VARIATION IN CHROMOSOME NUMBER IN THE *MANULEAE* (SCROPHULARIACEAE) AND ITS CYTOTAXONOMIC IMPLICATIONS

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Chromosome numbers for 11 genera and 36 species in the predominantly South African tribe *Manuleae* (Scrophulariaceae) are presented, the majority being first reports. The basic numbers of the genera form a dysploid series, x = 6, 7, 8, 9. The somatic number of most of the species is diploid, ranging from $2n = 12$ to $2n = 18$; that of *Jamesbrittenia*, $2n = 24$, is probably of polyploid derivation. The highest somatic number encountered in this survey is that in *Camptoloma lyperiiflorum*, with $2n$ = c.56, which, taken in conjunction with a published count of $2n = 28$ for another species of *Camptoloma*, is octoploid based on x = 7. This is the only example, so far, of intrageneric polyploidy in the *Manuleae*. The somatic number in certain genera appears to be constant over some subgeneric divisions (e.g. *Jamesbrittenia*, $2n = 24$), but variable in others (e.g. *Sutera*, $2n$ = 12, 14). The variation in chromosome number observed in this study parallels and corroborates the redefinition of generic boundaries proposed by O. M. Hilliard. One species of *Selago* (in the tribe *Selagineae*) was also included in this survey for cytological comparison with *Tetraselago* (*Manuleae*).

INTRODUCTION

The tribe *Manuleae* in the Scrophulariaceae is being revised by Dr O. M. Hilliard of the Royal Botanic Garden Edinburgh (Hilliard, 1994). The present paper is an interim report mainly on chromosome counts that have been made to complement that taxonomic revision. Additional cytological information will be published later.

Previously, there has been much confusion in the delimitation of genera in the *Manuleae*. The tribe as redefined by Hilliard now consists of some 344 species distributed among 17 genera, the majority being found in southern Africa.

Cytologically, little was hitherto known about the *Manuleae*, as reference to Table 1 will confirm; chromosome counts were available for only four species in two of the genera, namely one in *Camptoloma* Benth. and three in *Zaluzianskya* F. W. Schmidt.

In this study, the somatic chromosome numbers for a total of 36 species have been determined, including one species of *Selago* L. from the neighbouring tribe, the *Selagineae*. Except for two species of *Zaluzianskya*, all the counts are new, being also first reports for nine out of the 12 genera included in this survey.

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Dedication: I owe Mr B. L. Burtt an immense debt of gratitude for his botanical mentorship over all the years that I have known him, and for his support in my cytotaxonomic activities in recent times. It is a pleasure to dedicate this paper to him on his 80th birthday.

TABLE 1. Published chromosome numbers in the tribe *Manuleae*.

	2n	Authors
Camptoloma Benth.		
canariense (Webb & Berth.) Hilliard (as *Lyperia canariensis* Webb & Berth.)	**28**	Borgen, 1969
Zaluzianskya F. W. Schmidt		
capensis (L.) Walp. (as *Nycterinia capensis* (L.) Benth.)	**12**	Propach, 1934*
	12	Sugiura, 1936*
elgonensis Hedb.	**12**	Hedberg, 1970
villosa F. W. Schmidt (as *Nycterinia selaginoides* (Thunb.) Benth.)	**12**	Sugiura, 1936*

* In Bohkhovskikh et al., 1969.

MATERIALS AND METHODS

Apart from a few species that were already in cultivation at the Royal Botanic Garden Edinburgh, most of them at the specialist garden at Port Logan (Logan Botanic Garden, Port Logan, Stranraer, Scotland), cytological material was largely obtained from plants grown in Edinburgh from seed received from kind friends and collectors in South Africa. Table 2 lists the plants investigated, their locality, and other details.

Seeds were germinated in a seed compost of the John Innes type, and the seedlings grown on in individual pots. Root tips were harvested about 4–6 weeks after pricking out. These were mostly pretreated in a saturated aqueous solution of 1-bromonaphthalene for 2 hours at room temperature (c.20°C)—some roots were treated overnight for c.20 hours at 4.5°C—and then fixed in a freshly made 3:1 ethanol-propionic acid fixative. Following a 30 min hydrolysis in 5N HCl at room temperature, the root tips were satisfactorily stained after 2 hours in the Feulgen Reagent prepared according to Fox (1969). After a 5–10 min wash in tap water, the stained roots were transferred to a 1:1 enzyme mixture of 2% pectinase and 2% cellulase, for 10–15 min at room temperature. The roots were then rinsed in several changes of, and kept in, distilled water. For longer storage, the roots were transferred to 45% acetic acid and kept in a refrigerator at 6–7°C. Prolonged storage of the enzyme-treated roots is not advisable, as the root meristem tends to become detached, and subsequent handling may present difficulty. The squashes were mounted in 45% acetic acid or in a reinforcing stain either of 0.5% aceto-carmine or 0.9% acetic orcein.

Suitable slides were made permanent according to Conger & Fairchild's quick-freeze method (1953), using liquid nitrogen instead of dry-ice for freezing. Kodak Technical Pan rated at 100 ISO, and developed for 6 min in Dilution D of Kodak HC110, was used in the preparation of photomicrographs.

Voucher specimens are kept in the herbarium collection in E.

