The genus Rubus in South Africa. II. Meiotic chromosome behaviour

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ABSTRACT

Meiotic chromosome behaviour in the genus *Rubus* is relatively normal. Polyploidy occurs in both South African subgenera, i.e. *Eubatus* and *Idacobatus*. The subgenus *Eubatus* contains plants tending mostly towards autoploidy, whereas the subgenus *Idaeobatus* varies from autoploid, through segmental alloploid to alloploid. It is concluded that this apparent difference might be due to the study of a statistically insufficient number of plants and that alloploidy originated from intersubgeneric hybridization.

INTRODUCTION

The genus *Rubus* includes the blackberries, raspberries and dewberries and is regarded as one of the world's most complex taxonomic groups. In an attempt to solve the *Rubus* riddle, all available taxonomic methods should be exploited. Among these methods cytogenetics could play a major role. The cytogenetic contributions to the taxonomy of *Rubus* can be separated into two distinct types of data. The contribution may involve information regarding either the reproductive system or it may involve the ploidy status and genome analysis of the specimen.

The genus *Rubus* is subdivided into 12 subgenera, of which two are represented in South Africa. All species belonging to the subgenus *Eubatus* Focke are exotic in South Africa, whereas the subgenus *Idaeobatus* Focke contains two exotic and nine indigenous species (Spies & Du Plessis, 1985).

L. H. Bailey did not describe any Rubus as a hybrid in his classical work 'Species Batorum' (1941). His criteria for hybridization included the presence of both parents in close proximity to the hybrid and the hybrid must be morphologically intermediate between its parents. Strict application of these criteria resulted in the description of a multitude of different species. Another paper in this series will deal with the validity of these criteria. This study is an attempt to determine, through a study of meiotic chromosome behaviour, whether the indigenous polyploid species (Spies & Du Plessis, 1985) originated through allo-, segmental allo- or autoploidy. The chromosome behaviour of the indigenous species will also be compared with that of the introduced species.

MATERIALS AND METHODS

The plants used during this study were listed in a previous publication (Spies & Du Plessis, 1985). Aceto-carmine squashes of anthers (Darlington & LaCour, 1976) were used to study chiasma frequencies chromosome associations, metaphase I, ana-

phase 1 and telophase II. At least 20 cells per plant were studied.

Diakineses were used to study chiasma frequencies and chromosome associations. Chiasma frequencies were calculated as the average number of chiasmata per half bivalent. This frequency was calculated by dividing the total number of chiasmata observed by the somatic chromosome number. The expected frequencies of bivalent and multivalent formation for autoploid plants were calculated by using the method described by Spies (1984a).

RESULTS

The chiasma frequencies varied from 0.45 in a R. × protetus specimen to 1,41 in a R. apetalus specimen (Table 1). The low chiasma frequencies observed in all triploid (2n = 21) and pentaploid (2n = 35) plants, could be attributed to the high frequency of univalents. If a correction factor of Z was added for each univalent, values between 1,15 and 1,22 for triploid and 1,1 to 1,15 for pentaploids were obtained. The correction factor (Z) is obtained by dividing the sum of the number of univalents (U) and a third of the number of trivalents (T) per cell by the somatic chromosome number (N) {Z = [U + (T/3)]/N}. The variation in chiasma frequency, after correction, was decreased and varied between 1,01 and 1,41.

Chromosome associations were presented as the percentage of chromosomes bound as uni-, bi- or multivalents in Table I. Differences in chromosome behaviour between different species and even within a species were demonstrated by the number of multivalents formed. The number of univalents during metaphase I increased with an increase in ploidy level (Table 2). Anaphase I (Table 3) and telophase II (Table 4) were normal in almost all diploid (2n = 14) and tetraploid (2n = 28) plants, whereas abnormalities occurred in triploid (2n = 21) and pentaploid (2n = 35) plants. These abnormalities included laggards and a maldistribution of chromosomes during anaphase I, as well as the occurrence of additional micronuclei during telophase II.

The expected frequencies of bivalents and multivalents formed in autoploids were determined for all the triploid and tetraploid plants. These results are presented in Tables 5 & 6. Chromosome behaviour in the different species was as follows:

