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Nectar Sugars in Proteaceae: Patterns and Processes

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Abstract

The nectar sugar composition is presented for 147 species from 16 genera of South African and Australian Proteaceae. Patterns associated with flower age, different plants and populations, plant phylogeny and pollination have been examined. In addition to the usual three nectar sugars (sucrose, fructose and glucose), the nectar of *Protea* and *Faurea* contains the pentose sugar xylose at concentrations of up to 39% of total sugar. Xylose has not previously been reported from floral nectar and is absent from the nectar of *Adenanthos, Banksia, Brabejum, Dryandra, Grevillea, Hakea, Lambertia, Leucospermum, Macadamia, Mimetes, Orothamnus, Paranomus, Stenocarpus* and *Telopea*. Most genera and species have hexose-dominant nectar, but within the large genera *Banksia, Grevillea, Leucospermum* and *Protea* some of the seemingly more derived species have sucrose-dominant nectar. This interesting dichotomy of low versus high sucrose is of diagnostic value at the species level and indicative of phylogenetic relationships within the larger genera. At the generic level, the presence of xylose is a convincing synapomorphy for *Protea* and *Faurea*. Studies of physiological processes (e.g. enzyme activities) and ecological processes (e.g. pollination) may help to explain some of the conservative and taxonomically interesting nectar sugar patterns.

Introduction

Studies of nectar sugar composition have shown that floral nectars have only three major sugars (sucrose, fructose and glucose) and that the ratios between them are of ecological (Baker and Baker 1982, 1983) and taxonomic significance (Van Wyk 1993; Van Wyk *et al*. 1993). Recently, a new major nectar sugar, the pentose sugar xylose, has been found to occur at concentrations of up to 39% of total sugar in two genera of Proteaceae, namely *Protea* and *Faurea* (Van Wyk and Nicolson 1995).

This paper forms part of a general study of nectar sugar composition in various plant families. The aim is to evaluate the taxonomic and/or ecological significance of the ratios between the three main nectar sugars (Van Wyk 1993; Van Wyk *et al*. 1993; Barnes *et al*. 1995). The discovery of xylose as a major nectar sugar in *Protea* and *Faurea* species (Van Wyk and Nicolson 1995) suggested that a wider survey of genera within the Proteaceae may be useful. The various patterns observed in the nectar sugars of Proteaceae are described, with an attempt to offer some explanation of the processes involved. The most important aim is to evaluate the contribution that data on nectar sugars can make towards a better understanding of, firstly, phylogenetic relationships within the family and, secondly, plant–pollinator interactions.

Materials and Methods

Collection of Samples

Nectar samples were taken with micropipettes from cultivated plants in various botanical gardens (see Appendix) and from a few species in their natural habitats. Collecting voucher specimens was considered impractical. All identifications were carefully verified and localities are given in the Appendix. A total of 336 samples was analysed, including 147 species representing 16 genera of African and Australian Proteaceae. Small-flowered genera may be under-represented since their nectars, with small volumes and high viscosity, are more difficult to sample. However, samples were obtained from as many genera as possible.

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Care was taken to sample freshly secreted nectar from newly opened flowers. Where multiple samples from a single species were analysed, these samples were always collected from different plants. Although most samples had to be pooled from different florets within a single inflorescence to obtain sufficient nectar for routine analysis, the floret-to-floret variation was investigated in a few species (see Fig. 2 and Appendix).

Nectar Sugar Analyses

Nectar was sampled as spots (5–15 mm diameter) on filter paper (Whatman no. 1). After air-drying, the papers were stored at –18°C awaiting analysis. Nectar was recovered from the papers by repeated rinsing (3×) with 15 µL to 50 µL distilled water, followed by centrifugation. Samples were analysed by isocratic HPLC operating at 2.5 mL min^{-1}, with a 'Waters Sugarpack' column and acetonitrile : water (87 : 13) as eluent. For detection a refractive index detector was used. The quantities of xylose, fructose, glucose and sucrose were determined as percentages of total sugars, using peak area and 8 mg mL^{-1} of each sugar as external standard. Xylose was identified by optical rotation, melting point, HPLC, and gas chromatography of the trimethylsilyl derivative (Van Wyk and Nicolson 1995).

Results and Discussion

Nectar sugar analyses of samples from 147 species of 16 Proteaceae genera are presented in the Appendix. There are distinct differences between genera and species, which are highlighted in the discussion below. The overall pattern is an interesting dichotomy of low versus high levels of sucrose (i.e. sucrose is either the dominant sugar, or it is present in very small amounts). Most genera and species can be classified as either high sucrose or low sucrose taxa, with very few intermediates. The same dichotomy has been found in *Erica* nectars (Barnes *et al*. 1995). Examples of high sucrose genera are *Paranomus* and *Dryandra*; low sucrose genera are *Mimetes* and *Macadamia*. Note that the large genera (*Leucospermum, Protea, Banksia, Grevillea*) include high and low sucrose species, with relatively few

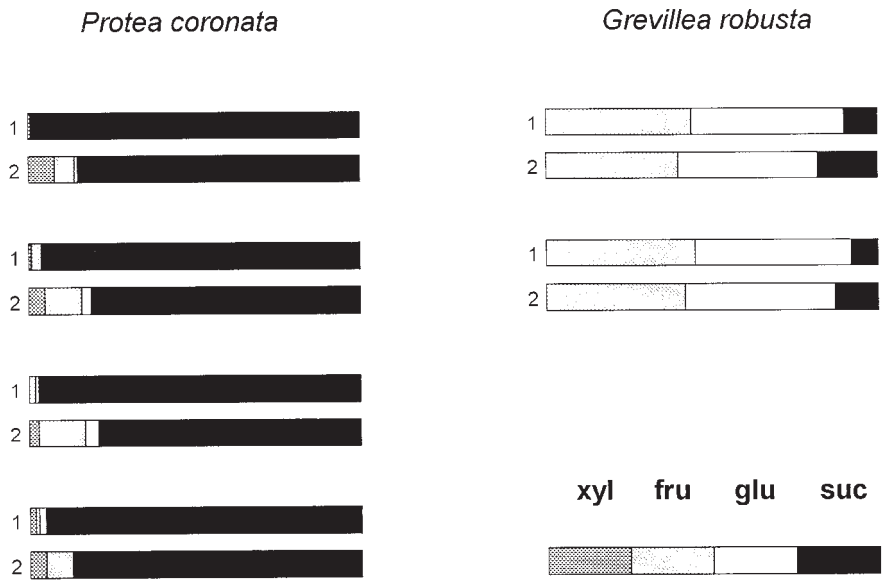

Fig. 1. Effect of flower age on nectar sugar composition in *Protea coronata* and *Grevillea robusta*. *Protea coronata* inflorescences were sampled when freshly picked and again after 2 days in water; data presented for 4 out of 9 inflorescences, showing the range of variation observed. Individual florets of *G. robusta* were sampled on the tree on the first and second days of nectar secretion; data presented for 2 out of 6 florets. 1 = young flowers, 2 = older flowers.

intermediates. *Protea* and *Faurea* are the only two genera with xylose as a nectar sugar. This pentose sugar occurs in high concentrations in some species (up to 39% in *Faurea rochetiana*, 36% in *Protea lanceolata* and *P. pendula*). The ratio between the two hexose sugars fructose and glucose is generally well balanced (the sugars occur in more or less equal proportions). Note, however, the distinct imbalance in some species of *Protea* (e.g. *P. acuminata, P. pityphylla*) where fructose is more abundant than glucose.