RESULTS AND DISCUSSION

Chromosome numbers have been determined in 36 species distributed amongst 11 of the 17 genera of the *Manuleae*. The results are presented in Table 2. The small genus *Tetraselago* Junell is considered to show some affinity to the genus *Selago* in the *Selagineae*, and hence observations were also made on a representative of that genus, *Selago thunbergii*. Table 3 (p. 370) summarizes the distribution of chromosome numbers among the different genera and their subdivisions, giving also an indication of the proportion of species examined (see Notes) to the total number of species in a given group.

SUTERA, *JAMESBRITTENIA*, *LYPERIA* AND *CAMPTOLOMA*

The four genera *Sutera*, *Camptoloma*, *Lyperia* and *Jamesbrittenia*, often treated as belonging to a single genus, *Sutera*, show variation in the basic chromosome number that parallels closely the recent taxonomic recognition of all four genera by Hilliard (1992): *Lyperia* has a basic number x = 8, *Jamesbrittenia*, $n = 12$, most probably x = 6, while *Sutera* has two basic numbers x = 7 and 6, and *Camptoloma* polyploid numbers, most probably based on x = 7. Although most of the species in the latter two genera share a common basic number x = 7, they can be readily discriminated on differences in the somatic chromosome number, *Sutera* with $2n = 14$ (Section 2), and two of the three species of *Camptoloma* form a polyploid series $2n = 28$ and c.56.

There are, however, other cytological features that provide additional evidence for the distinctness of the four genera: *Camptoloma* has the smallest chromosomes of all (in fact, amongst all the genera so far examined in the *Manuleae*), and those of *Jamesbrittenia* are next smallest. Those of *Sutera* are similar in size to those of most other *Manuleae*. Those of *Lyperia tristis* appear to be the largest among the four genera. Other karyotypic differences have also been noted; for example, in *Camptoloma*, *Jamesbrittenia* and *Sutera*, most of the chromosomes are metacentric to submetacentric, whereas in *Lyperia tristis* many of the chromosomes are acrocentric (Fig. 2A). This karyotype differs from that of *L. antirrhinoides* [= *Sutera ochracea*] which resembles the karyotypes of other *Manuleae*. A closer scrutiny of karyotypic variation is therefore warranted.

Because many of Hilliard's new sectional and subsectional names have yet to be published, they are referred to by numbers in Table 3 and the discussion below.

Sutera (x = 6, 7). Fig. 1A & B.

More than half of the 49 species of *Sutera* are found in the southern and western Cape; the others are found in the eastern half of southern Africa, with a few species occurring in both regions. Hilliard divides the genus into two sections: Section 1 (with 3 species) and Section 2 (with 46 species). The two basic numbers mentioned above occur in the two different sections of the genus: x = 6 in the type species *S. foetida* (an annual) in the small Section 1, and x = 7 in two species of Section 2, namely *S. archeri* and *S. roseoflava*, and one garden hybrid *S.* x 'Longwood', all herbaceous perennials. This hybrid (apparently from a cross between *S. hispida* and *S. caerulea* (Table 4, p. 370)) is named after Longwood Garden, Philadelphia, whence material was received at the Royal Botanic Garden Edinburgh.

TABLE 2. New chromosome numbers in the tribe *Manuleae*.

	2*n*	Coll. no.	RBGE no.	Origin
Camptoloma Benth.				
lyperiiflorum (Vatke) Hilliard	**c.56**	A. G. Miller et al. 8391		YEMEN: Socotra, c.6 km south east Qalansiyah, 450m
Glumicalyx Hiern				
goseloides (Diels) Hilliard & Burtt	**14**	H&B 8757	771190	NATAL: Sani Pass, 2130–2440m
montanus Hiern	**14**	Hilliard 4842A	682665	NATAL: Drakensberg, gully behind Giant's Castle hut, 2250m
Manulea L.				
chrysantha Hilliard	**16**	Vlok 2514		CAPE: Beaufort West Distr., road between Seekoeigat and Kruidfontein, farm 'Waterval', 85m
crassifolia Benth. subsp.?	**16**	H&B 16688	831337	CAPE: Elliot Distr. Bastervoetpad, 2196m
obovata Benth.	**18**	B&P 23	882608	CAPE: 20km from Port Alfred, farm 'Kleinemond', c.45m
parviflora Benth.	**16**	Balkwill 6514		TRANSVAAL: Johannesburg Distr., Westdene Koppies, Sima Eliovson Park, 1800m
thyrsiflora L.f.	**16**	B&P 25	890093	CAPE: Cape Town, Bloubergstrand, 10–20m
tomentosa (L.) L.	**16**	B&P 22	890092	CAPE: Cape Town, Bloubergstrand, 10–20m
Phyllopodium Benth.				
cephalophorum (Thunb.) Hilliard	**12**	Bokelmann 65	890097	CAPE: Atlantis, Silverstrand to Mamre road, 150m
cuneiifolium	**12**	Batten 1125		CAPE: East London, Kwelera Mouth
pumilum Benth.	**12**	Bean & Viviers 2551	912237	CAPE: Namaqualand, nr Wallekraal, near farm 'Baksteenhoek'
Lyperia Benth.				
antirrhinoides (L.f.) Hilliard [= *Sutera ochracea* Hiern]	**16**	B&P 9	882621	CAPE: Farm 'Eerste Hoop', between Villiersdorp and Bot Rivier
tristis (L.f.) Benth.	**16**	B&P 43	890099	CAPE: Potsdam, nr Cape Town, c.50m
Sutera Roth				
archeri Compton	**14**	Dean 960		CAPE: Prince Albert Div. Tierberg, 900m
foetida Roth	**12**	Batten 1107		CAPE: Clanwilliam Div., N of Graafwater
roseoflava Hiern	**14**	Batten 1000	900223	CAPE: Between Bushman's River and Cannon Rock, nr sea level
x 'Longwood'	**14**	(Garden hybrid)	781071	
Jamesbrittenia O. Kuntze				
breviflora (Schltr.) Hilliard	**24**	H&B 12642	791406	NATAL: Underberg Distr., Upper Polela Cave, 2075m
breviflora (Schltr.) Hilliard	**24**	H&B 19142	912205	NATAL: Underberg Distr. Bamboo Mt, c.1525m
filicaulis (Benth.) Hilliard	**24**	CDR 948	911655	CAPE: Top of Swaershoek Pass, 60km N of Somerset East, 1500m