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			Chiasma**	970	of chrom	osomes bo	und as:
Species	Specimen no.	2n=	frequency	1	11	111	IV
Eubatus			51 A.C3-		Calificato	-312	
R. affinis*	Stirton 5746	28	1,22(1,24)	0.75	76.57	2.25	20.43
R. cuneifolius*	Stirton 8102	14	1.04		100		
	Stirton 8154	14	1.09	-	100		
	Liengme 5.p.	21	0.85(1.18)	30.09	60.19	9.71	_
	Henderson & Gaum 93	28	1.19(1.27)	8.2	53.6	13.9	24 3
R. pascuus*	Henderson & Gaum 18	71	0.8(1.15)	31 3	58.8	10	
	Stirtun 9800	21	0.86(1.2)	30.48	58.1	11 43	_
	Stirton 9861	78	1(1.06)	4 46	86.07	3 75	5 71
	Stirton 9868	78	1 (19(1-17)	6 14	74 86	6 43	17 57
R. flagellaris"	Henderson & Gaum 2	28	1,08	0,36	99,64	-	
Ideobatur							
R operature	C. Hammen	14	1.11		100		
n. upcianas	G. Hommen h	24	1.71		70.4	_	20.6
	Handarson & Gaum 6	20	1 19(1 71)	1.75	27 14	2.75	7.94
	Wells 5000	10	1,10(1,21)	1,20	100	3,13	1,00
R longenedications	Henderson & Cours 32	20	1 12(1 14)	2.14	07.86	_	-
in longepennenuna	Handarson & Gaum 14	14	1.12(1,14)	2.14	97,00	_	1.4
	Stimon 0963	20	1 11(1 17)	0.57	90,4		1,4
	Handowan & Cause 36	20	1.11(1.12)	14.0	67.6	116	0
P. ninnatur	Henderson & Guum 30	35	0.99(1,19)	14,9	02,0	14,0	0
R. pinnulus	Anald 1225	14	1.09	0,7	99,5	-	15.4
P hudustail	Arnold 1355 Administrat & Dulifleaux 2040	28	1.24	0.57	84,0	_	15,4
n. iuuwigii	Admiraal & Drijjnout 2940	14	1,2	0.57	99,43	-	-
P M MARK	Henderson & Gaum 41	14	1.18	_	100		_
κ . × proteus	Henderson & Gaum 28	14	1.1		100	10.00	-
	Stirton 9866	21	0.88(1,22)	29.76	56,67	13,57	-
	Stirton 9798	28	0.45(1,01)	55,4	44,6		
	Henderson & Gaum 27	28	1,09(1,14)	2,5	86,4	7,5	3,6
	Henderson & Gaum 32	28	1.09(1,11)	1,6	96,8	1,6	2012
	Henderson & Gaum 51	28	1,18(1,19)	0,4	81,7	3	14.9
	Stirton 9865	35	1,05(1,15)	9,57	84,86	2,14	3,43
	Henderson & Gaum 20	35	0,91(1,1)	18	77,7	4,3	
	Henderson & Gaum 31	42	1,21(1,3)	4,4	68,6	14,6	12,4
R. transvaliensis ×							
R. longepedicellatus	Henderson & Gaum 10	28	1,07		100	-	
R species	Henderson & Goum 74	28	1 16(1 26)	8	57 1	7	27.9

TABLE 1. - Chiasma frequencies and chromosome associations in different Rubus species

*Exotic species

**Corrected values

a) The subgenus *Eubatus* Focke (all species are exotic)

Rubus affinis Wh. & N.

This tetraploid (2n = 28) species had an average chiasma frequency of 1,22 (*Stirton* 5746). The average chromosome association was 0.21_{I} 10, 71_{II} 0, 21_{III} 1,43_{IV} and it varied from 14_{II} to 8_{II} 31_V. Although early segregation of chromosomes was observed during metaphase I (3 cells had either 1 or 2 univalents), anaphase I was normal in all the cells studied. No additional micronuclei were observed during telophase II. The high correlation between the expected and observed chromosome association frequencies indicated that this species was autotetraploid.

R. cuneifolius Pursh.

This weedy polyploid species had chromosome numbers of 2n = 14, 21 and 28 (Spies & Du Plessis, 1985). The two diploid specimens, *Stirton* 8102 and 8154, had respectively average chiasma frequencies of 1,04 and 1,09. Both plants formed 7_{II} per cell during diakineses. No univalents were observed during metaphase I and a normal 7–7 anaphase I segregation was observed. Both plants exhibited normal telophase II cells.

The triploid plant, Liengme s.n., had an average chiasma frequency of 0.85 and an average chromosome association of $6.3_1 6.3_{11} 0.7_{111}$. Liengme s.n. had an average of 5.3 univalents (varying from 3 to 7 per cell) per cell during metaphase 1. Anaphase I showed an unequal segregation of chromosomes and laggards. The chromosome segregation varied from 11–10 to 13–8. Telophase II appeared normal. Trivalents occurred and the plant was found to be an autoploid.

The tetraploid specimen, *Henderson & Gaum* 93, had an average chiasma frequency of 1,19 and an average chromosome association of $2,3_{\rm I}$ 7,5₁₁ 1,3₁₁₁ 1,7_{1V}. An average of 2,3 univalents (varied from 0 to 4) per metaphase 1 cell was observed. Anaphase I occasionally had 1 to 2 laggards. One telophase II cell contained an additional micronucleus. The observed frequency of chromosome associations corresponded with the expected frequency for autotetraploid plants and *Henderson & Gaum* 93 could consequently be regarded as an autotetraploid.

R. pascuus Bailey

This species escaped from cultivation and became a weed in the eastern Transvaal. The two triploid specimens, *Henderson & Gaum* 18 and *Stirton* 9800,

Bothalia 15, 3 & 4 (1985)

Species	Specimen no,	2n=					Perce diff	entag Terent uni	e of c num valen	cells v ibers its	vith of			Average number of
			U	1	2	3	4	5	6	7	8	9	10	
Eubatus														
R. affinis*	Stirton 5746	28	85	10	5									0,2
R. cunefolius*	Stirton 8102	14	100											0
	Stirton 8154	14	100											0
	Liengme s.n.	21				10	10	30	40	10				5,3
	Henderson & Gaum 93	28		10	20	20	30	20						3,3
R. pascuus*	Henderson & Gaum 18	21			5	5	5	20	20	20	10	15		6,2
	Stirton 9800	21					10	50	20	20				5,5
	Stirton 9861	28	100											0
	Stirton 9868	28	100											0
R. flagellaris*	Henderson & Gaum 2	28	100											0
Idaeobatus														
R. apetalus	G. Hemmsn.a	14	100											0
	G. Hemmsn h	28	100											ő
	Henderson & Gaum 6	28	100											ã
	Wells 5000	28	100											ő
R. longepedicellatus	Henderson & Gaum 27	14	70		30									0.6
and the second sec	Henderson & Gaum 14	28	100											0,0
	Stirton 9862	28	100											ŏ
	Henderson & Gaum 36	35	100	5	5	10	20	15	15	20	to			51
R. ninnatus	Henderson & Gaum 15	14	100	1		10	20	-	10	20	10			0
	Arnold 1335	28	100											0 0
R. ludwieii	Admiraal & Dritthout 2940	14	100											õ
	Henderson & Gaum 41	14	100											ő
R. X proteus	Henderson & Gaum 28	14	100											ŏ
	Stirton 9866	21	100			10	70	40	20	10				5
	Stirton 9798	28		5		5	15	15	15	20	15	5	5	6
	Henderson & Goum 27	28	100	- 01		÷.	1.57	10	10	20	1.5	-	2	ő
	Henderson & Gaum 32	28	70	20	10									0.4
	Henderson & Gaum 51	28	100	10	10									0,1
	Stirton 9865	35	100	10	20	20	30	10	10					3.4
	Henderson & Gaum 70	35	20	R	20	12	12	R	10	8		8	4	34
	Henderson & Gaum 31	42	20	40	30	30		0		9			197	1.9
R. transvaliensis ×	same or a count of	12		10	20	50								A4.7
R. longepedicellatus	Henderson & Gaum 10	28	100											0
R. species	Henderson & Gaum 74	28	5	10	38	24	14	9						2.6