Patterns Associated with Flower Age

The normally equal amounts of glucose and fructose in floral nectars are assumed to result from the enzymatic breakdown of sucrose by invertase in the nectary (Pate *et al*. 1985). The proportion of sucrose in the nectar might be expected to decrease with flower age. In two separate experiments, conflicting results were obtained (Fig. 1): decreasing levels of sucrose in older flowers of *Protea coronata* (nine inflorescences); increasing levels in *Grevillea robusta* (six florets). The difference may be ascribed to higher invertase activity with age in *P. coronata* and to lower activity in *G. robusta*. It is important to note that these two species are fundamentally different in their nectar sugar ratios, the one sucrose-dominant, the other hexose-dominant. Decreasing levels of sucrose with age have been observed in *Dianthus caryophyllaceus* and in *Kalanchoe* species (B.-E.Van Wyk, unpublished data). The order of magnitude of these variations in sucrose levels is typically between 5% and 10% and is generally small when compared to the distinct high versus low sucrose dichotomy in most Proteaceae (see Appendix). The effect of age was also examined in nine inflorescences of *P. neriifolia*, which secretes a nectar containing no sucrose. No change was found, except for a slight increase in the proportion of xylose (data not shown). Xylose also increased in older flowers of *P. coronata* (Fig. 1). It therefore appears that the activity of invertase is genetically determined, resulting in either low sucrose or high sucrose taxa, and that flower age is relatively unimportant.

The re-absorption of nectar sugars has been recently demonstrated in *Grevillea robusta* flowers under field conditions (Nicolson 1995). Each flower secretes nectar for only 2 days, and the relatively constant nectar composition suggests that all three nectar sugars are re-absorbed. The final nectar reward is determined by a balance between secretion, re-absorption and evaporation (Nicolson 1995).

Patterns Associated with Populations and Individual Plants

Multiple samples from different plants and different populations showed that the nectar sugar ratios are surprisingly uniform within species and that only a small part of the variation can be ascribed to population and individual plant differences. Figure 2 gives a graphic summary of variation at the plant level in *Protea amplexicaulis* and *P. humiflora* in their natural habitat. For each of these species, nectar samples were collected in a nested design: three florets from each of three inflorescences from each of two plants. Variation within an inflorescence was greater than that between inflorescences and between plants. These two mammal-pollinated species of *Protea* were chosen because they produce nectar of a mixed sugar composition, which might be expected to show more variation. In a pure hexose nectar, such as that produced by many bird-pollinated species of *Leucospermum* and *Protea* (Appendix), variation between plants and populations is unlikely to occur. Very few studies along these lines have been undertaken, but little intraspecific variation was found by Lanza *et al*. (1995) in the mixed nectar of *Impatiens capensis.*

Variation at the plant and population level was also examined in other species (see Appendix). Examples are *Faurea rochetiana* (three populations, several different plants, example of a savanna species with xylose in the nectar), *Protea caffra* (three populations, four plants, example of a grassland species with xylose in the nectar), *Protea nitida* (three populations, three plants, a fynbos species with mainly hexoses in the nectar) and *Protea*

pruinosa (one population, three different plants, a fynbos species with more sucrose in the nectar). Note the subtle differences between populations, which may be partly due to climatic conditions (e.g. *Faurea rochetiana* trees in the National Botanic Gardens at Kirstenbosch have lower levels of nectar xylose than the natural populations). Only limited variation can be ascribed to different plants at the same locality. As with *P. amplexicaulis* and *P. humiflora*, there seems to be more variation within than between inflorescences (e.g. *P. caffra*, single floret versus whole inflorescence).

Many samples in the Appendix are of pooled nectar from large inflorescences (e.g. *Protea*, *Banksia*, *Dryandra*), because a single small floret may not produce sufficient nectar for

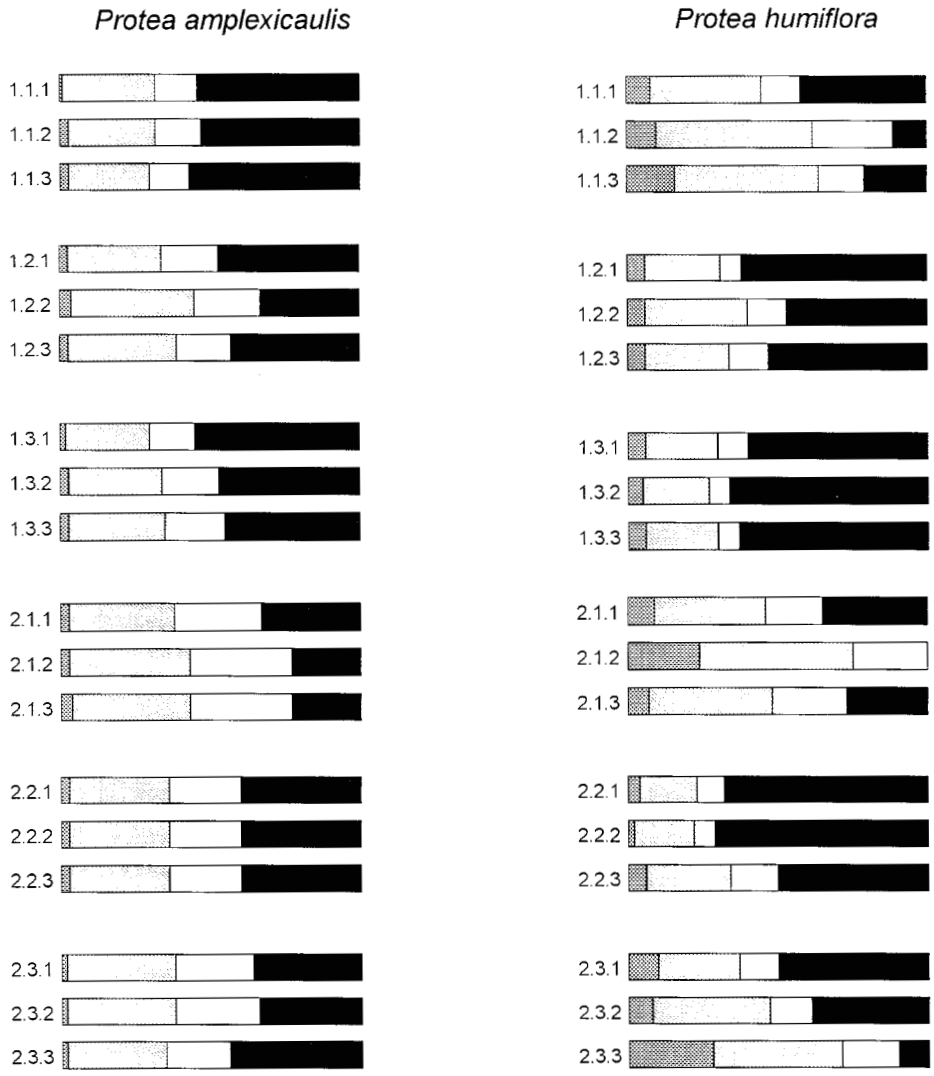

Fig. 2. Variation in nectar sugar composition at the inflorescence and plant level in two mammal-pollinated species of *Protea*, *P. amplexicaulis* and *P. humiflora*, sampled in their natural habitat on Jonaskop in the south-western Cape. Nectar samples were taken in a nested design ($2 \times 3 \times 3$; three florets from three inflorescences from two plants). For key to shading see Fig. 1.