TABLE 2. (Contd)

	2n	Coll. no.	RBGE no.	Origin
foliolosa (Benth.) Hilliard	**24**	CDR 936	911653	CAPE: Assegaibos, nr Cloete's Pass 50km NW of Mossel Bay
foliolosa (Benth.) Hilliard	**24**	Munich Botanic Garden		CAPE: Exact location not known
fruticosa (Benth.) Hilliard	**24**	Oliver 9468	912234	CAPE: Van Rhynsdorp Distr. Knersvlakte, NE of Kwagga Kop, 160m
phlogiflora (Benth.) Hilliard	**c.24**	Batten 1124		CISKEI: Midway between Keiskamma and Fish River on road to Peddie
grandiflora (Galpin) Hilliard	**24**	2092		TRANSVAAL: Emmarentia, 1825m
jurassica (Hilliard & Burtt) Hilliard	**24**	H&B 19148	912210	LESOTHO: Sani Top, 2890m
Tetraselago Junell				
natalensis (Rolfe) Junell	**14**	H&B 19168	912227	NATAL: Pinetown Distr., Everton, 610m
Trieenea Hilliard				
taylorii Hilliard	**12**	Taylor 11894	890710	CAPE: North Cedarberg, Moedersielshoek path up Groot Krakadouw, 1450–1600m
glutinosa (Schltr.) Hilliard	**14**	Vlok 2564	921320	CAPE: Ladismith Div. Klein Swartberg, just W of Seweweekspoort Peak, 2075m
Zaluzianskya F. W. Schmidt				
affinis Hilliard [*Z. villosa* var. *glabra* (Benth.) Hiern]	**12**	Batten 867	882342	CAPE: Namaqualand, Garies, between Rondawel and Gamoep, en route to Groen Rivier
benthamiana Walp.	**12**	Batten 1030	911633	CAPE: Namaqualand, 14km from top of Kamiesberg Pass, on road to Gamoep
capensis (L.) Walp.	**12**	Batten 1051	911636	CAPE: Port Elizabeth Div. Seaview, between Kini Bay and Lauries Bay, sea level
collina Hiern	**12**	Batten 1025	911630	CAPE: Namaqualand, Groenvlei, nr Garies
maritima (L.f.) Walp.	**12**	Batten s.n.	771985	EAST CAPE: Rocklyffe, S of East London, grassy hillsides nr the sea, 3–50m
peduncularis (Benth.) Walp.	**12**	Batten 1037	911635	CAPE: Namaqualand, 14km from top of Kamiesberg Pass, on road to Gamoep, 451m
villosa F. W. Schmidt	**12**	CDR 732	911646	CAPE: Nr Cape of Good Hope
Polycarena Benth.				
pubescens Benth.	**14**	Batten 1052	911637	CAPE: Namaqualand, 14km from top of Kamiesberg Pass, on road to Gamoep
Tribe *Selagineae*				
Selago L.				
thunbergii	**28**	Jong 407/45	870344	CAPE: Kirstenbosch National Botanic Gardens

Abbreviations: H&B, Hilliard & Burtt; B&P, Bokelmann & Paine; CDR, Compton, D'Arcy & Rix; Distr., District; Div., Division. An unfilled space under the RBGE no. column indicates that a number has not been allocated.

TABLE 3. Generic variation in basic chromosome number.

	2n	Presumptive basic no.	Section or Group	Subsection or Subgroup
Camptoloma 2/3	**28**	7		
	c.56	7		
Glumicalyx 2/6	**14**	7		
Jamesbrittenia 1/12	**24**	6	1a	1
Jamesbrittenia 3/12	**24**	6	1a	3
Jamesbrittenia 1/7	**24**	6	1b	1
Jamesbrittenia 2/11	**24**	6	2	5
Lyperia 2/6	**16**	8		
Manulea 2/25	**16**	8	1	1a
Manulea 1/11	**16**		2	
Manulea 2/28	**16**	8	3	
Manulea 1/28	**18**	9	3	
Polycarena 1/17	**14**	7	2	
Phyllopodium 1/12	**14**	7	1	1a
Phyllopodium 2/4	**12**	6	3	
Sutera 1/3	**12**	6	1	
Sutera 3/46	**14**	7	2	
Tetraselago 1/4	**14**	7		
Trieenea 1/9	**12**	6		
Trieenea 1/9	**14**	7		
Zaluzianskya 2/20	**12**	6	1	
Zaluzianskya 1/16	**12**	6	2	2a
Zaluzianskya 1/4	**12**	6	2	2b
Zaluzianskya 4/10	**12**	6	*Holomeria*	

Notes: Fractional figures next to genus name indicate number of species examined (including published counts) to number of species in that generic subdivision. Where no such subdivisions are given, the denominator refers to the total number of species at present recognized in that genus.

