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The genus *Rubus* (Rosaceae) in South Africa. IV. Natural hybridization

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ABSTRACT

The genus *Rubus* L. is represented in southern Africa by the subgenera *Eubatus* Focke and *Idaeobatus* Focke. A combination of morphological data, data on the reproductive systems of some collections and meiotic chromosome behaviour indicates that a hybrid swarm in the eastern Transvaal was formed subsequent to the hybridization between *R. cuneifolius* Pursh. taxon B (subgenus *Eubatus*) and *R. longepedicellatus* (C. E. Gust.) C. H. Stirton (subgenus *Idaeobatus*). Other examples of intra- and intersubgeneric hybridization were found during this study of the South African material. These instances, with examples found in the literature, indicate that the subgeneric subdivisions of *Rubus* are artificial.

Three different methods were used to analyse the meiotic chromosome configurations. The genome relationship system of Alonso & Kimber (1981) and Kimber & Alonso (1981) and the modification of the binomial system of Jackson & Casey (1980) by Spies (1984) proved to be the most sensitive for distinguishing between allo-, segmental allo- and autoploids.

UITTREKSEL

Die genus *Rubus* L. word in suidelike Afrika verteenwoordig deur die subgenera *Eubatus* Focke en *Idaeobatus* Focke. 'n Kombinasie van morfologiese data, data rakende die voortplantingsisteem van sommige eksemplare en meiotiese chromosoomgedrag het aangetoon dat 'n basterkompleks in die oostelike Transvaal gevorm is na die verbastering van *R. cuneifolius* Bailey takson B (subgenus *Eubatus*) en *R. longepedicellatus* (C. E. Gust.) C. H. Stirton (subgenus *Idaeobatus*). Ander voorbeelde van intra- en intersubgeneriese verbastering is tydens hierdie studie in Suid-Afrika gevind en in samehang met verdere voorbeelde in die literatuur toon dit aan dat die onderverdeling van die genus *Rubus* in subgenera kunsmatig is.

Drie verskillende metodes is gebruik om die meiotiese chromosoomgedrag van die plante te vergelyk. Die genoomverwantskapsisteem van Alonso & Kimber (1981) en Kimber & Alonso (1981) en die modifikasies op die binomiale sisteem van Jackson & Casey (1980) deur Spies (1984) toon aan dat hierdie twee metodes die sensitiefste is om tussen allo-, segmentele allo- en outoploïede plante te onderskei.

CONTENTS

INTRODUCTION

The genus *Rubus* is somewhat enigmatic in South Africa. It forms part of our indigenous flora but naturalized species also occur. Most taxa are considered weedy and yet they are included in a breeding programme to improve their agricultural production. The genus also contains agamic species as well as sexual species. In short, it is a taxonomist's nightmare.

The genus *Rubus* comprises 12 subgenera of which two are represented in South Africa: *Eubatus* Focke and *Idaeobatus* Focke. In South Africa the subgenus *Eubatus*, or true brambles or blackberries, includes only exotics, whereas the subgenus *Idaeobatus*, or raspberries, contains a few exotics and a number of indigenous species (Spies & Du Plessis 1985).

It has been proposed (Stirton 1981a & b; Spies & Du Plessis 1985) that the problems with *Rubus* taxonomy in South Africa are caused by the occurrence of apomixis, hybridization among indigenous species and between indigenous and exotic species, the variation produced by a breeding program with subsequent escape from cultivation and inadequately collected herbarium material.

Each paper in this series has dealt with a different aspect of the cytogenetics of *Rubus* in South Africa. The aim of this paper is to determine whether natural hybridization occurs in the South African *Rubus* complex and whether this hybridization, if it does occur, is restricted to intrasubgeneric taxa.

MATERIALS AND METHODS

The following specimens were collected in the veld, transplanted in the Pretoria National Botanical Garden

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and subsequently examined for this study [All the herbarium specimens are housed in the Pretoria National Herbarium (PRE)]:

R. cuneifolius Pursh taxon A*

NATAL.—2929 (Underberg): 14 km from Swartberg to Underberg (–CD), *Stirton 8154*. 2930 (Pietermaritzburg): 3 km from Midmar Dam to Lions River (–CB), *Henderson & Gaum 93*; 5 km from Pietermaritzburg to Mooi River (–CB), *Liengme s.n.*; Highlands Farm (–CD), *Beard 720*. 3029 (Kokstad): 40 km from Underberg to Swartberg (–BA), *Stirton 8157*; 11 km from Harding to Weza (–DB), *Stirton 8102*.

R. cuneifolius Pursh taxon B*

TRANSVAAL.—2329 (Pietersburg): 3 km from Haenertsburg to Boyne (–CC), *Stirton 8033*. 2330 (Tzaneen): Modderfontein (–CC), *Stirton 8013*. 2430 (Pilgrim's Rest): 1 km from Graskop to Sabie (–DD), *Stirton 9800, 9859, 9861, 9868, Henderson & Gaum 18*. 2530 (Lydenburg): 5 km from Lydenburg to Sabie (–AB), *Henderson & Gaum 37*; Dullstroom (–AC), *Stirton 7255*. 2628 (Johannesburg): Heidelbergkloof (–CA), *Bredenkamp 123*.

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

TRANSVAAL.—2329 (Pietersburg): 10 km from Tzaneen to Haenertsburg (–CC), *Stirton 5755*; near Pietersburg (–CD), *McCullum 13*. 2330 (Tzaneen): Pietersburg District (–CC), *McCullum 887*. 2430 (Pilgrim's Rest): Pilgrim's Rest (–DB), *Killick & Strey 2420*; Mariepskop (–DB), *Van der Schijff 4562*; Bourke's Luck (–DB), *Viljoen 27*; 1 km from Graskop to Sabie (–DD), *Henderson & Gaum 22, Stirton 9862*. 2530 (Lydenburg): 5 km from Lydenburg to Sabie (–AB), *Henderson & Gaum 36*; Brooklands (–BA), *Henderson & Gaum 14*; Nelspruit (–BD), *Mogg* (PRE 55710). 2531 (Komatipoort): Kruger National Park (–AB), *Van der Schijff 1228*.

NATAL.—3029 (Kokstad): Ngeli Forest (–DA), *Stirton 8135*.

R. × *proteus* sp. ined.

TRANSVAAL.—2329 (Pietersburg): 10 km from Tzaneen to Haenertsburg (–CC), *Stirton 5756, 5783*. 2430 (Pilgrim's Rest): Spekboom River, Burgersfort (–CB), *Henderson 319*; Bourke's Luck (–DB), *Henderson & Gaum 27, 28, 29, 31, 32*; Mac-Mac Waterfalls (–DD), *Henderson & Gaum 20*; 6 km from Pilgrim's Rest to Lydenburg (–DD), *Henderson & Gaum 33*; 1 km from Graskop to Sabie (–DD), *Stirton 9797, 9798, 9799, 9801, 9855, 9860, 9862, 9863, 9864, 9865, 9866, 9867, 9869*. 2530 (Lydenburg): 3 km from Brooklands to Hendriksdal (–BA), *Henderson & Gaum 12*; 33 km from Nelspruit to Sabie (–BD), *Henderson & Gaum 11*.

R. rigidus × *R. cuneifolius* taxon A

NATAL.—2929 (Underberg): 25 km from Himeville to Boesmansnek (–DC), *Henderson & Gaum 50, 51*.

This cytotaxonomic study concentrated upon a possible hybrid swarm in the area between Graskop and Sabie in the eastern Transvaal Lowveld (2430DD) (Stirton 1984). The cytogenetical methods and results were reported by Spies & Du Plessis (1985 & 1986) and Spies, Du Plessis & Liebenberg (1985). These investigations included meiotic analyses of aceto-carmine anther squashes and embryo sac studies.

In order to compare morphological characters of the plants, the following 18 characters were studied (Table 1): 1, inflorescence length; 2, flowers single or double; 3, flower colour; 4, petal length; 5, width of petal; 6, form of sepal apex; 7, ratio between length of petal and sepal; 8, rachis length; 9, length of petiole; 10, thorns straight or recurved; 11, leaf surface; 12, form of leaf apex; 13, leaf margin; 14, form of stipule; 15, number of leaflets per leaf in the floricane; 16, primocane leaves; 17, terminal leaf length and 18, form of base of terminal leaflet.

In an attempt to determine cytogenetically whether hybridization has occurred, three different methods were used to compare the observed chromosome configurations of polyploids with the expected values for autoploids. These methods included the genomic relationship system developed by Kimber and others (Kimber & Hulse 1978; Driscoll 1979; Driscoll, Bielig & Darvey 1979; Alonso & Kimber 1981; Espinasse & Kimber 1981; Kimber & Alonso 1981; Kimber, Alonso & Sallee 1981; Alonso & Kimber 1984), the binomial system developed by Jackson *et al.* (Jackson & Casey 1980 & 1982; Jackson & Hauber 1982) and the modification of this binomial system by Spies (1984). Computer programmes were used to calculate these values. The model with the smallest average sum of squares between the expected and observed frequencies, was considered as being the most appropriate model.