routine analyses. The assumption is made that the sugar composition of such species can be characterised by a single sample. It may be advisable to sample more than one floret or inflorescence, and more than one plant within a population, to get some idea of the variation within the species, and to reduce the chance of 'atypical' samples. Note the single outlier floret in *Protea humiflora* (2.1.2 in Fig. 2). Nevertheless, the variation among florets, inflorescences and plants is relatively unimportant in relation to the distinct species differences.

Patterns Associated with Phylogeny

The strongest phylogenetic signals from nectar sugars come from the generic and infrageneric levels. The discovery of xylose as a major nectar sugar in *Protea* and *Faurea* is remarkable, because this sugar was not detected in any of the other genera. There is only one other reference to xylose in nectar: small amounts in the extrafloral nectar of a grass (Bowden 1970). The range of variation in the level of xylose in *Protea* and *Faurea* nectar is graphically summarised in Fig. 3. The xylose provides strong supportive evidence for the proposed sister group relationship between *Protea* and *Faurea* (Rourke 1973; Johnson and Briggs 1975; Rebelo 1995) and may be considered a useful synapomorphy for these two genera. The absence of xylose in the nectar of other genera, such as *Adenanthos*, supports the idea that *Protea* and *Faurea* are not closely related to any of the superficially similar South African and Australian genera. This agrees with the distribution of phenolic lactones, which are present in all South African Proteoideae except *Protea*, *Faurea* and *Aulax* (Perold 1993).

Monosaccharides are able to undergo a considerable number of interconversions. These are important because all monosaccharides have to be converted into glucose or fructose (or one of their phosphate esters) before they can enter the glycolysis pathway. Two types of derivatives are usually involved in interconversions, namely phosphate esters and nucleoside diphosphate esters. Glucose can be converted to xylose via uridine diphosphate glucose (UDP-D-glucose) and uridine diphosphate glucuronic acid (UDP-D-glucuronic acid). The last step is the decarboxylation of UDP-D-glucuronic acid with the formation of UDP-D-xylose (Goodwin and Mercer 1983). The presence of xylose in *Protea* and *Faurea* nectar is more likely to be related to biochemical (enzymatic) processes in the plant than to selective advantage in terms of pollinator preferences. Studies on sugar preferences and sugar

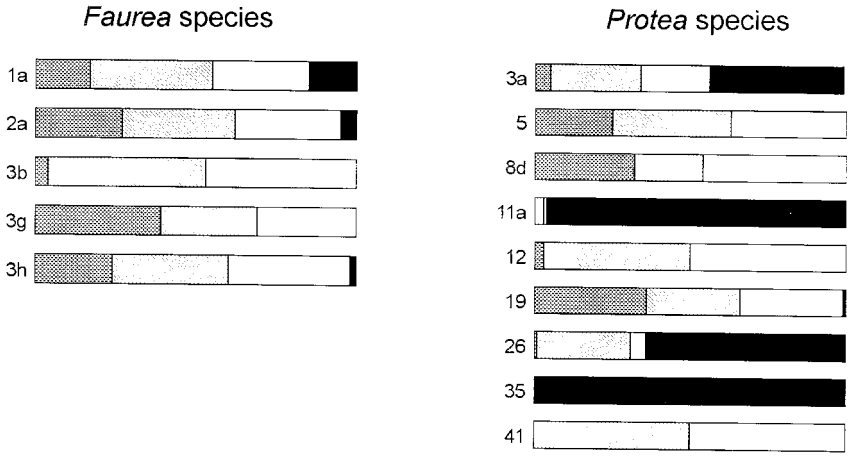

Fig. 3. Variation in the levels of xylose in the nectar of *Faurea* and *Protea* species. Numbering of species as in Appendix. For key to shading see Fig. 1.

absorption in passerine bird and bee pollinators of Proteaceae (Lotz and Nicolson 1996; Jackson *et al*. 1998*a*, 1998*b*; Allsopp *et al*. 1998) have shown that sunbirds, sugarbirds and honeybees avoid pure xylose, although they will drink mixtures containing xylose (as in nectar). Even when ingested, xylose appears to be poorly utilised, thus decreasing the value of the nectar as an energy source.

The overall pattern at the generic level is graphically summarised in Fig. 4. It is clear that the nectar sugars are generally conservative and that useful phylogenetic information can be obtained. The pattern suggests that hexose-rich nectar is the basal condition, with repeated increases in sucrose concentration in unrelated groups. The Grevilleoideae *sensu* Johnson and Briggs (1975) has predominantly hexose-rich nectar, despite the small flower size in most of the genera (e.g. *Macadamia* and *Brabejum* of the Macadamiinae and *Hakea* of the Grevilleeae). Small flower size tends to be associated with insect pollination and the production of sucrose-rich nectar. It seems reasonable to speculate that the nectar composition of *Grevillea robusta* (low sucrose) is the basal condition, with an increase in sucrose in the more derived shrubby species (McGillivray 1993). In the South African Proteoideae, by contrast, sucrose-rich nectar is generally found only in small-flowered genera and species (e.g. *Paranomus* and the small-flowered species of *Leucospermum*) but there are interesting exceptions at the sectional level in *Protea* (see below). Several genera of the Australian Proteoideae lack nectaries (Venkata Rao 1967).

Flower size and nectar sucrose levels are not logically correlated, suggesting that the high-sucrose nectar does not have adaptive significance in terms of pollination, but that the nectar sugar composition is perhaps a physiological necessity related to the plant's sugar economy and sugar metabolism. The presence of xylose is a useful synapomorphy for *Protea* and *Faurea*, while high sucrose concentrations appear to be convergent in various unrelated groups of Proteaceae.