TABLE 4. Infrageneric variation in chromosome number in *Sutera*.

	Chromosome no.	Habit	Section	No. of spp.
foetida (Fig. 1A)	**12**	A	1	3
archeri (Fig. 1B)	**14**	HP	2	46
roseoflava	**14**	HP	2	
x 'Longwood' (*S. hispida* x *caerulea*)	**14**	HP	2	

HP, Herbaceous perennial; A, annual herb.

Jamesbrittenia ($2n = 28$; $x = 6$). Fig. 1C.

Jamesbrittenia is a large genus of some 83 species, mostly perennial shrublets, distributed mainly in Africa from Angola and Zambia southwards; one species ranges from Egypt and the Sudan to India. The seven species examined all have $2n = 24$, which may be inferred to be based on $x = 6$. There appears to be no cytological discrimination, at least in terms of chromosome number, between the different subdivisions of the genus that are represented in this survey. Figure 1C is from *J. foliosa* (CDR 936).

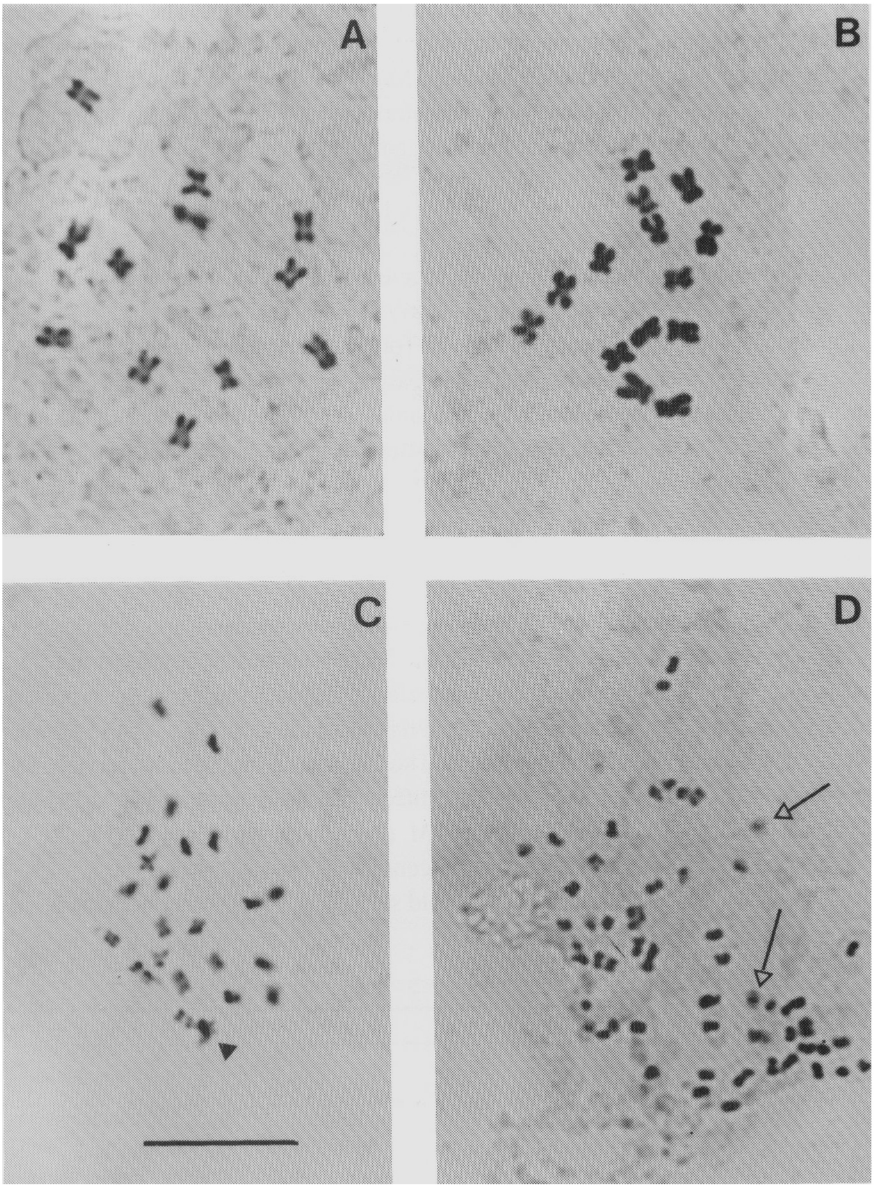

FIG. 1. A, *Sutera foetida*, $2n = 12$; B, *Sutera archeri*, $2n = 14$; C, *Jamesbrittenia foliosa*, $2n = 24$: arrowhead points to two chromosomes overlapping; D, *Camptoloma lyperiiflorum*, $2n$ = c.56: open arrows point to chromosomes markedly out of focus. Scale bar = 10μm.

Lyperia (x = 8). Fig. 2A.