RESULTS

Morphology

The two probable species participating in the formation of the apparent hybrid swarm were identified as *R. longepedicellatus* (C. E. Gust.) C. H. Stirton of the subgenus *Idaeobatus* Focke and *R. cuneifolius* Pursh taxon B belonging to the subgenus *Eubatus* Focke. It was assumed that these species formed morphologically distinct hybrids, referred to here collectively as, *R.* × *proteus* C. H. Stirton. The morphology of the different plants is summarized in Table 1.

In order to determine whether the *R.* × *proteus* specimens are intermediate between the putative parental species or fall within the normal infraspecific variation of these species, all *R. cuneifolius* B and *R. longepedicellatus* specimens in the National Herbarium (PRE) were scored for the selected characters listed in Materials and methods. These results are also summarized in Table 1 and clearly indicate that both these species are morphologically variable.

Nevertheless, several distinct morphological differences between *R. longepedicellatus* and *R. cuneifolius* B were observed. For example, the average petal length in *R. cuneifolius* B was 17,1 mm, compared to the average of 6,4 mm for *R. longepedicellatus*. *R. cuneifolius* B is separated from *R. longepedicellatus* mainly on flower colour, petal and rachis lengths, ratio between the lengths of the petal and the sepal and whether the primocane leaves are pinnate or pinnate/palmate. Characters that did not contribute to the separation of these species were double or single flowers, petiole length, straight or recurved thorns, con- or discolourous leaf surfaces, number of leaflets per leaf in the floricane and the terminal leaf length. It was therefore decided to use only those characters which contributed to the separation of the species, to determine a hybrid index (Figure 1) according to the method developed by Anderson (1949).

* For some time we have been aware that *R. cuneifolius* Pursh might comprise more than one taxon. The discovery of hybrid swarms in the eastern Transvaal confirmed this. We have been unable to clarify the identity of the Transvaal forms of *R. cuneifolius*, except that they may be conspecific with *R. pascuus* Bailey. However, the cytogenetic information would argue against recognizing *R. pascuus* at the species level. We feel, therefore, that until its status is resolved, we will refer to it as *R. cuneifolius* Pursh taxon B, whereas the Natal form (or typical form) of *R. cuneifolius* will be referred to as taxon A.

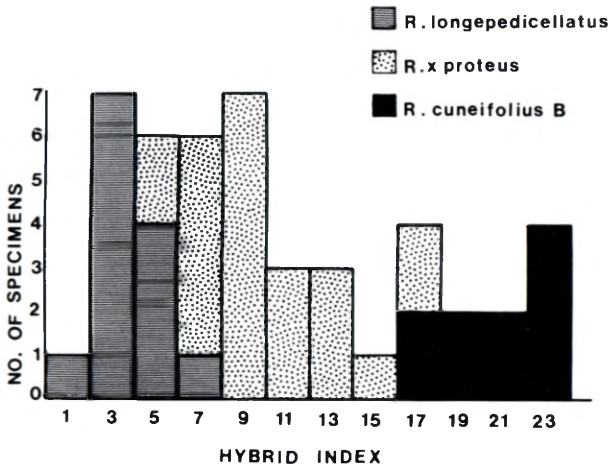

FIGURE 1.—Histogram of hybrid indices for specimens of *R. longepedicellatus* (area with horizontal lines), *R. cuneifolius* B (solid area) and *R.* × *proteus* (dotted area).

A scatter diagram (Figure 2) was constructed using the rachis and petal lengths on the X- and Y-axes respectively. Other morphological characters used in the scatter diagram were flower colour, the ratio between the lengths of the petals and sepals and whether the primocane leaves were pinnate or pinnate/palmate.

Reproductive system

The presence of both reduced (sexual) and unreduced (aposporic) embryo sacs was described in the triploid *R. cuneifolius* B specimens, *Henderson & Gaum 18* and *Stirton 9800* (Spies & Du Plessis 1986). However, all the reduced embryo sacs were observed to degenerate at maturity. The one tetraploid specimen, *Stirton 9861*, was 100 % sexual, whereas the other one, *Stirton 9868*, was only 35 % sexual. In addition to this sexual and asexual reproduction through seeds, all specimens reproduced vegetatively through stemtip-rooting.

In contrast to the apospory described in the *R. cuneifolius* B specimens, no apospory was observed at any ploidy level in the *R. longepedicellatus* sample studied, except that in the pentaploid *R. longepedicellatus* specimen, *Henderson & Gaum 36*, all the reduced embryo sacs degenerated at maturity and the plant was, therefore, sterile (Spies & Du Plessis 1986). Vegetative reproduction occurs through rhizomes.

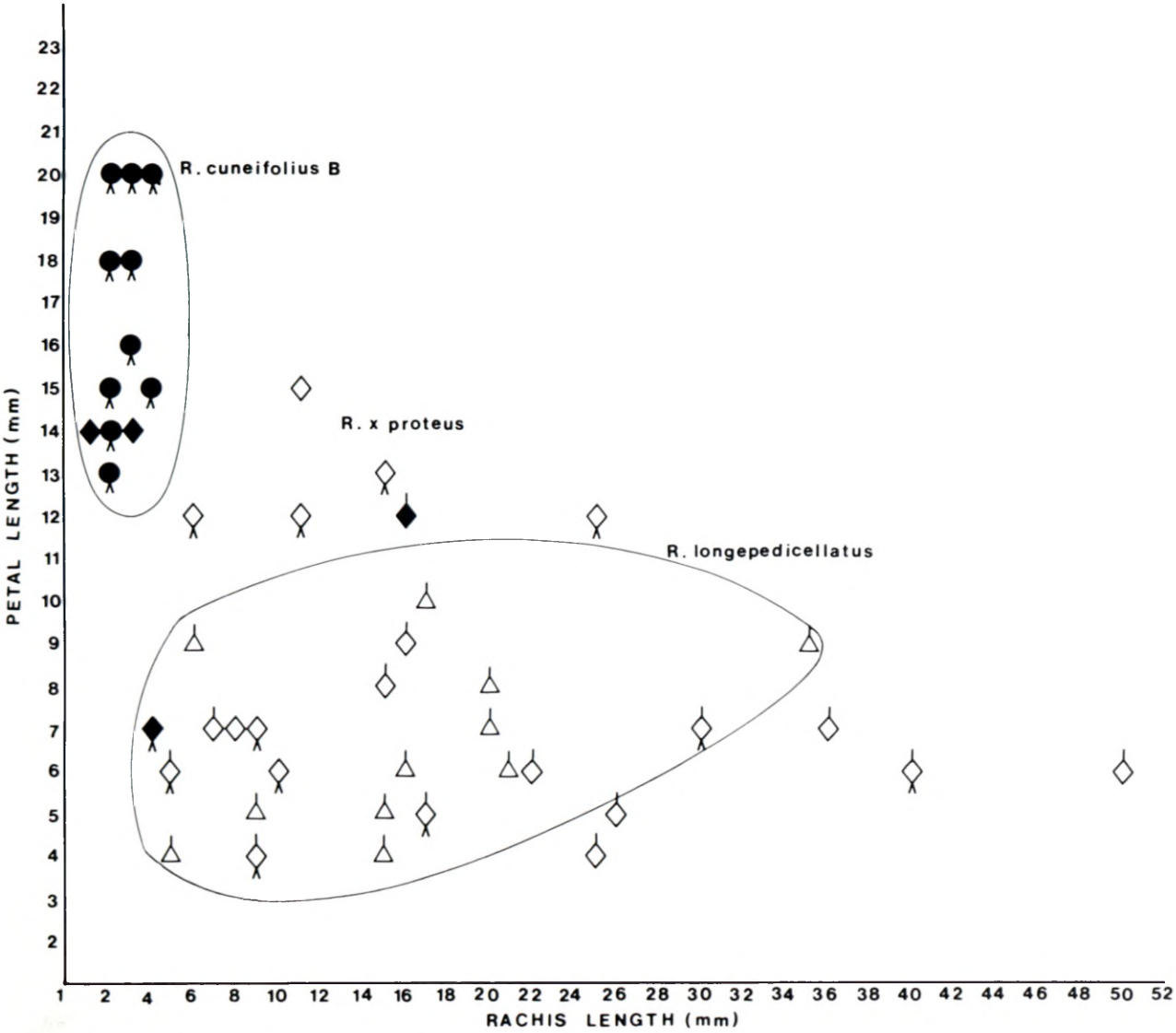

FIGURE 2.—Scatter diagram of *R. longepedicellatus* △, *R. cuneifolius* B ● and *R.* × *proteus* ◆ specimens. The occurrence of white flowers in a specimen is indicated by a solid character in contrast to the line character used for pink flowers. Specimens in which the petal length exceeds the sepal length are indicated by a Λ-sign under the character and pinnate leaves are indicated by a Λ-sign above the character.