At the sectional and species levels there are some interesting trends in *Leucospermum* and *Protea*. These results are illustrated in Figs 5 and 6 respectively. The patterns in *Leucospermum* are particularly clear, with hexose nectars in five sections, sucrose nectars in section Crinitae, and mixed nectars in two other sections. The exception is *L. muirii*, which morphologically fits comfortably into the section Tumiditubus (J. P. Rourke, pers. comm.), so that the high nectar sucrose is clearly a convergent character. In *Protea* section Ligulatae (Fig. 6), there is a clear increase in sucrose levels following the presumed evolutionary sequence in this section (Rourke 1980; Rebelo 1995; J. P. Rourke, unpublished data). Only two species in section Speciosae have high sucrose, namely *P. coronata* and *P. grandiceps*, suggesting an affinity between these two species, which is supported by morphological characters (J. P. Rourke, unpublished data). Section Pinifoliae is particularly interesting, as it is the only section where there is a distinct imbalance between the hexose sugars, fructose levels exceeding those of glucose (except in *P. canaliculata*).

The sequence of species in Rebelo (1995) is supposedly in phylogenetic order but there are no cladograms available at the species level. In evolutionary terms, higher sucrose levels may have evolved to increase the reward per flower in small flowers. In the Fabaceae, small volumes of nectar per flower are strongly correlated with high sugar concentrations and high sucrose levels (Van Wyk 1993). But then why do hexose sugars predominate in the small-flowered Australian (Grevilleoideae) genera (e.g. *Brabejum, Macadamia, Hakea*, etc.)? The nectar sugars of *Banksia* also do not seem to be related to pollination or other characters (Collins and Rebelo 1987; George 1981, 1996). The data include analyses of the nectar of three species from the *sphaerocarpa* group of series *Abietinae*, which produce unusual nectar which discolours and changes to a green mucilage within 1 or 2 days after secretion (Lamont 1980; Markey and Lamont 1996). These species are *B. leptophylla*, *B. telmatiea*, and *B. sphaerocarpa*, which have normal nectar sugar compositions (Appendix). None of the other *Banksia* nectar samples was green in colour, and all other nectars analysed were clear or

pale yellow. The increased fructose : glucose ratio in *Protea* section Pinifoliae is presumably due to enzymatic conversion of glucose to fructose. A similar imbalance occurs in three genera of the subfamily Alooideae (*Astroloba*, *Haworthia* and *Chortolirion*) and in *Pelargonium* (Van Wyk *et al*. 1993; B.-E.Van Wyk, unpublished data).

Patterns Associated with Pollination

The *Leucospermum* sections in Fig. 5 are associated with different pollen vectors (Rourke 1972). Hexose nectars are produced by the large bird-pollinated flowers (sections Crassicaudex, Conocarpodendron, Tumiditubus, Brevifilamentum and Cardinistylus).

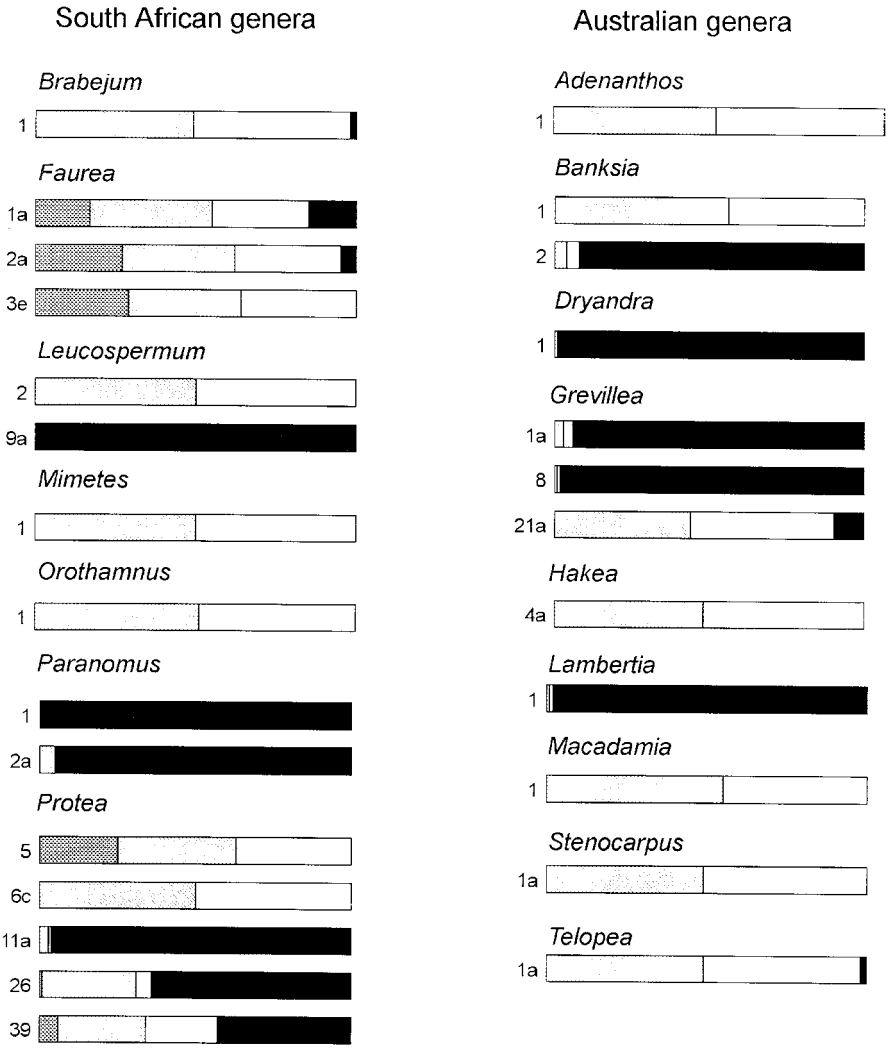

Fig. 4. General patterns of nectar sugar composition in 16 genera of African and Australian Proteaceae. Note the unique presence of xylose in *Protea* and *Faurea*, and both high and low sucrose levels in *Leucospermum*, *Protea*, *Banksia* and *Grevillea*. Numbering of species as in Appendix. For key to shading see Fig. 1.

Sucrose nectars are produced by the small insect-pollinated flowers (sections Leucospermum and Diastelloidea). There is less agreement in section Crinitae, which produces sucrose-dominant nectars but is thought to be pollinated by both birds and insects.