The two species studied, like all but one of the six species in the genus, are annuals. Both *Lyperia tristis*, the type species, and *L. antirrhinoides* have a diploid number of $2n = 16$. They are not considered to be taxonomically close, and this is also reflected in their very different karyotypes. In *L. tristis* the chromosomes are relatively large and predominantly acrocentric

(Fig. 2A), a karyotype unusual among the *Manuleae* examined. The chromosomes of *L. antirrhinoides*, on the other hand, are predominantly meta- to submetacentric, conforming more to the pattern found in many other *Manuleae*. Many of the species previously included by Bentham (1836) in this genus have been transferred by Hilliard (1992) to *Jamesbrittenia*. Cytologically, the differences between the two genera in chromosome number as well as size support Hilliard's transfers.

Camptoloma (x = 7). Fig. 1D.
The genus *Camptoloma* comprises only three species. The first chromosome count reported for the genus was $2n = 28$ for *C. canariense* (as *Lyperia canariensis*, Borgen, 1969), an endemic of Gran Canaria. The present count of $2n =$ c.56 for *C. lyperiiflorum* (Fig. 1D) from the island of Socotra (Yemen) is most probably octoploid based on a presumptive basic number of x = 7. As mentioned earlier, its chromosomes are the smallest so far encountered in the *Manuleae*.

From the foregoing, cytological data clearly offer strong support to Hilliard's (1992) recognition of these four genera.

MANULEA

Manulea (x = 8, 9). Fig. 2C.
Manulea is a large genus, mainly of the western and south-western Cape, consisting of shrubs and perennial to annual herbs. Hilliard places the 73 species now recognized in four sections, one of which (Section 1) comprises three subsections. Table 5 indicates the representation of cytologically examined species among the subdivisions of the genus. Two basic numbers occur, x = 8 and x = 9. The latter has been encountered so far in only one species, namely *M. obovata* which has a somatic number of $2n = 18$. This differs from the other two species belonging to the same group (Section 3), *M. chrysantha* and *M. thyrsiflora*, with $2n = 16$ (Fig. 2C), a diploid number occurring also in other sections of the genus.

The present survey has revealed that a dysploid series x = 8 and 9 occurs in the genus. So far, no polyploids have been detected.

TABLE 5. Infrageneric variation in chromosome number in *Manulea*.

	Chromosome no.	Habit	Section	Subsection	No. of spp.
			1		25
crassifolia	**16**	HP	1	1a	
parviflora	**16**	HP	1	1a	
tomentosa	**16**	WP	2		11
chrysantha	**16**	A	3		28
obovata	**18**	A-SP	3		
thyrsiflora	**16**	WP	3		

HP, herbaceous perennial; WP, woody perennial; A, annual; SP, short-lived perennial.

TRIEENEA, PHYLLOPODIUM AND *POLYCARENA*

Trieenea (x = 6 and 7). Fig. 2B.
In 1989, Hilliard described three new genera 'in the general affinity of *Phyllopodium* and *Polycarena*', namely *Glekia* Hilliard, *Melanospermum* Hilliard, and *Trieenea* Hilliard. Only *Trieenea* was available for cytological study.