TABLE 1.—List of morphological character values allocated to different *Rubus* specimens

Morphological character	Character value for different specimens																											
	R. cuneifolius A						*R. cuneifolius* B										*R. longepedicellatus*											
	Beard 720	*Henderson & Gaum 93*	*Liengme s.n.*	*Stirton 8102*	*Stirton 8154*	*Stirton 8157*	*Bredenkamp 123*	*Henderson & Gaum 18*	*Henderson & Gaum 37*	*Stirton 7255*	*Stirton 8013*	*Stirton 8033*	*Stirton 9800*	*Stirton 9859*	*Stirton 9861*	*Stirton 9868*	*Henderson & Gaum 14*	*Henderson & Gaum 22*	*Henderson & Gaum 36*	*Killick & Strey 2420*	*McCullum 13*	*McCullum 887*	*Mogg s.n.*	*Stirton 5755*	*Stirton 8135*	*Van der Schijff 1228*	*Van der Schijff 4562*	*Viljoen 27*
1. Inflorescence length	IN	IN	IN	IS	IS	IS	IN	IS	IS	IN	IN	IS	IN	IN	IS	IS	IN	IL	IN	IL	IL	IL	IL	IL	IL	IL	IL	IL
2 Flowers	FS	FS	FS	FS	FS	FS	FS	FD	FD	FS	FD	FS	FS	FS	FD	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS
3. Flower colour	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PW	PI	PI	PI	PI	PI	PI	PI	PI	PI	PI	PI	PI
4. Petal length (mm)	16	15	17	17	18	22	16	20	18	15	13	20	14	18	20	15	7	5	9	8	5	6	4	4	10	5	4	6
5. Petal width (mm)	5	8	16	11	14	11	11	10	10	7	9	15	11	10	11	10	6	3	6	4	4	4	3	4	6	4	3	3
6. Form of sepal apex	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AM	AM	AM	AM	AC	AM	AM	AM	AM	AC	AM	AC
7. Ratio between length of petal and sepal	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL	PS	PS	PE	PS	PE	PE	PS	PS	PS	PS	PS	PE
8. Rachis length (mm)	4	3	3	3	3	4	3	4	3	2	2	3	2	2	3	4	20	26	35	20	15	16	15	5	17	9	25	21
9. Petiole length (mm)	30	12	15	15	16	20	25	20	45	10	15	12	8	8	22	20	30	30	45	22	30	30	20	15	35	20	35	50
10. Thorns	–	TB	TR	TR	TR	TR	TR	TR	TR	TS	TR	–	TR	–	TR	TR	TR	TR	TS	TR	TR	TR	TR	TR	TS	TB	TB	TR
11. Leaf surface	CI	CC	CI	CC	CD	CI	CC	CC	CC	CI	CC	CD	CC	CC	CC	CC	CC	CC	CC	CD	CD	CD	CD	CD	CD	CD	CD	CD
12. Form of leaf apex	AC	AM	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AM	AM	AM	AC	AC	AM	AC	AM	AC	AM	AC	AC
13. Leaf margin	LP	LB	LP	LB	LB	LP	LP	LB	LB	LB	LB	LP	LB	LB	LB	LB	LS	LP	LS	LS	LS	LS	LS	LB	LB	LS	LS	LS
14. Form of stipule	SL	SL	SL	SL	SF	SL	SL	SF	SF	SF	SL	SF	SL	SF	SL	SL	SN	SN	SN	SN	SN	SN	SN	SN	SN	SN	SN	SN
15. No. leaflets/leaf in floricane	3	3	3	3	1–3	1–3	3	1–3	3	1–3	1–3	1–3	3	3	1–3	3	1–3	5–7	3	3–5	3	1–3	3	3	3–5	3	1–3	3
16. Primocane leaves	–	PB	PB	–	–	PB	–	PB	PB	PB	–	–	PB	PB	–	–	–	PB	PA	–	–	–	–	PA	PA	–	–	–
17. Terminal leaf length (mm)	60	50	55	33	40	40	70	80	55	30	50	40	45	60	80	70	70	55	90	45	50	66	65	30	70	40	75	53
18. Form of base of terminal leaflet	BO	BT–BN	BN	BN	BN	BN	BO	BN	BN	BC	BN	BT	BO	BN	BN	BN	BO	BT	BC	BO	BO	BC	BO	BT	BC	BT	BO	BC

AC, acute; AM, acuminate; BC, cordate; BN, cuneate; BO, obtuse; BT, truncate; CC, concolourous; CD, discolourous; CI, leaf slightly discolourous; FD, flower double; FS, flower single; IL, long; IN, intermediate; IS, short; LD, double serrate; LR, serrate; LS, serrulate; PA, pinnate; PB, pinnate/palmate; PE, petal = sepal; PI, pink; PL, petal > sepal; PP, pale pink; PS, petal < sepal; PW, white; SF, flabellate; SL, lanceolate, triangular or falcate; SN, needle, linear or filiform; TB, some thorns recurved and others straight; TR, recurved; TS, straight.

TABLE 1.—List of morphological character values allocated to different *Rubus* specimens (continued)

Morphological character	Character value for different specimens																						
	R. × *proteus*																						
	Henderson 319	*Henderson & Gaum 11*	*Henderson & Gaum 12*	*Henderson & Gaum 20*	*Henderson & Gaum 27*	*Henderson & Gaum 28*	*Henderson & Gaum 29*	*Henderson & Gaum 32*	*Henderson & Gaum 33*	*Stirton 5756*	*Stirton 5783*	*Stirton 9797*	*Stirton 9798*	*Stirton 9799*	*Stirton 9801*	*Stirton 9855*	*Stirton 9860*	*Stirton 9862*	*Stirton 9863*	*Stirton 9864*	*Stirton 9866*	*Stirton 9867*	*Stirton 9869*
1. Inflorescence length	IN	IL	IL	IL	IL	IL	IL	IS	IN	IN	IL	IL	IS	IN	IS	IL	IN	IL	IN	IN	IN	IN	IN
2. Flowers	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS	FS
3. Flower colour	PI	PP	PI	PI	PI	Pi	PI	PI	PP	PI	PP	PW	PI	PW	PI	PP	PP	PI	PI	PW	PW	PI	PI
4. Petal length (mm)	6	5	7	9	6	6	7	7	6	6	7	7	12	14	7	8	15	9	13	12	14	12	12
5. Petal width (mm)	4	6	4	6	4	6	4	5	4	4	5	6	–	11	6	5	7	6	9	10	11	7	9
6. Form of sepal apex	AC	AM	AC	AC	AM	AC	AM	AC	AM	AM	AM	AC	AM	AM	AM	AM	AM	AM	AC	AM	AM	AC	AM
7. Ratio between length of petal and sepal	PL	PE	PS	PE	PS	PS	PS	PL	PS	PS	PL	PL	PL	PL	PS	PS	PL	PE	PL	PS	PL	PL	PL
8. Rachis length (mm)	10	17	30	16	22	40	36	9	50	5	7	4	25	2	7	15	11	6	15	16	3	6	11
9. Petiole length (mm)	20	32	45	15	35	40	30	19	30	15	16	10	33	15	30	25	25	21	25	25	14	20	22
10. Thorns	TR	TR	TR	TR	TR	TB	TR	TR	TR	TR	TR	–	TR	TR	TS	TR	TS	TR	TR	TR	TR	TR	TR
11. Leaf surface	CC	CC	CC	CC	CC	CC	CC	CC	CC	CD	CD	CI	CC	CC	CI	CC	CI	CI	CI	CI	CC	CC	CC
12. Form of leaf apex	AM	AM	AC	AM	AM	AM	AM	AM	AM	AC	AC	AC	AM	AC	AC	AC	AC	AM	AM	AC	AC	AC	AC
13. Leaf margin	LP	LS	LB	LB	LS	LB	LP	LP	LS	LS	LS	LB	LP	LP	LP	LP	LP	LB	LB	LB	LB	LB	LP
14. Form of stipule	SN	SN	SN	SN	SN	SN	SN	SN	SL	SN	SN	SF	SL	SL	SL	SL	SN	SL	SL	SL	SL	SL	SN
15. No. leaflets/leaf in floricane	1–3	3	3	3	3	3	3	3	3–5	3	3	3	3	3	1–3	3	1–3	3	1–3	1–3	1–3	1–3	1–3
16. Primocane leaves	PB	PB	PB	PA	PB	PB	PB	PB	–	PB	PA	PA	PB	PA	–	–	–	PA	PB	–	–	PB	PB
17. Terminal leaf length (mm)	75	80	110	110	84	95	95	46	65	50	40	30	85	50	50	45	65	55	110	110	50	40	40
18. Form of base of terminal leaflet	BT	BN	BO	BO	BO	BO	BO	BO	BO	BO	BO	BC	BO	BN	BO–BN	BT	BT–BO	BC	BO	BC	BN	BC–BT	BT

AC, acute; AM, acuminate; BC, cordate; BN, cuneate; BO, obtuse; BT, truncate; CC, concolourous; CD, discolourous; CI, leaf slightly discolourous; FD, flower double; FS, flower single; IL, long; IN, intermediate; IS, short; LD, double serrate; LR, serrate; LS, serrulate; PA, pinnate; PB, pinnate/palmate; PE, petal = sepal; PI, pink; PL, petal > sepal; PP, pale pink; PS, petal < sepal; PW, white; SF, flabellate; SL, lanceolate, triangular or falcate; SN, needle, linear or filiform; TB, some thorns recurved and others straight; TR, recurved; TS, straight.