Figure 7 shows the sugar composition of *Protea* nectars according to pollinator type. The large and showy bird-pollinated species produce hexose nectars, while the species pollinated by small mammals all have balanced nectars. High sucrose levels in nectar may have evolved independently in different sections of *Protea*. The inflorescence characters associated with small-mammal pollination in *Protea* are considered to have evolved several times from bird-pollinated forms (Rourke and Wiens 1977; Wiens *et al*. 1983). If so, this supports the suggestion that hexose nectars were the original condition in the Proteaceae. Cowling and Mitchell (1981) analysed the nectar sugars of six species of *Protea* by gas–liquid chromatography, and similarly found hexoses dominating the bird-pollinated species, with more balanced nectars in the rodent-pollinated species (although *P. longifolia* was

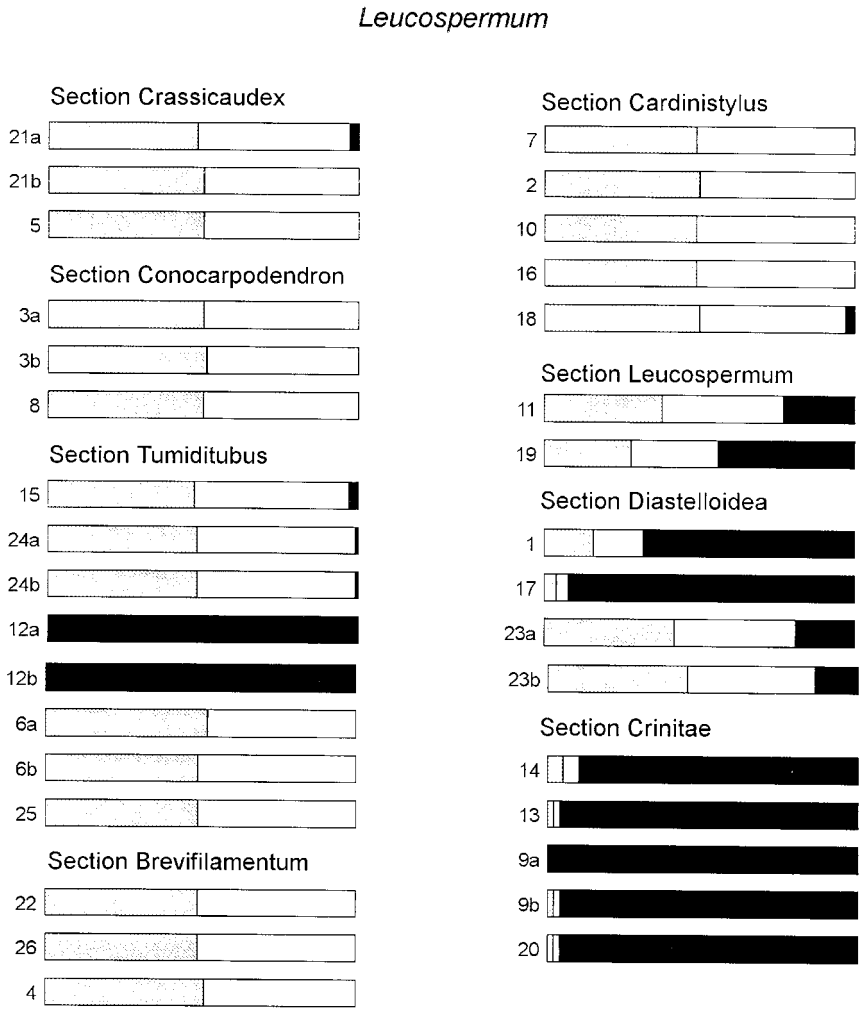

Fig. 5. Nectar sugar composition of *Leucospermum* at the sectional and species levels. Taxonomy as in Rourke (1972). Numbering of species as in Appendix. For key to shading see Fig. 1.

confusing). Wiens *et al*. (1983) give the sugar composition of nectar from four mammal-pollinated species of *Protea*. These analyses were done by H. G. Baker and I. Baker, using descending paper chromatography, and the maltose and melezitose is probably xylose. Since pollinators are apparently unable to utilise xylose in nectar, the high levels of xylose in *Faurea* spp. and in *P. caffra* are likely to be unrelated to pollination. Similarly, the lack of any preferences for sucrose or hexoses in sunbirds and sugarbirds (Lotz and Nicolson 1996; Jackson *et al*. 1998*a*) suggests that pollination is not the primary selective force behind switches in nectar sugar composition.

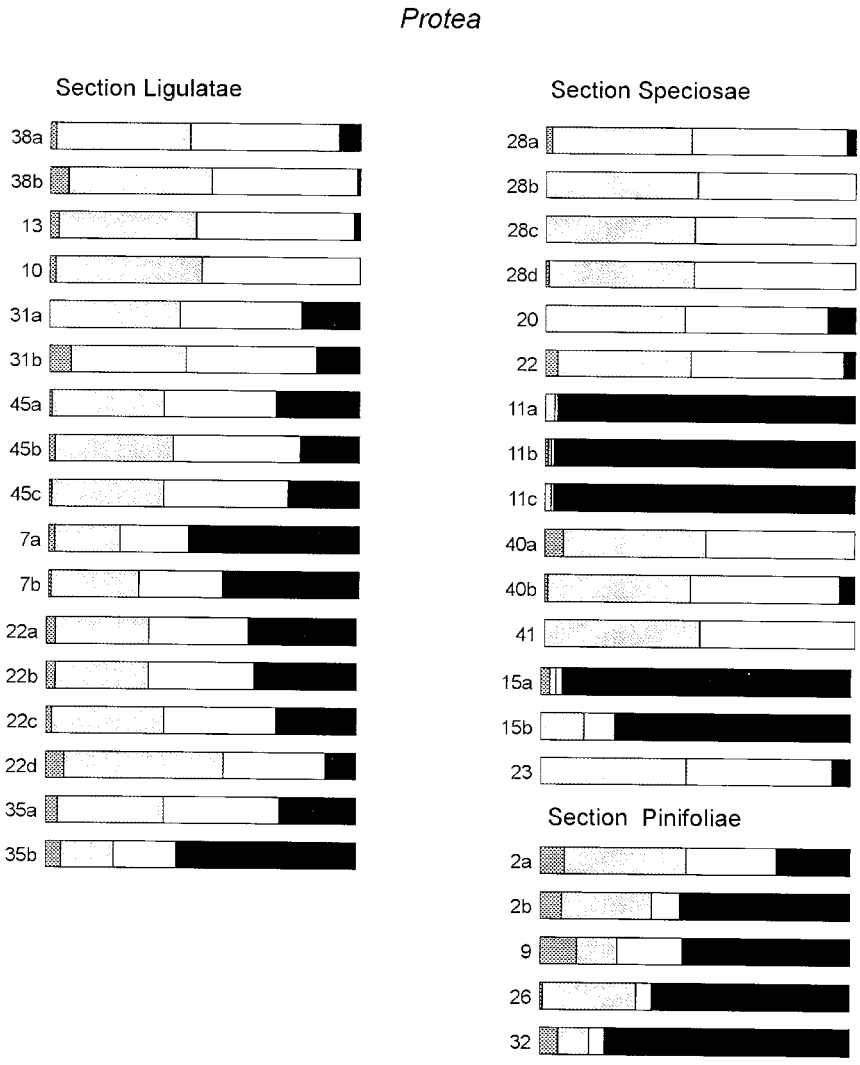

Fig. 6. Nectar sugar composition of *Protea* at the sectional and species levels. Taxonomy as in Rourke (unpublished data). Numbering of species as in Appendix. For key to shading see Fig. 1.