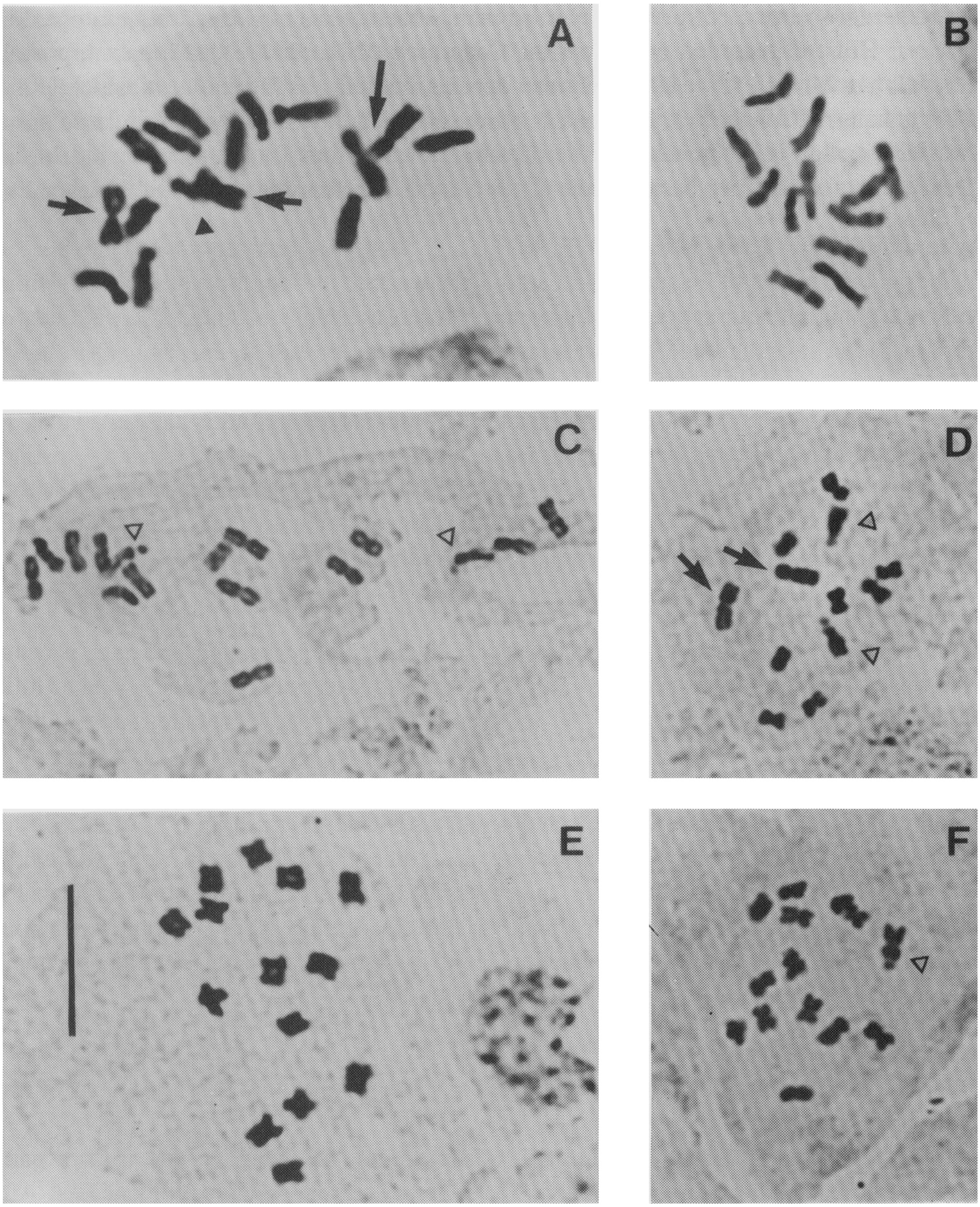

FIG. 2. A, *Lyperia tristis*, $2n = 16$: arrows indicate metacentric to submetacentric chromosomes. The rest of the genome consists of acrocentric chromosomes. Arrowhead points to two chromosomes overlapping; B, *Trieenea taylorii*, $2n = 12$; C, *Manulea thyrsiflora*, $2n = 16$; D, *Phyllopodium cephalophorum*, $2n = 12$: arrows indicate pair of heterozygous chromosomes, far left member with median centromere, the other submedian; E, *Polycarena pubescens*, $2n = 14$; F, *Glumicalyx montanus*, $2n = 14$. Scale bar = 10µm. Open arrowheads indicate SAT chromosomes.

Trieenea comprises at least nine species which are found only in the mountains of the western and southern Cape, occurring as shrublets to annual herbs. One of two species examined was *T. taylorii* Hilliard, recorded only from the Cedarberg (Hilliard, 1989). The plant, probably perennial, has $2n$ = 12 (Fig. 2B). The other species studied was *T. glutinosa*, which Hilliard (1989) transferred from *Phyllopodium* (*P. glutinosum* Schltr.). It can perennate, and has the widest distribution in the genus; it differs chromosomally from *T. taylorii* in having $2n$ = 14. *Trieenea* therefore has two basic numbers, x = 6 and x = 7, forming a dysploid series.

Phyllopodium (x = 6 and 7). Fig. 2D.
Phyllopodium consists of 26 species, mostly of the western and south-western Cape. Hilliard divides the genus into two subgenera. Three species were available for study: *P. cephalophorum* and *P. pumilum* have the same somatic number $2n$ = 12, x = 6, and *P. cuneifolium* has $2n$ = 14, x = 7. The two first-mentioned species belong to one subgenus, a small assemblage of only four species, all annuals. *P. cuneifolium* belongs to the other subgenus and is also an annual. It is interesting to note in *P. cephalophorum* that members of one chromosome pair are distinctly dimorphic (Fig. 2D), the only example found so far of structural heterozygosity not involving the satellite chromosomes. Meiotic analysis and a wider cytological sampling may throw further light on the extent and nature of structural change in the genus.

Polycarena (x = 7). Fig. 2E.
Polycarena is a genus of about 17 species which, like *Phyllopodium* and *Trieenea*, is centred in the western and south-western Cape. Within the genus, two groups of species, which Hilliard upholds, had earlier been recognized by Bentham (1846); each now contains many additional species.

The single species examined here is *P. pubescens* (which belongs to Hilliard's Group 2) with a somatic number of $2n$ = 14 (Fig. 2E), and hence a basic number x = 7.

From the foregoing, the same basic numbers occur in both *Trieenea* and *Phyllopodium*, viz. x = 6 and 7. These two genera, therefore, cannot be distinguished on chromosome number alone. *Polycarena* has x = 7, but this is based on only a single accession; further counts are obviously needed. It would be especially interesting to see if the subgeneric difference in basic number observed in *Phyllopodium* can be confirmed.

Zaluzianskya (x = 6). Fig. 3A.
Hilliard recognizes 55 species of *Zaluzianskya* and allocates them to four sections, one of which consists of two subsections.

Perennial to annual herbs, *Zaluzianskya* occurs mainly in the Cape and eastern part of southern Africa, with only one species, *Z. elgonensis*, extending into tropical East Africa (Hedberg, 1970). The seven species examined are all annuals, and include representatives from three sections and two subsections; all have a diploid number of $2n$ = 12, and thus a common basic number of x = 6. These counts conform to those already published under the synonym *Nycterinia* D. Don (Table 1), namely $2n$ = 12 for *Z. capensis* and *Z. villosa* (Fig. 3A). The same number occurs in *Z. elgonensis* from Mt Elgon, Kenya (Hedberg, 1970), the only tropical link in *Zaluzianskya* through the section *Holomeria* to which it belongs (Hilliard & Burtt, 1983).

Glumicalyx (x = 7). Fig. 2F.

Glumicalyx comprises six species of perennial herbs or subshrubs, all endemic to the Drakensberg area of south-eastern Africa.