	TABLE 2. — Number of	univalents per	metaphase I cell	in some South	African Rubus species
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Exotic species

corresponded in regard to their chiasma frequencies and chromosome associations (Table 1). They had an average chiasma frequency of 0,83 and an average chromosome association of $6,5_1 6,1_{II} 0,8_{III}$. The number of univalents during metaphase I varied from two to eight per cell with an average of 6,4. Anaphase I was only studied in *Henderson & Gaum* 18 and a chromosomal maldistribution (11–10 and 12–9) was observed, as well as up to five laggards. In spite of the abnormal anaphase I, 80% telophase II cells appeared normal in both plants. Both plants tended towards autoploidy.

The two tetraploid plants, Stirton 9861 and 9868, had slightly different chiasma frequencies. These differences are reflected in different chromosome associations where Stirton 9861, with its lower chiasma frequency, had more bivalents and less multivalents than Stirton 9868, with its higher chiasma frequency. Both plants had normal metaphase I, anaphase I and telophase II stadia and they represented autoploids.

R. flagellaris Willd.

The tetraploid plant representing this species, Henderson & Gaum 2, had a chiasma frequency of 1,08 and an average chromosome association of $0,1_{T}$. 13,9_{II} with a normal metaphase I, anaphase I and telophase II. This plant was found to be an alloploid.

b) The subgenus *Idaeobatus* Focke (with the exception of R. *phoenicolasius* and R. *niveus*, all species are indigenous).

R. apetalus Poir.

The diploid specimen, G. Hemm s.n., had an average of 1,11 chiasmata per half bivalent and all cells showed only bivalents. No abnormalities were observed during metaphase I, anaphase I and telophase II.

The three tetraploid plants, G. Hemm s.n., Henderson & Gaum 6 and Wells 5000, differed significantly in regard to their cytogenetic behaviour. The average chiasma frequencies varied from 1,06 in Wells 5000 to 1,41 in G. Hemm s.n. and the average chromosome association from 14_{II} to $11,1_{II}$ $1,4_{IV}$ in the two plants mentioned. Metaphase I, anaphase I and telophase II were normal in all three plants with the exception of one anaphase I cell of Wells 5000 where one laggard was observed. The differences in chromosome association indicate that G. Hemm s.n. and Henderson & Gaum 6 may be considered as segmental alloploids, whereas Wells 5000 may represent an alloploid.

Species	Specimen no.			Ch	romosome sa	gregation			
Diploid plants			7+7				6+6+2		
R. cuneifolius*	Stirton 8102		100					#1148(#J	
	Stirton 8154		100						
R. apetalus	G. Hemm s.n.		100						
R. longepedicellatus	Henderson & Gaum 22		90				10		
R. pinnatus	Henderson & Gaum 15		100						
R. ludwigu	Admiraal & Drijfhout 2940		100						
	Henderson & Gaum 41		100						
R. × proteus	Henderson & Gaum 28		100			-			
Triploid plants		11 + 10	12+9	13+8	8 10+10+	1 10+9+2	2 9+9+3	9+8+4	9+7+5
R. cuneifolius*	Liengme s.n.	60	30	10					
R. pascuus*	Henderson & Gaum 18	20	20		20	10	10	10	10
Tetraploid plants		14+14	14+13-	+1 1	4+12+2	18+10	14+11+3	13+11+4	13+12+3
R. affinis*	Stirton 5746	100		,					
R. cuneifolius*	Henderson & Gaum 93	70	20		10				
R. pascuus*	Stirton 9861	100							
	Stirton 9868	100							
R. flagellaris*	Henderson & Gaum 2	100							
R. apetalus	G. Hemm s.n. b	100							
	Henderson & Gaum 6	100							
	Wells 5000	90	10						
R. longepedicellatus	Henderson & Gaum 14	100							
n :	Surton 9862	100							
R. pinnatus	Arnold 1555	100				25	25	26	25
\mathbf{R} . \times proteus	Sunon 9/98	100				25	25	25	25
	Henderson & Gaum 21	100							
R. transvaliensis ×	nenuerson & Gaum 31	100							
R. longepedicellatus	Henderson & Gaum 10	100							
R. species	Henderson & Gaum 24	100							

TABLE 3. -- Chromosome segregation during anaphase I in some South African Rubus species

*Exotic species

R. longepedicellatus (C.E. Gust.) C. H. Stirton

This species contained diploid, tetraploid and pentaploid specimens. The diploid specimen, *Hender*son & Gaum 22, had an average of 1,12 chiasmata per half bivalent and an average chromosome association of 0.3_{I} 6,85_{II}. Three cells contained two univalents during metaphase I and one anaphase I cell contained two laggards. Telophase II was normal.