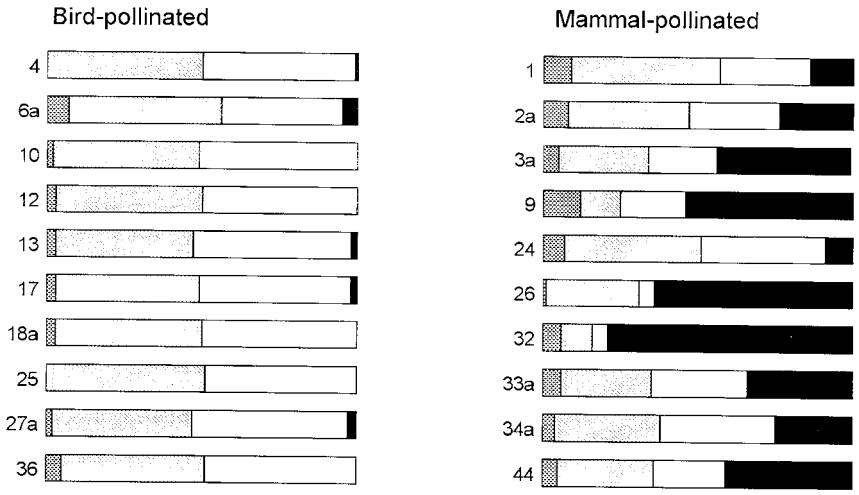

Fig. 7. Nectar sugars in some bird-pollinated and mammal-pollinated species of *Protea.* Numbering of species as in Appendix. For key to shading see Fig. 1.

Conclusions

It appears that no single driving force determines the nectar sugar composition in Proteaceae. A large part of the variation can be ascribed to phylogenetic (genetic) origin, at various levels (species, sections, genera and above). Of minor importance are patterns associated with pollination, flower age and other parameters. The data show that the Proteaceae is an interesting model to test various hypotheses regarding phylogeny, pollination and nectar sugar metabolism.

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Appendix. Nectar sugar composition in Proteaceae

South African genera are listed before Australian genera. Both genera and species are in alphabetical order for ease of reference. X, xylose; F, fructose; G, glucose; S, sucrose; –, no trace of the sugar was detected; tr, trace. Locality abbreviations: ELS = Fynbos Research Unit, Elsenberg; Flora 93 = 1993 flower show, Cape Town; FNR = Fernkloof Nature Reserve, Hermanus; MBG = Royal Botanic Gardens, Melbourne; MBG-C = Royal Botanic Gardens, Cranbourne; MU = Melbourne University; NBG = National Botanic Gardens, Kirstenbosch; NBI, PTA = National Botanical Institute, Pretoria; PBG = King's Park Botanic Garden, Perth; RNNP = Royal Natal National Park; UCT = University of Cape Town; UWA = University of Western Australia

Number	Genera and species		Locality	% total sugar			
				X	F	G	S
	Brabejum						
1	*B. stellatifolium* L.		Cape Town	–	49	49	2
	Faurea						
1a	*F. macnaughtonii* E.Phillips	sample 1	ex hort, Saasveld	17	38	30	15
1b		sample 2	ex hort, Saasveld	15	37	29	19
1c		sample 3	ex hort, Saasveld	12	36	28	24
2a	*F. saligna* Harv.	sample 1	The Wilds	27	35	33	5
2b		sample 2	The Wilds	17	38	40	5
3a	*F. rochetiana* (A.Rich.) Pic. Serm.	tree 1	NBG	7	45	48	–
3b		tree 2	NBG	4	49	47	–
3c		tree 3	NBG	7	47	47	–
3d		tree 4	NBG	4	51	45	–
3e		1 floret	NBI, PTA	29	35	36	–
3f		1 floret	NBI, PTA	36	35	29	–
3g		mixture	NBI, PTA	39	30	31	–
3h		sample 1	Saddleback Pass	24	36	38	2
3i		sample 2	Saddleback Pass	26	33	39	2
3j		sample 3	Saddleback Pass	20	38	42	–
3k		sample 4	Saddleback Pass	23	36	41	–
3l		sample 5	Saddleback Pass	20	40	40	–
	Leucospermum						
1	*L. calligerum* (Salisb. ex Knight) Rourke		NBG	–	16	16	68
2	*L. catherinae* Compton		NBG	–	50	50	–
3a	*L. conocarpodendron* (L.) H.Buek subsp. *conocarpodendron*		NBG	–	50	50	–
3b	*L. conocarpodendron* subsp. *viridum* Rourke		NBG	–	51	49	–
4	*L. cordifolium* (Salisb. ex Knight) Fourc.		NBG	–	51	49	–
5	*L. cuneiforme* (Burm. f.) Rourke		NBG	–	50	50	–
6a	*L. erubescens* Rourke	sample 1	NBG	–	52	48	–
6b		sample 2	NBG	–	49	51	–
7	*L. formosum* (Andrews) Sweet		NBG	–	49	51	–
8	*L. glabrum* E.Phillips		NBG	–	50	50	–
9a	*L. gracile* (Salisb. ex Knight) Rourke	sample 1	NBG	–	tr	tr	100
9b		sample 2	FNR	–	2	2	96
9c		1 floret	FNR	–	1	1	98
9d		1 floret	FNR	–	1	1	98
9e		1 floret	FNR	–	1	1	98
10	*L. grandiflorum* (Salisb.) R.Br.		NBG	–	49	51	tr
11	*L. hypophyllocarpodendron* (L.) Druce subsp. *canaliculatum* (H.Buek ex Meisn.) Rourke	Hopefield		–	38	39	23
12a	*L. muirii* E.Phillips	sample 1	NBG	–	–	–	100
12b		sample 2	NBG	–	–	–	100
13	*L. mundii* Meisn.		NBG	–	2	2	96
14	*L. oleifolium* (P.J.Bergius) R.Br.		NBG	–	5	5	90
15	*L. praecox* Rourke		NBG	–	47	50	3
16	*L. praemorsum* (Meisn.) E.Phillips		NBG	–	49	51	tr
17	*L. prostratum* (Thunb.) Stapf		NBG	–	4	4	92
18	*L. reflexum* H.Buek ex Meisn.		NBG	–	50	50	–

Appendix (*continued*)