The type species, *G. montanus* (Fig. 2F), and *G. goseloides* both have a diploid number of $2n = 14$. This differs notably from the uniform $2n = 12$, x = 6 recorded for eight species of *Zaluzianskya*, lending cytological endorsement to Hilliard & Burtt's (1977) transference of *Zaluzianskya goseloides* to *Glumicalyx*.

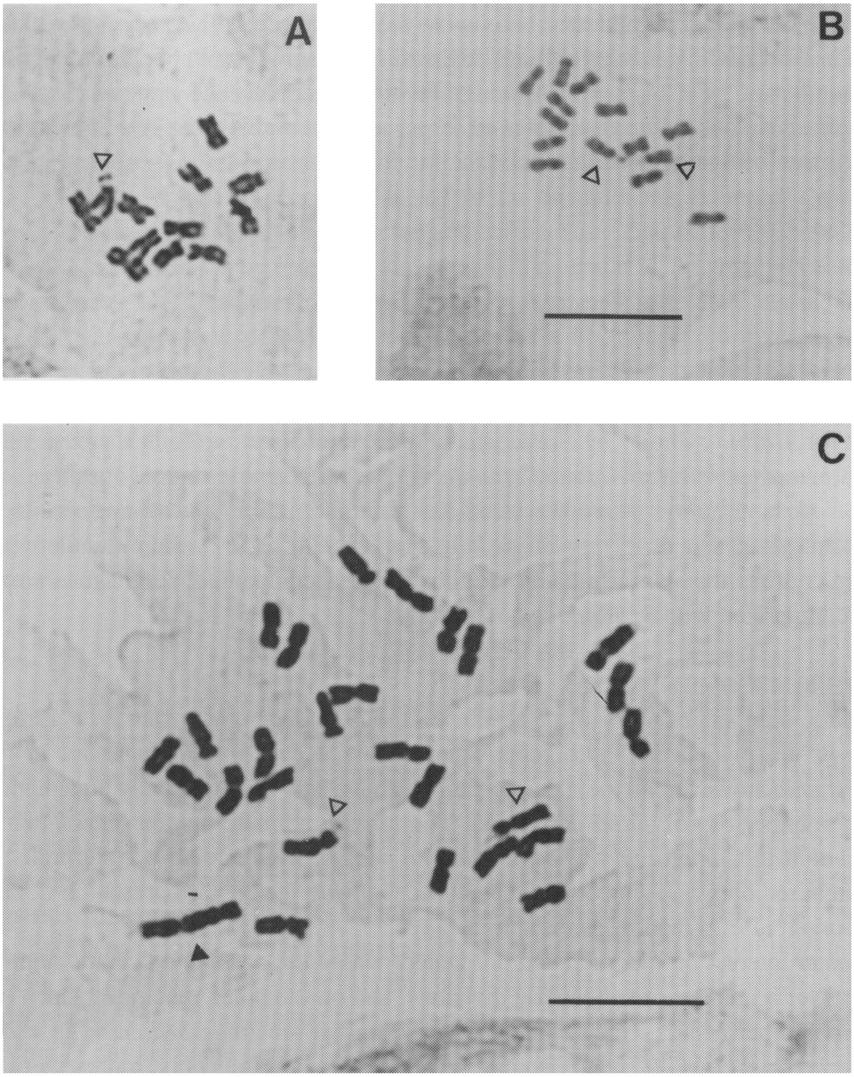

FIG. 3. A, *Zaluzianskya villosa*, $2n = 12$; B, *Tetraselago natalensis*, $2n = 14$, scale bar = 10μm; C, *Selago thunbergii*, $2n = 28$. Solid arrowhead indicates two chromsomes overlapping. Scale bar = 10μm, applies also to A. Open arrowheads indicate SAT chromosomes.

Tetraselago (x = 7). Fig. 3B.
A genus of only four species of tufted woody perennial herbs up to 1m high, *Tetraselago* is found only in the eastern part of South Africa, that is, the northern and eastern Transvaal, Swaziland and Natal (Hilliard & Burtt, 1977). This genus was created by Junell (1961) for a few species originally described in *Selago* but with two ovules in each loculus (*Selago* has only one). He suggested plausibly the route by which the *Tetraselago*-type ovary could have been derived from one similar to that of *Glumicalyx montanus*. For a more detailed discussion of this, see Hilliard & Burtt (1977).

The single collection studied here belongs to the type species, *T. natalensis*, which is quite common in the Natal Midlands (Hilliard & Burtt, 1977), growing in open grasslands. It has a diploid number of 2*n* = 14, x = 7. Although this is numerically the same as *Glumicalyx*, the chromosomes of *T. natalensis* appear to be larger and it remains to be seen to what extent the two genera can be distinguished karyotypically.

TRIBE *SELAGINEAE*

Selago (x = 7). Fig. 3C.
As stated earlier, species of *Tetraselago* were originally described in the genus *Selago* in the tribe *Selagineae*.

In this investigation, only one species of *Selago* was available for examination. This species, *S. thunbergii*, has a diploid number of 2*n* = 28. The only previous chromosome count is that recorded for *S. albida* Choisy by Nordenstam (1969), with 2*n* = 14. Thus, the basic number for *Selago* is presumed to be x = 7, and *S. thunbergii* is therefore a tetraploid species.