TABLE 2.—Average chiasma frequencies and average percentage of chromosome associations per polyploid level in the parental *Rubus* species and their putative hybrid

		R. cuneifolius B.		*R. longepedicellatus*			*R.* × *proteus*					
Somatic chromosome number		21	28	14	28	35	14	21	28*	28	35	42
Chiasma frequency		0,83	1,05	1,12	1,08	0,99	1,1	0,88	0,45	1,12	0,98	1,21
Chromosome Associations	I	30,98	5,30	2,14	1,34	14,90	–	29,76	55,40	1,50	13,79	4,40
	II	58,45	80,47	97,90	97,92	62,60	100	56,67	44,60	88,30	81,28	68,60
	III	10,72	5,09	–	–	14,60	–	13,57	–	4,03	3,22	14,60
	IV	–	9,14	–	0,70	8,00	–	–	–	6,17	1,72	12,40

* These frequencies are representative of *Stirton 9798* and are not included in the averages because this specimen deviates substantially from the other specimens.

In the putative hybrid, *R.* × *proteus*, a mixture of reproductive systems seems to operate in the specimens. Only reduced embryo sacs were observed in the diploid (*Henderson & Gaum 28*) and one tetraploid specimen (*Henderson & Gaum 27*), whereas a mixture of reduced and aposporic embryo sacs was observed in the remaining specimens (*Henderson & Gaum 20* and *31* and *Stirton 8135, 9798, 9865, 9866* and *9869*). Vegetative reproduction through stemtip-rooting and/or rhizomes was observed in the specimens studied.

Chromosome behaviour

Both putative parental species contain specimens on different polyploid levels. *R. cuneifolius* B has somatic chromosome numbers of 21 and 28 and *R. longepedicellatus* 14, 28 and 35, whereas their presumed hybrid, *R.* × *proteus*, has somatic chromosome numbers of 14, 21, 28, 35, 42, 49 and 56 (Spies & Du Plessis 1985; Spies *et al.* 1985).

The meiotic chromosome behaviour observed in *R. cuneifolius* B differs in some respects from that of the comparable ploidy level of *R. longepedicellatus* (Spies *et al.* 1985). The diploid *R. longepedicellatus (Henderson & Gaum 22)* specimen has a chiasma frequency of 1,12 per bivalent and that of the putative hybrid diploid specimen is similar, namely 1,1 (Table 2). Both diploid specimens usually formed bivalents, with the exception of two univalents in one *R. longepedicellatus* cell. The meiotic chromosome configurations in the triploid *R. cuneifolius* B and *R.* × *proteus* specimens were very similar (Table 2). No triploid *R. longepedicellatus* specimen has yet been found.

The method described by Spies (1984) for analysing the meiotic configurations in the pollen mother cells, indicates that the tetraploid *R. cuneifolius* B specimen is a segmental alloploid tending towards autoploidy, whereas the tetraploid *R. longepedicellatus* specimen is a segmental alloploid tending strongly towards alloploidy. Some of the tetraploid *R.* × *proteus* specimens appear to be segmental alloploids tending towards autoploidy (*Henderson & Gaum 27 & 32*), whereas one is probably an alloploid (*Stirton 9798*). The tetraploid *R. rigidus* × *R. cuneifolius* A specimen (*Henderson & Gaum 51*) seems to be a segmental alloploid tending towards autoploidy.

In a tetraploid *R.* × *proteus* specimen, *Stirton 9798*, asynapsis occurred in many pollen mother cells. In this specimen only 44,6 % bivalents were formed, whereas the remaining chromosomes were univalents (Table 2). The pentaploid *R. longepedicellatus* specimen tended to form less bivalents than the *R.* × *proteus* specimen. No higher ploidy levels than pentaploid were found in the parental species and comparison with the hexaploid *R.* × *proteus* specimens was, therefore, not possible. However, a surprisingly high frequency of multivalents (14,05 %) was observed in the higher ploidy levels of *R.* × *proteus* (Table 2).

The genome analysis indicated that there is no difference between the 2:1 and 3:0 models of Alonso & Kimber (1981), because the x-values in the 2:1 model were 0,5 for each triploid specimen, indicating that the two more closely related genomes are also closely related to the third genome. The model with 0–2 chiasmata of Jackson & Casey (1982) produced the same expected values as those obtained by using Kimber's models (Table 3). In all the specimens studied the average sum of squares increased from the 0–2 chiasmata model of Jackson & Casey (1982) to the 0–4 chiasmata model, indicating that the specimens studied have two or less chiasmata per chromosome pair (Table 4).

The genome analysis further indicated that the 2:2 model of Kimber & Alonso (1981) shows the best correspondence with the observed frequencies of chromosome associations in all the tetraploid *R. cuneifolius* B, *R. longepedicellatus* and *R.* × *proteus* specimens studied (Table 5). In each case the value of x was 1, indicating that two genomes are much more closely related to one another than to one of the other two genomes. The only exceptions were *Henderson & Gaum 93* (*R. cuneifolius* A) and *Stirton 9798 (R.* × *proteus)* in which the 3: 1 model fitted with x-values respectively of 0,5 and 0,501, indicating that the three closely related genomes have also a great affinity for the other genome. In both these cases the average sum of squares of the expected and observed frequencies of the accepted model varied very little from that of the 4:0 models.

In contrast to this phenomenon the model described by Jackson & Casey (1982) indicates that all the specimens

TABLE 3.—Comparison between observed chromosome configurations and the expected chromosome configurations in triploids according to the methods described by Alonso & Kimber (1981), Jackson & Casey (1980, 1982) and Jackson & Hauber (1982). Only the model with the lowest average sum of squares is given in this table

		Chromosome configuration						
		I	IIC	IIR	III	SS	X	C
R. cuneifolius A (*Liengme s.n.*)	O	6,32	6,07	0,25	0,68	–	–	–
	2:1	8,13	3,44	0,75	1,5	2,78	0,57	0,50
	0–2	8,13	3,44	0,75	1,5	2,78	–	–
R. cuneifolius B (*Stirton 9800*)	O	6,40	5,80	0,31	0,80	–	–	–
	2:1	8,02	3,43	0,77	1,53	2,25	0,50	0,57
	0–2	8,02	3,43	0,77	1,53	2,25	–	–
R. cuneifolius B (*Henderson & Gaum 18*)	O	6,57	5,38	0,79	0,70	–	–	–
	2:1	7,61	3,37	0,83	1,66	1,51	0,50	0,60
	0–2	7,61	3,37	0,83	1,66	1,51	–	–
R. × *proteus* (*Stirton 9866*)	O	6,25	5,95	0	0,95	–	–	–
	2:1	8,23	3,45	0,73	1,47	2,75	0,50	0,56
	0–2	8,23	3,45	0,73	1,47	2,75	–	–

O = observed frequency; 2:1 = Kimber's model where 2 genomes are more closely related to one another than to the third genome; 0–2 = Jackson's model where 0 to 2 chiasmata per bivalent are formed; I = univalents; IIC = rod bivalent; IIR = ring bivalent; III = trivalent; SS = average sum of squares of differences between observed and expected frequencies; X = value indicating the relative distance between the two homologous genomes and the third genome according to Kimber's models; C = chiasma frequency per half bivalent.

TABLE 4.—Comparison between the average sum of squares between the observed and expected values for chromosome configuration in triploids for different numbers of chiasmata according to Jackson's model (Jackson & Casey 1980, 1982; Jackson & Hauber 1982)

Species	Voucher No.	No. of chiasmata		
		0–2	0–3	0–4
R. cuneifolius A	*Liengme s.n.*	2,78	5,64	11,66
R. cuneifolius B	*Stirton 9800*	2,25	5,10	11,28
R. cuneifolius B	*Henderson & Gaum 18*	1,51	5,54	12,06
R. × *proteus*	*Stirton 9866*	2,75	5,00	10,59

studied are autotetraploids with 0–2 chiasmata per chromosome pair and with partly random chromosome associations. The model of Spies (1984) indicates that all the specimens are segmental alloploids but they vary from almost autoploid (*Henderson & Gaum 27, 51, 93* and *Stirton 9868*) to almost alloploid (*Henderson & Gaum 14, 32, Stirton 9798, 9861 & 9862*).

