes. There are also significant effects due to the source plants for length but not for arm ratio, and no evidence of plant $\times$ chromosome interactions for either characteristic.

Another test (Hinkley 1973) was used to compare chromosomes 1 and 2 in the hybrids. These are the two largest chromosomes, one from each parent, and were chosen because they too were reliably recognised by their satellites. In this test, it is assumed that the chromosomes have similar variances, but that since the two chromosomes are not clearly distinguishable occasional misclassifications will occur if the smaller is always allocated to one group and the larger to the other. Although, as Hinkley pointed out, the absence of an independent estimate of the variance limits the power of the test, the *t*-tests (Table 1) show that the differences between the chromosomes in arm ratio but not in length are greater than would arise by chance, refuting the null hypothesis that they are identical.

From these analyses it was con-

Table 1. *T* values for the differences between chromosomes 1 and 2 in the four hybrids*

	Hybrid			
	A	D	E	M
Length (μ m)	1.05	2.10	1.06	1.47
Arm ratio	3.78	11.48	2.63	3.90

* see Tables 3 and 4 for data on the chromosomes

cluded that the chromosomes were sufficiently identifiable to permit the study proposed. Although there were significant differences attributable to the plant from which the root tips were taken, these affected chromosome lengths and not arm ratios, and the failure to detect plant $\times$ chromosome interactions indicated that all the chromosomes were affected alike: the most likely explanation for this is that chromosome lengths were affected by minor variations in the preparation of the

Fig. 3. Morphological characteristics of the chromosomes of *R. idaeus* (open drawings) and *R. coreanus* (solid drawings). The position on the graph of the centromeres denote the chromosomes' relationships to the axes.

Table 2. Analyses of variance of the lengths and arm ratios of chromosomes* 1, 2, 5, 7, 10 and 12 in plants of the two species and their four hybrids

Source	d.f.	Mean squares	
		Length	Arm ratio
Plants allowing for chromosomes	5	5.09	0.008
Chromosomes allowing for plants	5	785.95	3.259
**Plant $\times$ chromosome interaction	19	0.33	0.009
Residual	396	0.58	0.019

* Each chromosome occurred in each hybrid, but only chromosomes 1 and 7 occurred in *R. idaeus* and only chromosomes 2, 5, 10 and 12 occurred in *R. coreanus*.

** The analysis is non-orthogonal because of the unequal distribution of the chromosomes.

squashes. Indeed, some cells appeared flat when photographed, making the chromosomes appear very long, while others were slightly tilted, giving the chromosomes a shorter appearance. For subsequent analyses cells were accordingly used as 'blocks', and the standard errors quoted are based on the residual variances of these analyses.

ii) *Chromosome karyotypes of R. idaeus, R. coreanus and their hybrids*

The karyotypes of the seven *R. idaeus* and seven *R. coreanus* chromosomes are shown in Fig. 3 and data on their lengths and arm ratios are given in Table 3. The differences found between the chromosomes of the two species are considerable, and it is not possible to determine which chromosomes of the two species are homologous to each other. Chromosome one of each species has a satellite on its long arm, but the chromosome is slightly shorter and has a greater short-arm ratio in *R. coreanus*. Chromosome two is similar in the two species but chromosome three of *R. idaeus* is shorter than chromosome three of *R. coreanus*, and it seems likely that it is homologous to chromosome four. Chromosome four of *R. coreanus* has a

Table 3. Lengths, short to long-arm ratios and satellite lengths for the chromosomes of *R. idaeus* and *R. coreanus*