Number	Genera and species		Locality	% total sugar X	F	G	S
19	*L. rodolentum* (Salisb. ex Knight) Rourke		Malmesbury	–	28	28	44
20	*L. saxatile* (Salisb. ex Knight) Rourke		NBG	–	2	2	96
21a	*L. saxosum* S.Moore	sample 1	NBG	–	48	49	3
21b		sample 2	NBG	–	50	50	–
22	*L. tottum* (L.) R.Br. var. *tottum*		NBG	–	49	51	–
23a	*L. truncatulum* (Salisb. ex Knight) Rourke	sample 1	FNR	–	42	39	19
23b		sample 2	FNR	–	45	41	14
24a	*L. truncatum* (H.Buek ex Meisn.) Rourke	sample 1	NBG	–	48	51	1
24b		sample 2	NBG	–	48	51	1
25	*L. utriculosum* Rourke		NBG	–	49	51	–
26	*L. vestitum* (Lam.) Rourke		NBG	–	49	51	–
	Mimetes						
1	*M. argenteus* Salisb. ex Knight		NBG	–	50	50	–
2	*M. capitulatus* R.Br.		NBG	–	65	35	–
3	*M. cucullatus* (L.) R.Br.		NBG	–	51	49	–
4	*M. fimbriifolius* Salisb. ex Knight		NBG	–	51	49	–
5	*M. hirtus* (L.) Salisb. ex Knight		NBG	–	52	48	–
6	*M. saxatilis* E.Phillips		NBG	–	49	51	–
	Orothamnus						
1	*O. zeyheri* Pappe ex Hook. f.		NBG	–	51	49	–
	Paranomus						
1	*P. longicaulis* Salisb. ex Knight		NBG	–	–	–	100
2a	*P. reflexus* (E.Phillips & Hutch.) Fourc.	plant 1	NBI, PRE	–	5	–	95
2b		plant 2	NBI, PRE	–	–	–	100
2c		plant 2	NBI, PRE	–	3	3	94
2d		mixture	NBG	–	–	–	100
	Protea						
1	*P. acaulos* (L.) Reichard		Flora 93	9	48	29	14
2a	*P. acuminata* Sims	sample 1	NBG	8	39	29	24
2b		sample 2	Kaaimansgat	7	29	9	55
3a	*P. amplexicaulis* (Salisb.) R.Br.	sample 1	Flora 93	5	29	22	43
3b	(see Fig. 2)	1 floret	Jonaskop	1	31	14	54
3c		1 floret	Jonaskop	3	35	29	33
4	*P. aristata* E.Phillips		NBG	tr	50	49	1
5	*P. aspera* E.Phillips		Flora 93	25	38	37	tr
6a	*P. aurea* (Burm. f.) Rourke subsp. *aurea*	sample 1	Montagu Pass	7	49	39	5
6b		sample 2	Montagu Pass	8	51	34	6
6c	*P. aurea* subsp. *potbergensis* (Rourke) Rourke		NBG	tr	50	50	–
7a	*P. burchellii* Stapf	sample 1	NBG	2	21	22	55
7b		sample 2	NBG	1	28	27	44
8a	*P. caffra* Meisn.	sample 1	Melville Koppies	23	40	37	tr
8b		sample 2	Melville Koppies	20	21	58	1
8c		sample 3	The Wilds	10	34	56	–
8d		sample 4	The Wilds	32	22	46	–
8e		1 floret	The Wilds	7	35	50	8
8f		sample 5	NBG	3	47	49	1
9	*P. canaliculata* Andrews		Swartberg Pass	12	13	21	54
10	*P. compacta* R.Br.		NBG	2	47	51	–
11a	*P. coronata* Lam.	sample 1	NBG	–	3	1	96
11b	(see Fig. 1)	sample 2	NBG	1	1	1	97
11c		sample 3	NBG	–	2	1	97
12	*P. cynaroides* (L.) L.		NBG	3	47	50	–
13	*P. eximia* (Salisb. ex Knight) Fourc.		NBG	3	44	51	2
14	*P. glabra* Thunb.		Clanwilliam	10	45	45	tr
15a	*P. grandiceps* Tratt.	sample 1	NBG	3	2	2	93
15b		sample 2	NBG	tr	14	10	76
16a	*P. humiflora* Andrews	mixture	Jonaskop	5	16	8	71
16b	(see Fig. 2)	1 floret	Jonaskop	8	37	13	42
16c		1 floret	Jonaskop	5	32	18	45
16d		1 floret	Jonaskop	9	37	19	35
17	*P. lacticolor* Salisb.		NBG	3	46	49	2
18a	*P. laetans* L.E.Davidson	sample 1	NBG	3	47	50	–
18b		sample 2	NBG	2	52	46	–
18c		sample 3	NBG	3	46	48	3

Appendix (*continued*)

Number	Genera and species		Locality	% total sugar X	F	G	S
19	*P. lanceolata* E.Mey. ex Meisn.		NBG	36	30	33	1
20	*P. lepidocarpodendron* (L.) L.		NBG	tr	45	46	9
21a	*P. longifolia* Andrews	sample 1	NBG	3	30	32	35
21b		sample 2	NBG	2	30	34	34
21c		sample 3	NBG	2	36	36	26
21d		sample 4	FNR	6	51	33	10
22	*P. lorifolia* (Salisb. ex Knight) Fourc.		NBG	4	43	49	4
23	*P. magnifica* Link		NBG	–	47	47	6
24	*P. montana* E.Mey. ex Meisn.		Spitskop	7	44	40	9
25	*P. mundii* Klotzsch		NBG	tr	51	49	–
26	*P. nana* (P.J.Bergius) Thunb.		NBG	1	30	5	64
27a	*P. neriifolia* R.Br.	sample 1	NBG	2	45	50	3
27b		sample 2	NBG	–	49	51	–
27c		sample 3	NBG	–	48	52	–
27d		sample 4	NBG	1	47	52	–
28a	*P. nitida* Mill.	sample 1	NBG	6	46	48	tr
28b		sample 2	George	4	46	48	2
28c		sample 3	Du Toit's Kloof	3	46	48	3
28d		sample 4	Du Toit's Kloof	4	47	48	1
29a	*P. nubigena* Rourke	sample 1	RNNP	8	39	38	15
29b		sample 2	RNNP	11	43	44	2
30a	*P. obtusifolia* H.Buek ex Meisn.	sample 1	NBG	tr	42	39	19
30b		sample 2	NBG	7	37	42	14
31	*P. pendula* R.Br.		Cedarberg	36	35	26	3
32	*P. pityphylla* E.Phillips		NBG	6	10	5	79
33a	*P. pruinosa* Rourke	sample 1	Swartberg	6	29	31	34
33b		sample 2	Swartberg	7	24	28	41
33c		sample 3	Swartberg	7	20	22	51
34a	*P. pudens* Rourke	sample 1	NBG	4	34	37	25
34b		sample 2	NBG	5	17	20	58
35	*P. punctata* Meisn.		NBG	–	tr	tr	100
36	*P. repens* (L.) L.		NBG	5	46	49	–
37a	*P. roupelliae* Meisn. subsp. *roupelliae*	sample 1	NBG	2	43	48	7
37b		sample 2	Soutpansberg	6	46	47	1
38	*P. rubropilosa* Beard		NBG	4	39	45	12
39	*P. scabra* R.Br.		Flora 93	6	28	23	43
40a	*P. speciosa* (L.) L.	sample 1	NBG	6	46	48	–
40b		sample 2	NBG	1	46	48	5
41	*P. stokoei* E.Phillips		NBG	–	50	50	–
42	*P. subulifolia* (Salisb. ex Knight) Rourke		Kaaimansgat	tr	52	40	8
43	*P. subvestita* N.E.Br.		NBG	tr	43	52	5
44	*P. sulphurea* E.Phillips		NBG	5	31	23	41
45a	*P. susannae* E.Phillips	sample 1	NBG	1	36	36	27
45b		sample 2	ex hort	2	38	41	19
45c		sample 3	ex hort	1	36	40	23
46a	*P. venusta* Compton	sample 1	NBG	7	35	35	23
46b		sample 2	NBG	2	39	41	18
46c		sample 3	Spitskop	4	47	48	1
46d		sample 4	Spitskop	4	46	48	2
	Adenanthos						
1	*A.* x *cunninghamii* Meisn.		PBG	–	49	51	–
2	*A. sericeus* Labill.		MBG	–	43	41	16
3	*A. pungens* Meisn.		PBG	–	46	48	6
	Banksia						
1	*B. aemula* R.Br.		NBG	–	56	44	–
2	*B. baxteri* R.Br.		ELS	–	4	4	92
3	*B. burdettii* Baker f.		ELS	–	50	50	–
4	*B. coccinea* R.Br.		ex hort	–	tr	5	95
5	*B. elderiana* F.Mueller & Tate		ELS	–	48	52	–
6	*B. ericifolia* L. f. var. *ericifolia*		ex hort	–	49	51	–
7a	*B. hookeriana* Meisn.	sample 1	ex hort	–	44	45	11
7b		sample 2	Eneabba	–	49	50	1
7c		sample 3	Eneabba	–	48	52	–
8	*B. integrifolia* L. f. subsp. *integrifolia*		Auckland, NZ	–	42	29	29
9a	*B. leptophylla* A.S.George var. *leptophylla*	sample 1	Eneabba	–	37	45	18
9b		sample 2	Eneabba	–	33	33	34