DISCUSSION

Morphology

Different methods can be used to ascertain whether a given specimen represents a true species or a hybrid. During this study several of these methods were used to determine the degree of hybridization in the genus *Rubus*. The first method used was based on morphological characters and in this process a hybrid index was determined and a scatter diagram constructed.

A study of morphological characters revealed that *R. cuneifolius* B has a short to medium length inflorescence (Table 1:1) with white flowers, whereas *R. longepedicellatus* has a medium to long inflorescence with pink flowers (2 & 3). *R.* × *proteus* has a short to long inflorescence with pink, pale pink or white flowers. The petal length (4) varied from 13 to 20 mm in *R. cuneifolius* B, from 4 to 10 mm in *R. longepedicellatus* and from 4 to 15 mm in *R.* × *proteus*. The petal width (5) varies from 7 to 15 mm in *R. cuneifolius* B, from 3 to 6 mm in *R. longepedicellatus* and from 3 to 11 mm in *R.* × *proteus*. The same intermediate arrangement position is observed when the ratio between the lengths of the petals and sepals (7) is compared; in *R. cuneifolius* B the petal is always longer than the sepal, whereas in *R. longepedicellatus* the petal is as long or shorter than the sepal and *R.* × *proteus* has the whole range of ratios. *R. cuneifolius* B, has acute petal apices compared to the acuminate apices with an occasional acute apex in *R. longepedicellatus* and both acute and acuminate apices found in *R.* × *proteus*. The leaf apex (12) is always acute in *R. cuneifolius* B and the leaf margin (13) is usually serrate with a double serrate margin in exceptional cases. *R. longepedicellatus* and *R.* × *proteus* have acute or acuminate leaf apices and serrulate, double serrate or serrate leaf margins. The stipules (14) vary from lanceolate/triangular/falcate to flabellate in *R. cuneifolius* B, from needle/linear/filiform to occasionally lanceolate/triangular/falcate in *R. longepedicellatus*, with all these different shapes being represented in *R.* × *proteus*. In contrast to the pinnate/palmate leaves on the floricanes of *R. cuneifolius* B, *R. longepedicellatus* has pinnate leaves and both forms occur in *R.* × *proteus*. These morphological data indicate that *R. cuneifolius* B and *R. longepedicellatus* are morphologically separate species, and the intermediate nature of the *R.* × *proteus* specimens suggests a hybrid origin.

The hybrid index diagram (Figure 1) indicates that only one specimen had all the characters associated with *R. longepedicellatus*, whereas four specimens had all the characters associated with *R. cuneifolius* B. The hybrid index also indicates that *R. longepedicellatus* and *R. cuneifolius* B are clearly separated morphologically. However, a continuous bridge of morphological characters spans the gap between them in the form of the very variable hybrid species, *R.* × *proteus* (Figures 1, 2, 3 &

TABLE 5.—Comparison between observed chromosome configurations and the expected chromosome configurations in tetraploids according to the methods described by Kimber & Alonso (1981), Jackson & Casey (1980, 1982) and Jackson & Hauber (1982). Only the two models with the lowest average sum of squares of each method are shown in the table

		Chromosome configuration								
		I	IIC	IIR	III	IVC	IVR	SS	X	C
R. affinis (*Stirton 5746*)	O	0,21	9,42	1,29	0,21	0,80	0,63	–	–	–
	4:0	4,49	3,15	1,87	1,33	1,69	0	10,06	–	0,62
	3:1	4,50	3,05	1,78	1,33	1,69	0,69	10,22	0,50	0,62
	0–2	3,54	4,39	1,53	1,04	1,69	0,69	6,31	–	–
	0–2R	4,06	3,88	1,79	1,04	1,69	0,69	7,88	–	–
R. cuneifolius A (*Henderson & Gaum 93*)	O	2,30	6,76	0,74	1,30	0,75	0,95	–	–	–
	4:0	4,85	3,26	1,78	1,38	1,63	0	3,59	–	0,60
	3:1	4,87	3,16	1,69	1,37	1,63	0,62	3,55	0,50	0,60
	0–2	3,87	4,54	1,43	1,07	1,63	0,62	1,47	–	–
	0–2R	4,41	4,01	1,70	1,07	1,63	0,62	2,31	–	–
R. cuneifolius B (*Stirton 9861*)	O	1,25	11,88	0,17	0,35	0,40	–	–	–	–
	4:0	7,37	3,82	1,28	1,50	1,19	0,62	17,66	–	0,50
	2:2	6,88	7,00	3,56	0	0	0	14,96	1,00	0,50
	0–2	6,30	5,23	0,90	1,17	1,19	0,30	11,93	–	–
	0–2R	6,88	4,65	1,19	1,07	1,19	0,30	14,41	–	–
R. cuneifolius B (*Stirton 9868*)	O	1,72	9,92	0,56	0,60	0,62	0,26	–	–	–
	4:0	6,39	3,63	1,45	1,48	1,36	0,30	10,57	–	0,54
	2:2	5,91	6,95	4,09	0	0	0	9,90	1,00	0,54
	0–2	5,33	5,02	1,08	1,15	1,36	0,40	6,36	–	–
	0–2R	5,91	4,45	1,36	1,15	1,36	0,40	8,17	–	–
R. flagellaris (*Henderson & Gaum 2*)	O	0,10	12,85	1,10	0	0	0	–	–	–
	4:0	6,47	3,65	1,44	1,48	1,34	0,40	21,58	–	0,54
	2:2	5,99	6,96	4,04	0	0	0	17,33	1,00	0,54
	0–2	5,41	5,04	1,06	1,15	1,34	0,39	15,41	–	–
	0–2R	5,99	4,47	1,35	1,15	1,34	0,39	18,05	–	–
R. apetalus (*G. Hemm s.n.*)	O	0	7,87	3,25	0	0,46	0,99	–	–	–
	4:0	2,78	2,54	2,38	1,04	1,93	0,39	6,75	–	0,70
	2:1:1	3,19	2,76	4,14	0,85	1,37	0,69	6,46	0,86	0,70
	0–2	2,05	3,50	2,11	0,81	1,93	1,15	4,57	–	–
	0–2R	2,45	3,10	2,31	0,81	1,93	1,15	5,42	–	–
R. apetalus (*Henderson & Gaum 6*)	O	0,35	10,93	1,27	0,35	0,33	0,22	–	–	–
	4:0	5,57	3,45	1,62	1,44	1,50	1,15	14,47	–	0,57
	2:2	5,11	6,85	4,59	0	0	0	12,13	1,00	0,57
	0–2	4,55	4,79	1,25	1,12	1,50	0,50	9,56	–	–
	0–2R	5,11	4,23	1,53	1,12	1,50	0,50	11,60	–	–
R. apetalus (*Wells 5000*)	O	0	13,15	0,85	0	0	0	–	–	–
	4:0	6,66	3,69	1,40	1,49	1,31	0,50	23,06	–	0,53
	2:2	6,18	6,97	3,94	0	0	0	18,63	1,00	0,53
	0–2	5,60	5,09	1,02	1,16	1,31	0,37	16,59	–	–
	0–2R	6,18	4,51	1,31	1,16	1,31	0,37	19,37	–	–
R. longepedicellatus (*Henderson & Gaum 14*)	O	0,60	12,85	0,65	0	0,10	0	–	–	–
	4:0	7,05	3,76	1,33	1,50	1,24	0	21,35	–	0,52
	2:2	6,56	6,99	3,73	0	0	0	17,55	1,00	0,52
	0–2	5,98	5,17	0,95	1,16	1,24	0,33	15,13	–	–
	0–2R	6,56	4,59	1,24	1,16	1,24	0,33	17,81	–	–
R. longepedicellatus (*Stirton 9862*)	O	0,16	12,28	1,64	0	0	0	–	–	–
	4:0	6,00	3,55	1,53	1,46	1,43	0,33	19,10	–	0,56
	2:2	5,53	6,91	4,32	0	0	0	15,10	1,00	0,56
	0–2	4,96	4,92	1,16	1,14	1,42	0,45	13,49	–	–
	0–2R	5,53	4,35	1,44	1,14	1,42	0,45	15,87	–	–

O = observed frequency; 4:0 = Kimber's model where all 4 genomes are homologous; 3:1 = Kimber's model where 3 genomes are more closely related to one another than to the fourth genome; 2:2 = Kimber's model where 2 genomes are more closely related to one another than to any of the other two genomes, which are also related to one another; 2:1:1 = Kimber's model where 2 genomes are more closely related to one another than to the third genome and the third and fourth genomes are not closely related; 0–2 = Jackson's model where 0 to 2 chiasmata per bivalent are partially randomly formed; 0–2R = Jackson's model where 0 to 2 chiasmata per bivalent are randomly formed; I = univalents; IIC = rod bivalent; IIR = ring bivalent; III = trivalent; IVC = rod quadrivalents; IVR = ring quadrivalents; SS = average sum of squares of differences between observed and expected frequencies; X = value indicating the relative distance between the different genomes according to Kimber's models; C = chiasma frequency per half bivalent.