The two tetraploid specimens, Henderson & Gaum 14 and Stirton 9862, had respectively an average of 1,04 and 1,11 chiasmata per half bivalent and an average chromosome association of $0,59_{I}$ 13,5_{II} $0,1_{IV}$ and $0,16_{I}$ 13,92_{II}. Metaphase I, anaphase I and telophase II were normal in both plants. Henderson & Gaum 14 was found to be a segmental alloploid, whereas Stirton 9862 represented a true alloploid.

The pentaploid plant, Henderson & Gaum 36, had an average of 0,99 chiasmata per half bivalent and an average chromosome association of $5,2_1 10,95_{11} 1,7_{111}$ $0,7_{1V}$. Metaphase I had an average of 5,1 (1-8) univalents per cell. Additional micronuclei were observed during telophase II. The absence of pentavalents and low frequency of multivalents suggested that this plant was a segmental alloploid.

R. pinnatus Willd.

Henderson & Gaum 15, the diploid specimen, had an average chiasma frequency of 1,09 and an average chromosome association of $0,1_1,6,95_{II}$. Metaphase I, anaphase I and telophase II were normal. Arnold 1335, the tetraploid specimen, had an average of 1,24 chiasmata per half bivalent and an average chromosome association of $11,84_{II}$ 1,08_{IV}. Metaphase I, anaphase I and telophase II were normal. The observed frequency of multivalents was lower than the expected and, therefore, this plant was a segmental alloploid.

R. ludwigii Eckl. & Zeyh.

The specimens representing this diploid species, Admiraal & Drijfhout 2940 and Henderson & Gaum 41, had average chiasma frequencies of 1,2 and 1,18 respectively and average chromosome associations of $0,08_1$ 6,96₁₁ and 7₁₁. All studied metaphase I, anaphase I and telophase II cells were normal.

$R. \times proteus$

The diploid specimen studied, *Henderson & Gaum* 28, had an average chiasma frequency of 1,1 and all cells contained 7_{II} . No abnormalities were seen during metaphase I, anaphase I or telophase II.

The triploid specimen, *Stirton* 9866, had an average chiasma frequency of 0,88 and an average chromosome association of $6.2_1 5.95_{11} 0.9_{111}$. There were between three and seven univalents (average is 5) during metaphase I. No additional micronuclei were observed during telophase II. This plant tended towards autoploidy.

Four tetraploid specimens, Stirton 9798, Henderson & Gaum 27, 32 and 51, were included in this

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Species Specimen no,	Specimum no	200	Percentage of cells with micronuclei					
	specifien no,	211=	Normal	Num	icronuclei			
			telophase II	1	2	3		
Eubatus				1.25				
R. affinis*	Stirton 5746	28	100					
R. cuneifolius*	Stirton 8102	14	100					
	Stirton 8154	14	100					
	Liengme s.n.	21	100					
	Henderson & Gaum 93	28	90	10				
R. pascuus*	Henderson & Gaum 18	21	80	20				
	Stirton 9800	21	80	20				
	Stirton 9861	28	100					
	Stirton 9868	28	100					
R. flagellaris*	Henderson & Gaum 2	28	100					
daeobatus								
R. apetalus	G. Hemm s.n. a	14	100					
	G, Hemm s.n. b	28	100					
	Henderson & Gaum 6	28	100					
	Wells 5000	28	100					
R. longepedicellatus	Henderson & Gaum 22	14	100					
	Henderson & Gaum 14	28	100					
	Stirton 9862	28	100					
	Henderson & Gaum 36	35	60	30	10			
R. pinnatus	Henderson & Gaum 15	14	100					
	Arnold 1335	28	100					
R. ludwigii	Admiraal & Drijfhout 2940	14	100					
	Henderson & Gaum 41	14	100					
R. × proteus	Henderson & Gaum 28	14	100					
	Stirton 9866	21	100					
	Stirton 9798	28		50	30	20		
	Henderson & Gaum 27	28	100					
	Henderson & Gaum 32	28	100					
	Henderson & Gaum 51	28	100					
	Stirton 9865	35	80	20				
	Henderson & Gaum 20	35	100					
	Henderson & Gaum 31	42	50	30	10	10		
l. transvaliensis ×								
t. longepedicellatus	Henderson & Gaum 10	28	100					
 species 	Henderson & Gaum 24	28	100					

TABLE 4. - Number of micronuclei during telophase II in some South African Rubus species

*Exotic species

TABLE 5. - Comparison between expected and observed chromosome association frequencies in triploid Rubus specimens

Binomial Configuration		R. cuneifolius Liengme s.n.		R. pascuus Henderson d	R. pascuu: Stirton 980	5 00	R. × proteus Stirton 9866		
		Ĕ	0	E	0	E	0	E	0
p ⁷	7,0	0	0	0	0	0	0	0	0
7p ⁶ q	$1_1 1_{11} 6_{11}$	0	0	0	0	0	0	0,001	0
21p ⁵ q ²	$2_1 2_{11} 5_{111}$	0,003	0	0,001	0	0,004	0	0,006	0
35p4q3	31 311 4ut	0,02	0	0,011	0	0,027	0,05	0,036	0
35p ³ q ⁴	41 411 3nt	0,092	0	0,063	0	0,112	0	0,132	0,1
21p ² q ⁵	51 511 2m	0,251	0,12	0.213	0,167	0,272	0,1	0,289	0,15
7pq ⁶	61 611 IIII	0,383	0,44	0,396	0.333	0,369	0.4	0,352	0,35
q ⁷	7, 7 ₁₁	0,251 q=0,8206 p=0,1794	0,44	0,316 q=0,8481 p=0,1519	0.5	0,215 q=0,8027 p=0,1973	0,45	0,184 q=0,7853 p=0,2147	0,4