Tetraselago and *Selago* share the same basic number, x = 7, but they seem to differ in chromosome size, that of *S. thunbergii* being larger (Fig. 3C). A more detailed analysis is needed to confirm this. Unfortunately, no illustration accompanied Nordenstam's report, and hence a comparison with *S. albida* is not possible.

BASIC CHROMOSOME NUMBERS
The somatic numbers of most of the species in the *Manuleae* range from 2*n* = 12 to 2*n* = 18, so far with no evidence of intrageneric polyploidy except in *Camptoloma*. In the genus *Jamesbrittenia*, however, all the species examined have 2*n* = 24, a relatively high number which is most probably of polyploid derivation. The highest somatic number encountered in this survey is that in *Camptoloma lyperiiflorum*, 2*n* = c.56, which could be interpreted as being octoploid based on x = 7, which would be consistent with the 2*n* = 28 present in *C. canariense* which Borgen (1969) regards as tetraploid.

Of special interest on the one hand are genera where the basic and somatic numbers seem constant over a number of subgeneric divisions, and on the other, genera in which more than one basic number occurs. Good examples of the former are *Jamesbrittenia* (2*n* = 24, x = 6) and *Zaluzianskya* (2*n* = 12, x = 6) from a reasonably broad infrageneric representation of each genus (Table 3). More than one basic number has been observed within several genera: *Manulea* x = 8, 9; *Phyllopodium* x = 6, 7; *Sutera* x = 6, 7, and *Trieenea* x = 6, 7, that is, the presence of a dysploid series within each of these genera. They clearly merit further in-depth cytological study to ascertain the interrelationship of these interspecific basic numbers and their karyotypes, which may shed some light on the direction of chromosomal change. This in turn may be of value to a taxonomic appraisal of evolutionary relationships.

Bearing in mind that the species representation among the genera examined is uneven, as indeed is the size of the different genera, the basic numbers at the tribal level form a dysploid series of x = 6, 7, 8 and 9. The most frequently encountered basic numbers in the present sample are x = 6 and 7, whereas x = 9 has been encountered only once, and this in a species of *Manulea*, namely *M. obovata* with a somatic number of $2n = 18$ in a genus where the majority of species have x = 8. It is interesting to note the evident rarity of x = 9 in the *Manuleae*, a number that seems to be characteristic of certain genera elsewhere in the Scrophulariaceae, for example *Diascia* and *Nemesia* in the *Hemimeridae* (Jong, 1989). The relatively high number $2n = 24$ of *Jamesbrittenia* may have been derived from x = 6.

Even where the chromosome number appears to be stable, it is quite possible that cytological diversification might have occurred, involving structural change without altering the basic number. Evidence for this conjecture is based so far on only one example, *Phyllopodium cephalophorum*, in which members of the longest chromosome pair differ in shape as well as size. This heterozygosity may possibly have been the result of an unequal chromosome interchange, but unfortunately material is not available for a more analytical study. Differences in the size of the satellite in satellited chromosomes have been observed in many *Manuleae*. Such polymorphisms may be another manifestation of heterozygosity. The distinctive karyotype of *Lyperia tristis* that consists of a majority of acrocentric chromosomes may also have arisen through structural rearrangements.

While the cytological data presented and discussed above have proved to closely parallel the current generic concepts of Hilliard's revision of the *Manuleae*, they are, however, insufficient at this juncture to allow a meaningful assessment of possible evolutionary direction of chromosomal change.

CONCLUSIONS AND SUMMARY

The main aim here is to highlight the significance of cytological information, primarily chromosome number in this report, to the taxonomic revision of the *Manuleae*. Karyotypic differences have also been noted, but only brief reference has been made where appropriate, pending detailed investigation.

The present cytological survey of 11 genera and some 36 species of the *Manuleae* reveals a close parallelism between variation in chromosome number and generic (in some cases infrageneric) divisions of the tribe. Such a parallelism has also been noted in the *Antirrhineae* (Sutton, 1988). The sample of taxa examined is very small, and with this limitation in mind, it can be seen that cytology provides a valuable and independent source of evidence vindicating Hilliard's realignment of taxonomic boundaries within the *Manuleae*.

Variation in chromosome number is taxonomically most useful at the generic level, and in certain genera (e.g. *Sutera*, *Phyllopodium*) possibly at the subgeneric level. In a few cases, interspecific differences in chromosome number have been observed, for example *Manulea obovata* with $2n = 18$, whereas the other species have $2n = 16$, and the two species of *Trieenea* having $2n = 12$ and $2n = 14$ respectively, but it is uncertain how common such differences are. It is clear, however, that there exists in the *Manuleae* a dysploid series of basic numbers, x = 6, 7, 8 and 9. The somatic chromosome number ranges from $2n = 12$ to $2n = c.56$. Intrageneric polyploidy appears to be rare, occurring so far only in *Camptoloma* (*C. canariense*, $2n = 28$, *C. lyperiiflorum*, $2n = c.56$). All the seven species of *Jamesbrittenia* are uniformly $2n = 24$; polyploidy is most likely implicated in the history of this genus, and perhaps more clearly so in *Camptoloma*. Otherwise, diversification in the *Manuleae* seems to have proceeded largely at the diploid level, possibly involving structural modifications to the chromosomes.

As far as *Selago* and *Tetraselago* are concerned, their common basic number $x = 7$ may suggest a close relationship that bridges two major tribes in the Scrophulariaceae. This inference, however, demands a much more substantive foundation.

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