TABLE 5.—Comparison between observed chromosome configurations and the expected chromosome configurations in tetraploids according to the methods described by Kimber & Alonso (1981), Jackson & Casey (1980, 1982) and Jackson & Hauber (1982). Only the two models with the lowest average sum of squares of each method are shown in the table (continued)

		Chromosome configuration								
		I	IIC	IIR	III	IVC	IVR	SS	X	C
R. pinnatus (*Arnold 1335*)	O	0	9,98	1,86	0	0,60	0,48	–	–	–
	4:0	4,42	3,13	1,89	1,33	1,70	0,45	11,57	–	0,62
	2:2	4,00	6,58	5,42	0	0	0	11,00	1,00	0,62
	0–2	3,48	4,37	1,55	1,03	1,70	0,70	7,67	–	–
	0–2R	4,00	3,85	1,81	1,03	1,70	0,70	9,31	–	–
R. × *proteus* (*Stirton 9798*)	O	15,50	6,25	0	0	0	0	–	–	–
	2:1:1	17,14	3,69	0,28	0,72	0,16	0,01	1,65	0,583	0,22
	3:1	17,14	3,72	0,26	0,72	0,16	0,01	1,61	0,501	0,22
	0–2	16,61	4,41	0,09	0,56	0,16	0,01	0,83	–	–
	0–2R	16,90	4,13	0,23	0,56	0,16	0,01	1,14	–	–
R. × *proteus* (*Henderson & Gaum 27*)	O	0,70	11,10	1	0,70	0,10	0,15	–	–	–
	4:0	6,15	3,58	1,50	1,47	1,40	0	14,79	–	0,55
	2:2	5,67	6,93	4,24	0	0	0	11,97	1,00	0,55
	0–2	5,10	4,96	1,13	1,14	1,40	0,43	9,84	–	–
	0–2R	5,67	4,39	1,41	1,14	1,40	0,43	11,98	–	–
R. × *proteus* (*Henderson & Gaum 32*)	O	0,45	12,15	1,40	0,15	0	0	–	–	–
	4:0	6,29	3,61	1,47	1,48	1,37	0,43	18,46	–	0,54
	2:2	5,81	6,94	4,15	0	0	0	14,63	1,00	0,54
	0–2	5,23	5,00	1,10	1,15	1,37	0,41	12,86	–	–
	0–2R	5,81	4,42	1,38	1,15	1,37	0,41	15,24	–	–
R. × *proteus* (*Henderson & Gaum 51*)	O	0,12	10,03	1,41	0,28	0,10	0,94	–	–	–
	4:0	4,38	3,12	1,90	1,32	1,71	0,41	11,69	–	0,62
	2:2	3,96	6,57	5,45	0	0	0	11,07	1,00	0,62
	0–2	3,45	4,35	1,56	1,03	1,71	0,71	7,75	–	–
	0–2R	3,96	3,84	1,82	1,03	1,71	0,71	9,41	–	–
R. transvaliensis × *R. longepedicellatus* (*Henderson & Gaum 10*)	O	0	13,05	0,95	0	0	0	–	–	–
	4:0	6,57	3,67	1,42	1,49	1,33	0,71	22,63	–	0,53
	2:2	6,09	6,97	3,99	0	0	0	18,20	1,00	0,53
	0–2	5,50	5,07	1,04	1,16	1,32	0,38	16,22	–	–
	0–2R	6,08	4,49	1,33	1,16	1,32	0,38	18,95	–	–
Rubus sp. (*Henderson & Gaum 24*)	O	2,24	7,47	0,52	0,65	1,45	0,50	–	–	–
	4:0	5,47	3,42	1,64	1,43	1,52	0,38	4,78	–	0,58
	3:1	5,48	3,33	1,56	1,43	1,52	0,52	4,89	0,50	0,58
	0–2	4,45	4,76	1,28	1,11	1,52	0,52	2,17	–	–
	0–2R	5,01	4,20	1,55	1,11	1,52	0,52	3,27	–	–

O = observed frequency; 4:0 = Kimber's model where all 4 genomes are homologous; 3:1 = Kimber's model where 3 genomes are more closely related to one another than to the fourth genome; 2:2 = Kimber's model where 2 genomes are more closely related to one another than to any of the other two genomes, which are also related to one another; 2:1:1 = Kimber's model where 2 genomes are more closely related to one another than to the third genome and the third and fourth genomes are not closely related; 0–2 = Jackson's model where 0 to 2 chiasmata per bivalent are partially randomly formed; 0–2R = Jackson's model where 0 to 2 chiasmata per bivalent are randomly formed; I = univalents; IIC = rod bivalent; IIR = ring bivalent; III = trivalent; IVC = rod quadrivalents; IVR = ring quadrivalents; SS = average sum of squares of differences between observed and expected frequencies; X = value indicating the relative distance between the different genomes according to Kimber's models; C = chiasma frequency per half bivalent.

4). It is also indicated that the hybrid species overlaps morphologically with both parental species. The five major characters described above (i.e. flower colour, petal and rachis lengths, ratio between length of petal and sepal and whether the primocane leaves are pinnate or pinnate/palmate) are, therefore, essential for distinguishing between the true species and the different hybrids.

The pictorialized scatter diagram (Figure 2) indicates that more hybrid specimens overlap with *R. longepedicellatus* than with *R. cuneifolius* B. *R. longepedicellatus* is completely surrounded by *R.* × *proteus* specimens in this diagram. Distinguishing between them will, therefore, be more difficult than between *R. cuneifolius* B and *R.* × *proteus*.

It is evident from these two diagrams that *R. cuneifolius* B and *R. longepedicellatus* represent the two extremes of a very variable population of plants (Figure 3). It is further evident that *R.* × *proteus*, which constitutes the morphologically intermediate population (Figure 4), resulted from hybridization between *R. cuneifolius* B and *R. longepedicellatus* and subsequent backcrosses and intercrosses to produce a continously variable hybrid swarm.

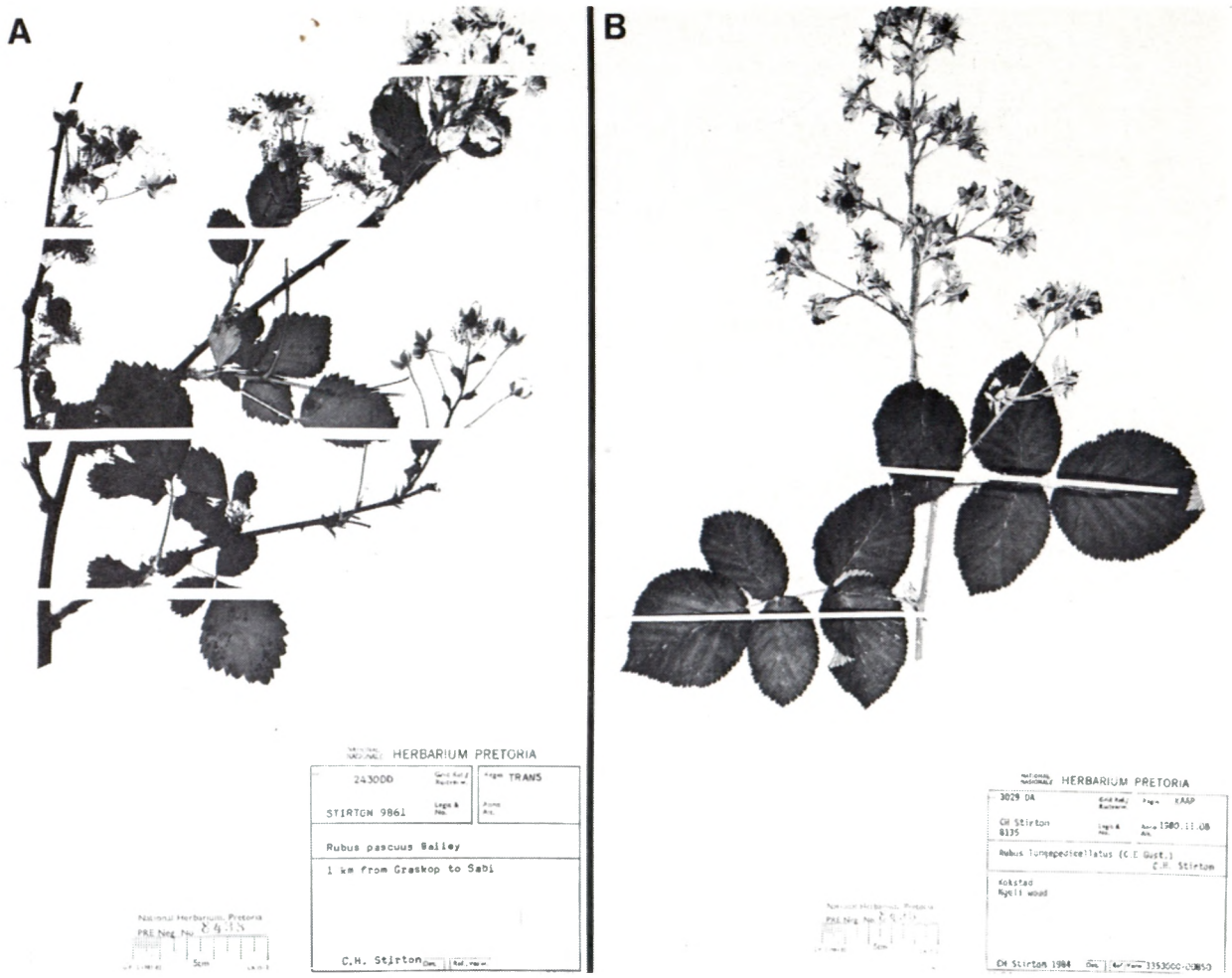

FIGURE 3.—Specimens of A, *Rubus cuneifolius* B (*Stirton 9861*); B, *R. longepedicellatus (Stirton 8135)*.