E Expected frequency

O Observed frequency

species. The average chiasma frequency varied from 0,45 in *Stirton* 9798 to 1,18 in *Henderson & Gaum* 51. The low chiasma frequency in *Stirton* 9798 might be attributed to asynapsis. In a number of cells no chiasmata were formed and 28 univalents were seen. This phenomenon resulted in an average chromosome association of $11.6_{\rm I}$ 4,7_{II} in *Stirton* 9798 com-

pared to an average of $0.2_{I} 8.6_{II} 0.2_{HI} 0.8_{IV}$ in Henderson & Gaum 51. The asynapsis further resulted in an average of 6 (1-10) univalents per metaphase I cell. The only other plant having univalents during metaphase I was Henderson & Gaum 32 with an average of 0.4 per cell. Anaphase I and telophase II were normal in Henderson & Gaum 27, 32 and 51, TABLE 6.—Comparison between expected and observed chromosome association frequencies in tetraploid Rubus specimens

Binomial	p7	7p6q	21p ⁵ q ²	35p ⁴ q ³	35p3q4	21p2q5	7pq6	q7		
Configuration	71V	6 _{IV} 2 _{II}	$5_{\rm IV}4_{\rm II}$	4 _{IV} 6 _{II}	$_{\rm IV} _{\rm NI}$	$2_{\rm IV} 10_{\rm II}$	$1_{\mathrm{IV}}12_{\mathrm{II}}$	1411	p	ġ.,
R. affinis* Stirton 5746 E O	0	0,001 0	0,008 0	0,046 0	0,152 0,3	0,302 0,25	0,333 0,35	0,158 0,1	0,2318	0,7682
R. cuneifolius* Henderson & Gaum 93 E O	0	0,003 0	0,021 0,056	0,087 0	0,215 0,333	0,319 0,333	0,262 0,278	0,092 0	0,2886	0,7114
R. pascuus* Stirton 9861 E O	0	0 0	0	0	0,007 0	0,061 0,2	0,301 0,35	0,63 0,45	0,0638	0,9362
<i>Stirton 9868</i> E O	0 0	0 0	0,002 0	0,017 0	0,082 0,12	0,24 0,32	0,389 0,36	0,27 0,2	0,1704	0,8296
R, flagellaris* Henderson & Gaum 2 E O	0	0 0	0 0	0,001 0	0,015 0	0,096 0	0,348 0	0,541 1	0,0841	0,9159
R. apetalus G. Hemm s.n. E	0,001	0,01	0,053	0,157	0,275	0,29	0,17	0,043	0,3626	0,6374
Henderson & Gaum 6 E O	0	0	0,005 0	0,032 0	0,20 0,122 0,053	0,08 0,281 0,105	0,36 0,361 0,368	0,28 0,199 0,473	0,1973	0,8027
Wells S000 E O	0 0	0 0	0	0 0	0,007 0	0,061 0	0,301 0	0,63 1	0,0638	0,9362
R, longepedicellatus Henderson & Gaum 14 E O	0	0	0	0 0	0,007 0	0,061	0,301	0,63	0,0638	0,9362
Stirton 9862 E O	0	0	0	0,005 0	0,039 0	0,166 0	0,392 0	0,397 1	0,1235	0.8765
R. pinnatus Arnold 1335 E O	0 0	0 0	0,008 0	0,046 0	0.152 0	0,302 0,16	0,333 0,76	0,158 0,08	0,2318	0,7682
R. X proteus Stirton 9798 E O	0	0	0	0	0	0,002	0,071	0,926	0,0109	o,9891
Henderson & Gaum 27 E O	0	0	0,001 0	0,009 0	0,055 0,05	0,198 0,1	0,397 0,6	0,341 0,25	0,1426	0,8574
Henderson & Gaum 32 E O Vandemon & Gaum 51	0 0	0 0	0 0	0,004 0	0,032 0	0,149 0	0,386 0,15	0,429 0,85	0,1138	0,8862
E O O D Itempolitanio V D Iongenedicalitati	0 0	0 0	0,003 0	0.024 0	0,102 0,083	0,262 0,292	0,377 0,375	0,232 0,25	0,1884	0,8116
Henderson & Gaum 10 E O	0	0	0	0	0,01 0	0,078 0	0,327 0	0,584 1	0,074	0.926

Exotic species
p and q were used to determine the expected values
E Expected frequency
O Observed frequency

Bothalia 15, 3 & 4 (1985)

whereas Stirton 9798 had either laggards or a chromosomal maldistribution during anaphase I. These abnormalities resulted in additional micronuclei during telophase II and no normal telophase II cells were seen. The differences in chromosome pairing among these plants are reflected in the fact that Henderson & Gaum 51 is considered as an autoploid, Henderson & Gaum 27 as a segmental alloploid tending towards autoploidy, Henderson & Gaum 32 as a segmental alloploid tending towards alloploidy and Stirton 9798 as an alloploid.

The pentaploid specimens, *Stirton* 9865 and *Henderson & Gaum* 20, had respectively average chiasma frequencies of 1,05 and 0,91 and chromosome associations of $3,3_{\rm I}$ 14,85_{II} $0,25_{\rm III}$ $0,3_{\rm IV}$ and $6,3_{\rm I}$ 13,6_{II} $0,5_{\rm III}$. Although both plants had an average of 3,4 univalents per cell during metaphase I, the number of univalents varied from 1 to 6 in *Stirton* 9865 and 0 to 10 in *Henderson & Gaum* 20. During telophase II *Stirton* 9865 had two cells containing an additional micronucleus. *Stirton* 9865 is regarded as a segmental alloploid and *Henderson & Gaum* 20 as a segmental alloploid tending towards alloploidy.