No. of chromo. in parent	No. of chromo. in hybrids	Total length (μm)	Short/long arm ratio	Satellite length (μm)
<i>R. idaeus</i>				
1	1	2.84	0.37	0.53
2	4	2.42	0.60	—
3	6	2.13	0.93	—
4	8	2.03	0.74	—
5	7	1.97	0.55	—
6	9	1.87	0.88	—
7	13	1.37	0.84	—
Mean		2.29	0.70	
S.E.D.		0.05	0.041	
<i>R. coreanus</i>				
1	2	2.71	0.60	0.45
2	3	2.55	0.96	—
3	5	2.39	0.53	—
4	10	2.16	0.88	0.34
5	11	1.79	0.99	—
6	12	1.47	0.67	—
7	14	1.29	0.82	—
Mean		2.24	0.77	
S.E.D.		0.04	0.044	

satellite on its short arm and is not matched by a similar one in *R. idaeus*; its length and arm ratio are closer to chromosome three than to chromosome four of *R. idaeus*, though this would not be so if it lost its satellite. Chromosome five and six of the two species differ in both lengths and arm ratios, while the two shortest chromosomes of the two species are similar.

Data on the karyotypes of the four hybrids (Table 4) show that the chromosome complements of each hybrid were essentially similar and that the 14 chromosomes present in each hybrid represent seven from each parent. A key to the numbering of the chromosomes in the parents and hybrids is given in Table 1.

Table 4. Lengths, short to long-arm ratios and satellite lengths for the chromosomes of four F_1 hybrids of *R. idaeus* $\times$ *R. coreanus*

No. of chromo.	Hybrid A				Hybrid D				Hybrid E				Hybrid M			
	Length μm	Arm ratio	Sat. length	Length μm	Arm ratio	Sat. length	Length μm	Arm ratio	Sat. length	Length μm	Arm ratio	Sat. length	Length μm	Arm ratio	Sat. length	Sat. length
1	2.84	0.38	0.53	2.87	0.37	0.50	2.87	0.38	0.53	2.89	0.36	0.53	2.89	0.36	0.53	0.53
2	2.68	0.58	0.45	2.66	0.64	0.45	2.68	0.59	0.45	2.68	0.60	0.45	2.68	0.60	0.45	0.45
3	2.61	0.55	—	2.61	0.54	—	2.63	0.52	—	2.61	0.58	—	2.61	0.58	—	—
4	2.39	0.51	—	2.42	0.50	—	2.39	0.54	—	2.39	0.47	—	2.39	0.47	—	—
5	2.39	0.95	—	2.37	0.94	—	2.39	0.93	—	2.39	0.95	—	2.39	0.95	—	—
6	2.13	0.94	—	2.11	0.96	—	2.08	0.98	—	2.11	0.93	—	2.11	0.93	—	—
7	1.92	0.55	—	1.89	0.56	—	1.89	0.52	—	1.89	0.53	—	1.89	0.53	—	—
8	2.03	0.71	—	2.05	0.69	—	1.97	0.71	—	1.97	0.67	—	1.97	0.67	—	—
9	1.87	0.89	—	1.89	0.86	—	1.84	0.93	—	1.84	0.86	—	1.84	0.86	—	—
10	2.13	0.87	0.34	2.16	0.86	0.34	2.11	0.86	0.34	2.13	0.90	0.34	2.13	0.90	0.34	0.34
11	1.82	0.90	—	1.82	0.95	—	1.76	0.91	—	1.82	0.92	—	1.82	0.92	—	—
12	1.47	0.64	—	1.50	0.66	—	1.45	0.61	—	1.50	0.67	—	1.50	0.67	—	—
13	1.37	0.85	—	1.37	0.87	—	1.34	0.79	—	1.34	0.89	—	1.34	0.89	—	—
14	1.26	0.94	—	1.26	0.90	—	1.29	0.93	—	1.29	0.88	—	1.29	0.88	—	—
Mean	2.16	0.73	—	2.16	0.74	—	2.14	0.73	—	2.16	0.73	—	2.16	0.73	—	—
S.E.D.	0.03	0.036	—	0.035	0.035	—	0.037	0.034	—	0.041	0.032	—	0.041	0.032	—	—

iii) *Pollen and meiotic studies*

Pollen grains of both parents and hybrids took up stain to the same extent and were similar in size and shape; there was no evidence of abortive or malformed grains.