The above hybridization hypothesis is also supported by the geographical distribution of the species concerned. *R. cuneifolius* B is restricted to the Transvaal, whereas *R. longepedicellatus* specimens were collected in the Transvaal and Natal, with the majority of them collected in the Transvaal. The hybrids are restricted to the Transvaal. The low frequency of *R.* × *proteus* and *R. longepedicellatus* specimens from Natal in the collection may be attributed to an insufficient number of *Rubus* collections from Natal. The absence of *R. cuneifolius* B specimens from Natal in the National Herbarium may be due to inadequate collecting or to its non-occurrence in this province. If the latter is true, the paucity of *R.* × *proteus* specimens from Natal is explained. The specimen resembling *R.* × *proteus (Henderson & Gaum 51)* from Natal rather represents a hybrid between *R. cuneifolius* A and *R. rigidus* than *R.* × *proteus* itself. The morphological differences between *R. cuneifolius* B and *R. cuneifolius* A are very slight and hybrids between any one of these taxa and *R. longepedicellatus* will result in morphologically similar hybrids. The only differences observed between these taxa are small differences in the leaf texture and leaf margin, as well as the frequent occurrence of double flowers in *R. cuneifolius* B. No *R. cuneifolius* A specimen with double flowers was observed. Since all *R.* × *proteus* specimens have single flowers, it is possible that *R. cuneifolius* A and B are interchangeable as parents with *R. longepedicellatus*.

Reproductive system

The embryo sac study indicated that both putative parents produce reduced embryo sacs and may, therefore, participate in hybridization. It was further demonstrated that a number of hybrids also produced reduced embryo sacs and so backcrossing to either parent is also possible. In addition to reduced reproduction all hybrid specimens had the potential to reproduce asexually, either through agamospermy or vegetatively. This apomictic reproduction provides all plants with the potential to reproduce even when meiotic chromosome pairing fails after interspecific hybridization. Although the embryo sac study cannot prove the occurrence of hybridization, it indicates that hybridization is possible and that interspecific hybrids may either reproduce sexually or perpetuate themselves apomictically.

Chromosome behaviour

The somatic chromosome numbers of 21 and 28 in *R. cuneifolius* B and 14, 28 and 35 in *R. longepedicellatus* (Table 2) seem to contradict hybridization because, although a diploid hybrid specimen exists, no diploid *R. cuneifolius* B specimen has yet been observed. However, the occurrence of triploid *R. cuneifolius* B specimens with meiotic chromosome behaviour resembling autoploids, suggests that these triploids are formed by pollination of autotetraploids by diploids, both containing

FIGURE 4.—Specimens of *Rubus* × *proteus*. A, *Stirton 9799*; B, *Stirton 9869*; C, *Stirton 5783*; D, *Henderson & Gaum 20*.

similar genomes. Therefore, it is suggested that diploid *R. cuneifolius* B specimens do exist and that they could have hybridized with diploid *R. longepedicellatus* specimens to form diploid hybrids. The occurrence of a diploid hybrid *R.* × *proteus* specimen (*Henderson & Gaum 28*), with normal chromosome pairing during meiosis (Table 2), indicates that the genomic differences between the parental species are insignificant. The two diploid parents of *R.* × *proteus* probably differ only in a few gene loci and as such must be considered varieties of the same species.

This homology between the genomes of *R. cuneifolius* B and *R. longepedicellatus* is also manifested at higher ploidy levels. However, differences in the meiotic chromosome behaviour of polyploid *R.* × *proteus* specimens was observed. These differences include a variation in chromosome pairing from the multivalent formation expected in autoploids to that expected in alloploids. These differences can be attributed to either pre- or post-hybridization chromosomal evolution.

Pre-hybridization chromosomal evolution would suggest that structural chromosome differences were present in some plants of the parental populations. Hybridization between such plants followed by polyploidization would give rise to segmental alloploids with meiotic chromosome pairing resembling that of alloploids. The normal meiosis found in a diploid hybrid specimen (*Henderson & Gaum 28*), indicates that only very small structural differences exist at the diploid level between the genomes of at least some plants of the parental taxa.

The results of Spies *et al.* (1985) indicate that the polyploids of *R. cuneifolius* B may have had an autoploid origin in contrast to the presumed segmental alloploid origin of *R. longepedicellatus* polyploids. The morphological similarity between the diploid and the segmental alloploids of *R. longepedicellatus* indicates that the structural chromosome changes in a genome were not accompanied by gene mutations which could produce morphological changes. The differences in chromosome pairing observed in different *R.* × *proteus* specimens at higher ploidy levels (Tables 3 & 5), might consequently be attributed to repeated hybridization between different *R. cuneifolius* B and *R. longepedicellatus* plants which differ in their structural chromosome changes.

Post-hybridization chromosomal evolution is due to structural changes in some chromosomes after hybridization. The occurrence of multivalents tends to increase meiotic instability and to lower fertility. Chromosome changes that will inhibit multivalent formation will, therefore, have a selective advantage due to the increased number of bivalents and the consequent increase in seed viability. These changes form part of the diploidization process. Different *R.* × *proteus* specimens may, therefore, represent different stages of diploidization and their meiotic chromosome pairing may consequently differ. However, the post-hybridization hypothesis only provides for autopolyploidization, whereas the pre-hybridization chromosome evolution hypothesis allows repeated hybridization between different ploidy levels or between plants at the same ploidy level but with different genomic constitutions. The pre-hibridization hypothesis is also supported by the greater morphological variation in *R. longepedicellatus* when compared with *R. cuneifolius* B. This larger morphological variation might be the result of the segmental alloploid origin of the *R. longepedicellatus* polyploids.

Other interspecific hybrids and intersubgeneric hybrids have been described in the literature (See discussion under hybridization). In addition to the examples cited in the literature, the hybrid origin of certain taxa was inferred from their meiotic chromosome pairing. These taxa include *R. cuneifolius* A, *R. flagellaris, R. apetalus* and *R. pinnatus*. Chromosome pairing indicated that *R. flagellaris, R. apetalus (Henderson & Gaum 6* and *Wells 5000*) and *R. pinnatus* are true alloploids; the 2:2 model of Kimber & Alonso (1981) was applicable and an x-value of 1 was obtained (Table 5). The tetraploid *R. cuneifolius* A specimen tends towards autoploidy, because the 3:1 model was applicable and the reduced x-value of 0,5 implied an affinity between the two sets of genomes. The other *R. apetalus* specimen, *G. Hemm s.n.*, conforms with the 2:1:1 model and has an x-value of 0,86. A specimen that appears to be an amphiploid between *R. transvaliensis* and *R. longepedicellatus* had an x-value of 1 when the 2:2 model was applied. No indication of a hybrid origin could be found for *R. affinis*, where the 4:0 model of Kimber & Alonso (1981) was applicable.

The results obtained by using the method described by Alonso & Kimber (1981) and Kimber & Alonso (1981) to a certain extent correspond with the results obtained by using the method described by Spies (1984). According to the latter method no specimens are true autoploids and *Arnold 1335, G. Hemm s.n., Henderson & Gaum 6, 14, 24, 27, 32, 51, 93, Stirton 5746, 9861* and *9868* are segmental alloploids and *Henderson & Gaum 2, 10, Stirton 9798, 9862* and *Wells 5000* are alloploids (Spies *et al.* 1985). The method of Spies (1984) further distinguishes between the segmental alloploids and indicates that *Arnold 1335, Henderson & Gaum 14, 32* and *Stirton 9861* tend towards alloploidy, whereas *Henderson & Gaum 24, 27, 51, 93, Stirton 5746* and *9868* tend towards autoploidy. The rest of the specimens are intermediate segmental alloploids.