The hexaploid specimen studied, Henderson & Gaum 31, had an average chiasma frequency of 1,21 and an average chromosome association of $1,85_{1}$ 14,4₁₁ 2,05₁₁₁ 1,3_{1V}. All the metaphase I cells had at least one univalent (average = 1,9). Due to the high chromosome number anaphase I could not be studied. Half of the studied telophase II cells were abnormal, containing 1 to 3 additional micronuclei. The absence of higher order configurations than quadrivalents and the relatively low frequency of triand quadrivalents seemed to indicate that this specimen has been a segmental alloploid.

R. transvaliensis \times R. longepedicellatus

This tetraploid hybrid specimen, *Henderson & Gaum* 10, had an average chiasma frequency of 1,07 and 100% bivalents were formed. No abnormalities were observed in any meiotic stage. The chromosomal behaviour indicated that this specimen represented an alloploid.

Rubus species

The herbarium personnel were unable to identify *Henderson & Gaum* 24. This plant was tetraploid and had a chiasma frequency of 1,16 and an average chromosome association of $2,24_{I},7,99_{II},0,65_{III},1,95_{IV}$. Anaphase I and telophase II were normal, whereas metaphase I had an average of 2,6 univalents per cell. According to the number of multivalents formed, this specimen represented a segmental alloploid tending towards autoploidy.

DISCUSSION

Most *Rubus* chromosomes have the ability to participate in the formation of more than one chiasma. During diplotene/carly diakinesis most bivalents occur as ringbivalents (Figs 1a & 2a), whereas later diakinesis stages have a majority of chainbivalents (Figs 1b & 2b-2f). This might possibly be due to chiasma terminalization. The formation of multivalents may suppress the effect of chiasma terminalization and thus increases the chiasma frequency.

It is sometimes difficult to determine whether metaphase I univalents originated from asynapsis or early segregation (Figs 1c & 2g & h). The occurrence of univalents during diakinesis would suggest asynapsis. This segregation of univalents might be random (for example see Fig. 1c: only one univalent can be seen on one side of the metaphase plate and three on the other side of the camera lucida drawing). The effect of univalents during metaphase I is not very serious, because their occurrence is restricted to either high ploidy levels or uneven polyploid levels, where it is presumed that apomixis will occur.

Chromosome laggards are occasionally observed in diploid and tetraploid *Rubus* plants. The number of laggards increases drastically in triploid plants where a maldistribution of chromosomes is also found.

An exceptionally high frequency of apparently normal telophase II cells was observed (Figs 1f & 2j). This may be due to the formation of microspores



FIG. 1.—Camera lucida drawings of different meiotic stages in *R. × proteus (Stirton* 9865). a, diplotene/early diakinesis; b, diakinesis; c, metaphase I; d, telophase I; e, anaphase II; f, telophase II: C. chainbivalent; H, horizontat division; L, univalents or early segregating chromosomes; M, metaphase chromosomes; O, quadrivalent; R. ringbivalent; S, secondary chromosome association; T, trivalent; U, univalent. (× 1600.) with varying numbers of chromosomes rather than excluding laggards through micronuclei. During anaphase II chromatid segregation might occur without being preceded by the formation of a cell wall (Fig. 1d & 2i). Chromosome laggards from anaphase I might thus be incorporated into the tetrad nuclei. A study of pollen fertility might prove interesting.

The meiotic chromosome behaviour mentioned above indicates differences in different *Rubus* species complexes. These differences in chromosome pairing (Table 1) indicate either differences in genome homology or the existence of genes inhibiting chromosome pairing in certain species.

All species belonging to the subgenus Eubatus occur as exotics in South Africa. Both naturalized South African polyploid Eubatus species studied, R. affinis and R. cuneifolius, were apparently autoploid. All published chromosome numbers for R. affinis indicated a somatic chromosome number of 28 (Gustafsson, 1933, 1939 & 1943; Heslop-Harrison, 1953; Spies & Du Plessis, 1985) and, therefore, it is not known if the diploid form still exists. An exception from the general autoploid situation was found in the octoploid *R. cuneifolius* specimen, *Henderson* & *Gaum* 50, which was a segmental alloploid and in *R. flagellaris* where the only studied specimen represented an alloploid. The fact that the South African specimen is tetraploid, whereas extra-African specimens were either octoploid or nonaploid (Einset, 1947; Faasen & Nadeau, 1976) is an indication that further studies of this group are necessary before any conclusions can be made.

The existence of triploid and tetraploid R. pascuus forms might suggest that the diploid form may still be present in South Africa. The fact that all specimens represented autoploidy or tended towards au-



FIG. 2.—Microphotograph of different meiotic stages in different Rubus species. a-c, R. × proteus (Stirton 9865): a, Diplotene/early diakinesis; b, diakinesis; c, late diakinesis (note the decrease in the number of ringbivalents). d-f, diakinesis: e, R. × proteus, Henderson & Gaum 32; d & f, R. cuneifolius, respectively Liengme s.n. and Henderson & Gaum 93, g, metaphase I (R. × proteus, Stirton 9865); h, early anaphase I (R. × proteus, Henderson & Gaum 31); i, late anaphase I (R. apetalus, Henderson & Gaum 6); j, telophase II (R. pinnatus, Arnold 1335). (× 1800.)

toploidy supports this suggestion. It is further supported by the fact that a diploid R. × proteus specimen was observed. Since R. × proteus originated as a hybrid between R. pascuus and R. longepedicellatus, a diploid hybrid specimen suggests diploidy in both parents.

The subgenus *Idaeobatus* contains two exotic and nine indigenous species in South Africa. The chromosomal behaviour of these species is variable and autoploidy, segmental alloploidy and alloploidy were observed. Different systems might be operating in some species, resulting in either autoploidy and alloploidy (R. × proteus) or alloploidy and segmental alloploidy (R. longepedicellatus & R. apetalus) in the same species.

It would seem that the majority of exotic *Rubus* plants in South Africa tends towards autoploidy (77,8%), whereas the minority of indigenous *Rubus* specimens tends towards autoploidy (23,5%). The suggested ploidy types of different *Rubus* species are summarized in Table 7.