Studies of meiosis in both parents and hybrids showed completed synapsis (Fig. 2) and generally regular separation of chromosomes with only rare irregularities, the most common of which was the occurrence of six bivalents and two univalents, but this occurred just as commonly in the parents as in the hybrids. The only recurring factor present in the hybrids and not in the parents was early separation of one of the bivalents at early anaphase, but this did not result in irregularity of the meiotic products. The early separation may have resulted from failure in chiasma formation in one bivalent and this in turn may have been a consequence of an overall reduction in the number of chiasmata. However, preparations suitable for chiasma analysis were rare. Good preparations were obtained only for late stages of meiosis where chromosome contraction precluded their separate identification: it was therefore not possible to ascertain which chromosomes were pairing together to form bivalents.

Discussion

In contrast to experience with some other *Rubus* species, the seven chromosomes of *R. idaeus* and *R. coreanus* were reliably identified from their lengths, arm ratios and presence or absence of satellites. Moreover, morphological dissimilarities were detected between four of the seven chromosomes of the two species and also between the chromosomes of these species and those which Bammi (1965) described for *R. parvifolius*. The dissimilarities between *R. idaeus* and *R. coreanus* were so marked that it is surprising that the pollen was so well formed and that chromosome pairing at meiosis was so regular. The hybrids were sub-fertile, and though the fertility reductions may have been due to post-meiosis factors rather than to irregularities at meiosis, it should be noted that the bivalents must have contained morphologically diverse chromosomes, and that this would have resulted in the formation of gametes lacking in some of the genetic material.

The variation in number of chromosomes with satellites may not have great significance, because *Rubus* species show amphiplasty in this characteristic and the number of satellites present is not a good indication of species relationships. Heslop-Harrison (1953), for example, did not find more than two satellite chromosomes in a large number of *Rubus* species studied from diploids to hexaploids; and Gustafsson (1943) found satellite numbers of from one to three in species up to pentaploid. However, the differences found in length and arm ratio between chromosomes of *R. idaeus* and *R. coreanus* clearly indicate that the structural changes associated with the differentiation of these species are not inconsiderable, even if chromosomal conjugation remains unimpaired.

Meiotic studies have provided plenty of evidence of structural diversity between the chromosomes of other *Rubus* species: translocational heterozygosity occurs in the diploid blackberries *R. tomentosus* and *R. ulmifolius* (Gustafsson 1943) and

R. ulmifolius also differs from *R. argutus* by one or more translocations (Hull 1968); translocational heterozygosity might well occur commonly in the tetraploid blackberries too, because they frequently have only one quadrivalent at meiosis and almost exactly 50% pollen sterility, and both these observations are explained more easily by the presence of heterozygosity for a chromosome translocation than by autopolyploidy. Structural diversity caused by chromosome inversions has also been found frequently, for example in such polyploid blackberries as *R. nessensis*, *R. caesius* and *R. dissimulans* (Gustafsson 1943). Thus both the studies of meiosis in blackberries and the studies of somatic chromosomes reported here support the idea that considerable chromosome diversity exists in *Rubus* and, in spite of the limited success of previous workers, it seems possible that karyotype analyses can contribute to the understanding of species relationships in this difficult genus.

Summary

Previous workers have reported that *Rubus* chromosomes are too small for karyotype analyses at mitosis, and also that meiotic studies indicate no major structural differences between *Rubus* genomes. However, it was found possible to identify the seven chromosomes of Glen Clova, a cultivar of *R. idaeus*, and of *R. coreanus*. The chromosomes of *R. idaeus* differed from those of *R. coreanus*, four of them appreciably, and it was possible to identify each of the 14 chromosomes of four F_1 hybrids between the two species. In spite of these differences, meiosis and pollen formation in the hybrids appeared normal. It is suggested that genome differentiation in the genus is greater than realised, and that further karyotype analyses may aid studies of species relationships.

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