In contrast to the methods described by Alonso & Kimber (1981) and Kimber & Alonso (1981) and Spies (1984) the method described by Jackson & Casey (1982) and Jackson & Hauber (1982) suggests that all the plants are autoploids with partial random chromosome associations and 0–2 chiasmata per chromosome pair (Table 5). The reason why the latter method did not distinguish between different chromosomes in the specimens studied is that the initial assumption of the method, that the formation of chiasmata is random, does not apply in the genus *Rubus*. From random chiasma formation and a maximum of two chiasmata per chromosome pair, frequencies of 0,25, 0,5 and 0,25 are expected for chromosome pairs with no chiasmata, one chiasmata and two chiasmata respectively. In the genus *Rubus* these figures are 0,08, 0,79 and 0,13. This deviation from the expected values indicates that this method is not applicable in the genus *Rubus*.

Hybridization

Hybridization in the genus *Rubus* is a topic as controversial as the taxonomy of the genus. Taxonomists

usually adhere to one of two extremes. Either every entity not fitting the species description exactly is regarded as a hybrid, or the occurrence of hybrids in the genus is totally ignored.

Bailey (1941–1945) described over 500 different species of *Rubus* for North America without the recognition of hybrids. He considered three points as essential for hybridization:

(1) both parents must be in the vicinity of the hybrid;

(2) hybrids occur in small numbers as incidental or as exceptions to the main population and

(3) characters appear to belong to the parents in various degrees of combinations.

We support Bailey in his plea that all unidentifiable examples should not be regarded as hybrids. However, the validity of his three criteria for hybridization must be discussed before any conclusions can be made. His claim that both parents must be in the vicinity of the hybrid was usually fulfilled in the present study as the hybrids and the parental taxa often occurred together. However, hybrids were sometimes found with no parental form in the vicinity. This phenomenon may be attributed to one or more of several factors. Pollination by insects over large distances might occur and in such cases only the maternal parent need be in the vicinity. Seed could also have been transported from the mother plant by birds or man, dropping it far from the parental forms. This may be a common means of dispersal in southern Africa as the fruits of *Rubus* are relished by birds and man. One or both parents may die and only the hybrid may survive, especially in a weedy taxon like *Rubus* where hybrids might be very aggressive. Only one or neither parent need therefore be in the vicinity of the hybrid. The first of Bailey's criteria for hybridization is therefore invalid.

The second criterion claims that hybrids occur in small numbers as incidental or exceptions to the main population. This will be valid only for newly formed hybrids or weakly developed hybrids or species which have good barriers against hybridization. *Rubus* hybrids are often aggressive (Bammi 1964) and, due to hybrid vigour, they may exceed their parents and could become more abundant than either parental taxon. This is definitely the case with *R.* × *proteus* in the Graskop and Sabie areas of the Transvaal where the hybrids are exceptionally vigorous and are more abundant than the putative parents.

Characters do not have to be intermediate in the hybrids. They may exceed the ranges of both parents, new traits may be present in the hybrid or the traits of one parent may be absent in the hybrids due to dominance or epistasis. An example of the hybrid's trait exceeding that of its parents is found in the *R. trifidus* × *R. hirsutus* hybrid which has a larger flower diameter than either parent (Jinno 1957). In the present study it was observed that some hybrid specimens had longer rachises than either parent.

The three criteria for the determination of hybridity described by Bailey are, consequently, not always valid. These criteria are all based on morphological characters. Therefore, cytogenetic studies seem to be the only positive way of identifying hybrids. However, even this field is beset with problems and must be handled with extreme care to obtain meaningful results. This is illustrated by the different results obtained when using the different methods described for analysing genome homology.

The consequences of hybridization in *Rubus* described in this paper are not restricted to the South African material. Interspecific and even intersubgeneric hybridization, giving rise to progeny that varies from completely fertile to totally infertile, has been described elsewhere (Crane & Darlington 1927; Crane & Thomas 1949; Heslop-Harrison 1953; Jinno 1955, 1957, 1958, 1959, 1961, 1963; Britton & Hull 1959; Haskell & Tun 1961; Thompson 1961; Bammi 1964; Naruhashi 1971, 1976, 1979; Naruhashi & Masaki 1980).

Morphological, reproductive and cytogenetic evidence indicates that hybridization does occur in the South African *Rubus* complex. Futhermore hybridization appears to take place on both the present taxonomic intrasubgeneric and intersubgeneric levels. The progeny derived from certain intersubgeneric hybridizations are fertile (Jinno 1958; Newton 1975).

Taxonomic implications of hybridization

In general, F_1 hybrids and their offspring cannot be considered to be separate species because they are sterile due to the failure of normal chromosome pairing during the meiotic process of sporogenesis. However, when hybridization is associated with, or followed by chromosome doubling, amphiploids are produced with normal chromosome pairing and good fertility. These new self-reproducing entities may be regarded as new species (Davis 1958) because the amphiploids are reproductively isolated from their parents. In the event of hybridization resulting in apomixis, each apomictic hybrid might represent a different genotypic combination of the sexual parents and a multitude of different self-reproducing entities can be formed. An increase in the degree of heterozygosity of the parental forms will result in an increase in the number of different recombinant entities. This array of apomictic self-reproducing entities, which are morphologically different from each other and genetically isolated, may on superficial study be regarded as separate species or microspecies. It is, however, unpractical to consider each of these apomictic hybrids as separate species, even if only obligate apomixis exists. In fact, they belong to an agamic complex without species boundaries which rests on pillars of sexual diploid (and polyploid) species. Only cytogenetical studies can distinguish between the true sexual species and the array of apomicts forming the agamic complex.

The fact that many *Rubus* species are restricted to very small geographical areas (Bailey 1941–1945; Davis & Davis 1951) could indicate that they represent either newly formed species or the abovementioned amphiploid apomicts. Apomixis is restricted to a small number of *Rubus* specimens in South Africa, i.e. the subgenus *Eubatus*. The tendency to describe a sexual hybrid as a separate species is frequently encountered in this genus. As an example the diploid species *R. toyorensis* and *R. nishimuranus* can be cited (Jinno 1957; Naruhashi 1971). The F_1 hybrid between the diploid species *R. trifidus* and *R. hirsutus* is regarded as a separate species, *R. toyorensis*, and the backcross of *R. toyorensis* to one of its parents is regarded as *R. nishimuranus*. In our opinion many of the described *Rubus* 'species' are only

hybrids. This has resulted in a totally artificial classification of the genus *Rubus*, where different morphological entities are regarded as separate biological species.

An example of the hybridization between different morphological 'species' is found in the species *R. apetalus* Poir., *R. exsuccus* Steud., *R. adolfi-friederici* Engl. and *R. ecklonii* Focke. Although these four 'species' are morphologically distinct, hybridization among them has produced more intermediate fertile specimens than typical specimens. In our opinion these four species belong to one biological species.

Spontaneous hybridization is less common among the indigenous *Rubus* species of southern Africa. It occurs between *R. rigidus* J. E. Sm. and *R. pinnatus* Willd. wherever these species are sympatric, e.g. *G. Hemm s.n.* in PRE and was described by Focke (1914). Hybridization between indigenous and introduced *Rubus* species is observed much more frequently. Such hybridization takes place between *R. fruticosus* L. agg. and *R. pinnatus* in disturbed areas of the Cape Peninsula (Adamson & Salter 1950). Other examples are *R. cuneifolius* A and *R. pinnatus* in Natal (*G. Hemm s.n.*) and *R. affinis* and *R. rigidus* described by Gustafsson (1933). All these cases involve hybridization between indigenous *Idaeobatus* and introduced *Eubatus* species. No hybrid swarms of any of these examples have been recorded to date.

CONCLUSIONS

The combination of morphological, geographical, reproductive and cytogenetic evidence revealed that natural hybridization occurs in the South African *Rubus* complex and also indicated that the hybridization is not restricted to intrasubgeneric hybridization, but that intersubgeneric hybridization also occurs. The progeny derived from certain intersubgeneric hybridizations are fertile.

The application of the genome analysis method of Kimber & Alonso (1981) on the meiotic data indicated that all the tetraploid plants of *R. cuneifolius* B, *R. flagellaris*, *R. apetalus*, *R. longepedicellatus*, *R. pinnatus* and *R.* × *proteus* have two genomes that are more closely related to each other than to the other two genomes which are also related. This model indicates that all the plants are segmental alloploids with a tendency towards alloploidy. The model of Jackson & Casey (1982), on the other hand, indicates that all the plants are autoploids with partly random chromosome association. Totally different conclusions can, therefore, be drawn from the same meiotic data. Neither of the two models mentioned above distinguishes between any of the specimens studied. However, the chromosome configurations indicate that chromosome pairing varies between the different plants. These differences are accentuated by the model of Spies (1984). It is, therefore, concluded that the latter model is the most applicable for plants with very short chromosomes which have a low chiasma frequency, as is the case in the genus *Rubus* (Spies *et al.* 1985).

Finally, interspecific hybridization in the genus *Rubus*, without loss of fertility in the progeny, indicates that several of the morphological 'species' described in the past, belong to the same biological species. Since the difference in fertility levels between 'intersubgeneric' hybrids and 'interspecific' hybrids is negligible, it was concluded that the present classification of the genus *Rubus* is very artificial and urgently needs a biosystematic revision.

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