The high frequency of autoploidy found in this *Eubatus* population, does not support an intersubgeneric hybridization theory (Spies & Du Plessis, 1985) or even interspecific hybridization within the subgenus. However, it must be remembered that the specific delimitation in *Rubus* does not conform with the biological species concept. Therefore, 'interspecific hybridization' within a *Rubus* subgenus could lead to autoploidy. A prerequisite for this assumption is that the current classification system must represent natural relationships. Plants within a subgenus must, therefore, be more closely related to one another than to any species in another subgenus. It is further assumed that karyotipic evolution, although not directly correlated with morphological divergence, progresses along the same lines. Large genomic differences are, therefore, more likely to be expected in different subgenera than within a subgenus. Consequently, hybridization within a subgenus is more likely to involve smaller chromosomal differences and even interspecific hybridization within the subgenus would be more likely to produce segmental alloploids tending towards autoploidy.

In contrast to the South African Eubatus species, the Idueobatus species have only 23,5% autoploids or segmental alloploids tending towards autoploidy and 35,3% alloploids or segmental alloploids tending towards alloploidy. These figures indicate that hybridization might occur more frequently in the subgenus Idaeobatus than in the subgenus Eubatus in South Africa.

Crane & Thomas (1949) described preferential pairing in *Eubatus* \times *Idaeobatus* hybrids when each genome is represented twice. Their finding is not supported by this study in which tetraploid R. \times proteus [a hybrid between R. pascuus (Eubatus) and R. longepedicellatus (Idaeobatus)] specimens varied from alloploid to autoploid. Crane & Thomas (1949) also described intergenomic pairing with little restriction when each genome is represented only once. This finding is supported by the chromosome behaviour of Henderson & Gaum 28.

The preferential pairing in *Rubus* is due to minor alterations of the genetic material. The degree of

Specimen no.	2n=		Autoploid	ty	Alloploidy		
		1	2	3	4	5	
Stirton 5746	28	X					
Liengme s.n.	21		Х				
Henderson & Gaum 93	28	X					
Henderson & Gaum 18	21	10.41	х				
Stirton 9800	21		x				
Stirton 9861	28		x				
Stirton 9868	28	x					
Henderson & Gaum 2	28					Х	
G. Hemms.n.b	28			x			
Henderson & Gaum 6	28			x			
Wells 5000	28			1000		x	
Henderson & Gaum 14	28			X			
Stirton 9862	28			27970		x	
Henderson & Gaum 36	35			x			
Arnold 1335	28			x			
Stirton 9866	21		х	100			
Stirton 9798	28					х	
Henderson & Gaum 27	28		x				
Henderson & Gaum 32	78		5.5		x		
Henderson & Gaum 51	28	X					
Stirton 9865	35			x			
Henderson & Gaum 20	35				x		
Stirron 8135	56			x	23		
GINDA DIDD				~			
Henderson & Gour 10	28					x	
Henderson & Gaum 74	28	x				25	
	Specimen no. Stirton 5746 Liengme s.n. Henderson & Gaum 93 Henderson & Gaum 18 Stirton 9800 Stirton 9861 Stirton 9868 Henderson & Gaum 2 G. Hemm s.n. b Henderson & Gaum 6 Wells 5000 Henderson & Gaum 14 Stirton 9862 Henderson & Gaum 36 Arnold 1335 Stirton 9798 Henderson & Gaum 27 Henderson & Gaum 27 Henderson & Gaum 21 Stirton 9798 Henderson & Gaum 51 Stirton 9865 Henderson & Gaum 20 Stirton 8135 Henderson & Gaum 10 Henderson & Gaum 14	Specimen no. 2n= Stirton 5746 28 Liengme s.n. 21 Henderson & Gaum 93 28 Henderson & Gaum 18 21 Stirton 9800 21 Stirton 9861 28 Stirton 9868 28 Henderson & Gaum 2 28 G. Herm s.n. b 28 Henderson & Gaum 6 28 Wells 5000 28 Henderson & Gaum 6 28 Wells 5000 28 Henderson & Gaum 14 28 Stirton 9862 28 Henderson & Gaum 36 35 Arnold 1335 28 Henderson & Gaum 36 35 Stirton 9798 28 Henderson & Gaum 27 28 Henderson & Gaum 32 28 Henderson & Gaum 51 28 Stirton 9865 35 Henderson & Gaum 20 35 Stirton 8135 56 Henderson & Gaum 10 28 Henderson & Gaum 10 28 <t< td=""><td>Specimen no. 2n= 1 Stirton 5746 28 X Liengme s.n. 21 1 Henderson & Gaum 93 28 X Henderson & Gaum 18 21 3 Stirton 9800 21 5 Stirton 9861 28 X Henderson & Gaum 18 21 3 Stirton 9861 28 X Henderson & Gaum 2 28 X Henderson & Gaum 2 28 X Henderson & Gaum 6 28 X Wells 5000 28 4 Henderson & Gaum 14 28 5 Wetls 5000 28 4 Henderson & Gaum 36 35 28 Henderson & Gaum 36 35 28 Henderson & Gaum 27 28 4 Henderson & Gaum 27 28 4 Henderson & Gaum 51 28 X Stirton 9865 35 5 Henderson & Gaum 20 35 5</td><td>Specimen no. $2n =$ Autoploid Stirton 5746 28 X Liengme s.n. 21 X Henderson & Gaum 93 28 X Henderson & Gaum 18 21 X Stirton 9800 21 X Stirton 9861 28 X Stirton 9868 28 X Henderson & Gaum 6 28 X Stirton 9868 28 X Henderson & Gaum 6 28 X Henderson & Gaum 6 28 X Henderson & Gaum 6 28 X Henderson & Gaum 14 28 S Stirton 9862 28 X Henderson & Gaum 36 35 X Stirton 9798 28 X Henderson & Gaum 27 28 X Henderson & Gaum 32 28 X Henderson & Gaum 51 28 X Henderson & Gaum 51 28 X Henderson & Gaum 20 35 35 <tr< td=""><td>Specimen no. $2n =$ Autoploidy 1 2 3 Stinton 5746 28 X Liengme s.n. 21 X Henderson & Gaum 93 28 X Henderson & Gaum 93 28 X Henderson & Gaum 18 21 X Stirton 9800 21 X Stirton 9861 28 X Stirton 9868 28 X Henderson & Gaum 2 28 X Henderson & Gaum 6 28 X Wells 5000 28 X Henderson & Gaum 14 28 X Stirton 9862 28 X Stirton 9866 21 X Stirton 9866 21 X Stirton 9798 28 X Henderson & Gaum 31 28 X Henderson & Gaum 51 28 X Henderson & Gaum 51 28 X Henderson & Gaum 51 28 X Henderson & Gaum 20</td><td>Specimen no.$2n =$Autoploidy AutoploidyAllStirton 574628XLiengme s.o.21XHenderson & Gaum 9328XStirton 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Gaum 9328XStirton 980021XStirton 986128XStirton 986828XHenderson & Gaum 628XStirton 986828XHenderson & Gaum 628XWells 500028XHenderson & Gaum 1428XStirton 986228XHenderson & Gaum 3635XStirton 986621XStirton 986621XStirton 986621XStirton 986621XStirton 986528XHenderson & Gaum 3228XHenderson & Gaum 5128XHenderson & Gaum 5535XStirton 885335XHenderson & Gaum 5128XHenderson & Gaum 1028Henderson & Gaum 2428Henderson & Gaum 2428</td></tr<>	Specimen no. $2n =$ Autoploidy 1 2 3 Stinton 5746 28 X Liengme s.n. 21 X Henderson & Gaum 93 28 X Henderson & Gaum 93 28 X Henderson & Gaum 18 21 X Stirton 9800 21 X Stirton 9861 28 X Stirton 9868 28 X Henderson & Gaum 2 28 X Henderson & Gaum 6 28 X Wells 5000 28 X Henderson & Gaum 14 28 X Stirton 9862 28 X Stirton 9866 21 X Stirton 9866 21 X Stirton 9798 28 X Henderson & Gaum 31 28 X Henderson & Gaum 51 28 X Henderson & Gaum 51 28 X Henderson & Gaum 51 28 X Henderson & Gaum 20	Specimen no. $2n =$ Autoploidy AutoploidyAllStirton 574628XLiengme 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 TABLE 7. — The polyploid classification of some South African Rubus species (1 represents autoploidy, 2-4 segmental alloploidy, with 2 tending towards autoploidy and 4 tending towards autoploidy and 5 represents alloploidy)