Appendix (*continued*)

Number	Genera and species		Locality	% total sugar X	F	G	S
9c		sample 3	Eneabba	–	32	36	32
10a	*B. media* R.Br.	sample 1	ELS	–	39	53	8
10b		sample 2	ELS	–	36	64	tr
11a	*B. menziesii* R.Br.	sample 1	ex hort	–	3	5	92
11b		sample 2	E Perth	–	15	13	72
11c		sample 3	E Perth	–	15	13	72
12	*B. occidentalis* R.Br.		ELS	–	2	2	96
13	*B. pilostylis* Gardner		ELS	–	11	13	76
14	*B. prionotes* Lindley		ELS	–	49	47	4
15	*B. serrata* L. f.		NBG	–	15	16	69
16	*B. speciosa* R.Br.		ELS	–	51	49	–
17a	*B. sphaerocarpa* R.Br. var. *sphaerocarpa*	sample 1	S Eneabba	–	25	26	49
17b		sample 2	S Eneabba	–	22	22	56
18	*B. spinulosa* Smith var. *collina* (R.Br.) A.S.George		Taupo, NZ	–	15	20	65
19a	*B. telmatiea* A.S.George	sample 1	E Perth	–	19	19	62
19b		sample 2	E Perth	–	26	25	49
19c		sample 3	E Perth	–	23	25	52
	Dryandra						
1	*D. formosa* R.Br.		ex hort	–	1	tr	99
2	*D. plumosa* R.Br.		PBG	–	–	–	100
3	*D. quercifolia* Meisn.		MBG–C	–	4	2	94
4	*D. sessilis* (Knight) Domin		Perth	–	5	5	90
5	*D. trifontinalis*		PBG	–	tr	1	99
	Grevillea						
1a	*G. banksii* R.Br.	sample 1	Paarl	–	3	3	94
1b		sample 2	Paarl	–	4	5	91
1c		sample 3	ex hort	–	6	3	91
2	*G. barklyana* F.Muell. ex Benth.		MBG	–	2	2	96
3	*G. bipinnatifida* R.Br.		ex hort	–	1	1	98
4	*G. calliantha* Makinson & Olde		PBG	–	2	3	95
5	*G. deflexa* F.Muell.		PBG	–	50	47	3
6	*G. depauperata* R.Br.		PBG	–	–	1	99
7	*G. fistulosa* A.S.George		PBG	–	–	–	100
8	*G. insignis* Kippist ex Meisn.		UWA	–	1	1	98
9	*G. jephcottii* J.H.Willis		MBG	–	tr	2	98
10	*G. juniperina* R.Br.		ex hort	–	1	1	98
11	*G. lavendulacea* Schlechtd.		ex hort	–	1	1	98
12	*G. nana* C.Gardner		PBG	–	1	1	98
13	*G. obtusifolia*		PBG	–	tr	1	99
14	*G. olivacea* A.S.George		PBG	–	1	2	97
15	*G. petrophiloides* Meisn.		PBG	–	47	49	4
16	*G. pinaster*		PBG	–	1	1	98
17	*G. pinifolia* Meisn.		PBG	–	1	1	98
18	*G. speciosa* (Knight) McGillivray subsp. *speciosa*		ex hort	–	tr	tr	100
19	*G. pythara* Olde & Marriott		PBG	–	41	42	17
20	*G. ripicola* A.S.George		PBG	–	3	3	94
21a	*G. robusta* A.Cunn. ex R.Br.	1 floret	ex hort	–	44	46	10
21b	(see Fig. 1)	1 floret	ex hort	–	44	46	10
21c		1 floret	ex hort	–	40	42	20
22	*G. rosmarinifolia* A.Cunn.		MBG–C	–	3	3	94
23	*G. saccata* Benth.		PBG	–	tr	1	99
24	*G. tripartita* Meisn.		ex hort, Perth	–	2	2	96
25	*G. wilsonii* A.Cunn.		ex hort, Perth	–	1	1	98
	Hakea						
1	*H. bucculenta*		MBG–C	–	50	50	tr
2a	*H. coriacea* Maconochie	sample 1	MBG–C	–	50	50	tr
2b		sample 2	MBG–C	–	52	48	tr
3	*H. cucullata* R.Br.		MBG–C	–	48	52	–
4a	*H. petiolaris* Meisn.	sample 1	UCT	–	48	52	–
4b		sample 2	UCT	–	49	51	–
4c		sample 3	UCT	–	49	51	–

Appendix (*continued*)

Number	Genera and species		Locality	% total sugar X	F	G	S
	Lambertia						
1	*L. echinata* R.Br.		UWA	–	1	1	98
2	*L. formosa* Sm.		MU	–	1	tr	99
3a	*L. multiflora* Lindl.	sample 1	MBG–C	–	2	2	96
3b		sample 2	PBG	–	4	4	92
	Macadamia						
1	*M. integrifolia* Maiden & Betche		ex hort, Cape Town	–	55	45	–
	Stenocarpus						
1a	*S. sinuatus* (Loudon) Endl.	sample 1	ex hort, Cape Town	–	49	51	–
1b		sample 2	ex hort, Westdene	–	51	49	–
1c		sample 3	ex hort, Westdene	–	49	51	–
	Telopea						
1a	*T. speciosissima* (Sm.) R.Br.	sample 1	ex hort	–	49	49	2
1b		sample 2	NBG	–	49	48	3

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