*Exotic species

chromosomal alterations varies in different Rubus species complexes. Due to these genomic differences in the hybrids, the hybrids varied from sterile to fertile in some interspecific crosses involving plants on the same (Jinno, 1958; Britton & Hull, 1959; Haskell & Tun, 1961) or at different (Crane & Darlington, 1927; Crane & Thomas, 1949; Shoemaker & Sturrock, 1959; Bammi, 1964) ploidy levels. The implications of these phenomena are that intersubgeneric hybridization will apparently result in alloploidy at tetraploid level and segmental alloploidy or alloploidy at other ploidy levels. However, according to the present study, intersubgeneric hybrids may even represent autoploidy. Therefore, the type of ploidy in Rubus can make only a limited contribution to the knowledge of hybridization among different species. The reason for this phenomenon might be that the current classification of this genus is artificial and does not represent the true phylogenetic relationship between species.

The relatively normal meioses observed in diploid plants also seems to contradict hybridization. However, intergenomic pairing with little restriction was described in such cases by Crane & Thomas (1949). Consequently, seemingly normal diploid plants might represent intersubgeneric hybrids.

Longley & Darrow (1924) described the subgenus Idaeobatus as being diploid with almost no reproductive isolation between the different species. Therefore, hybridization among Idaeobatus species does not require chromosome doubling for the restoration of fertility. Consequently, the high polyploidy frequency and especially the alloploid situation in the South African Idaeobatus species suggest hybridization at intersubgeneric level. The fact that apparently all hybrids represent their Idaeobatus parents could be attributed to several factors. Either the hybridization hypothesis is incorrect, or the occurrence of matrocliny (Markarian & Olmo, 1959) combined with oneway hybridization (introgression), suppressed the presence of hybrids representing the subgenus Eubatus morphologically. The chances of collecting only F_1 matroclinous hybrids is extremely small and introgression was not described in any other intersubgeneric Rubus hybrids (Crane & Thomas, 1949; Jinno, 1958; Britton & Hull, 1959; Haskell & Tun, 1961; Thompson, 1961). It is, therefore, concluded that the assumption that hybridization occurs only within Idaeobatus species is erroneous due to statistically insufficient material studied and that the original assumption of intersubgeneric hybridization is still valid.

UITTREKSEL

Meiotiese chromosoomgedrag in die genus Rubus is relatief normaal. Poliploïdie kom in beide Suid-Afrikaanse subgenera, nl. Eubatus en Idaeobatus, voor. Die subgenus Eubatus bevat plante wat merendeels na outoploïdie neig, terwyl die subgenus Idacobatus varieer van outoploïed, deur segmenteel alloploïed tot by alloploïed. Uit die data word afgelei dat hierdie skynbare verskil toegeskryf kan word aan 'n statisties onvoldoende aantal plante en dat alloploïdie ontstaan het na intersubgeneriese verbastering.

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