RELATIONSHIPS OF SOUTH-EAST AUSTRALIAN SPECIES OF SENECIO (COMPOSITAE) DEDUCED FROM STUDIES OF MORPHOLOGY, REPRODUCTIVE BIOLOGY AND CYTOGENETICS

- by -

Margaret Elizabeth Lawrence, B.Sc. (Hons.)
Department of Botany, University of Adelaide

Thesis submitted for the Degree of
Doctor of Philosophy, University of Adelaide

May, 1981

Awarded 4 December 1981

TABLE OF CONTENTS

Volume 2

CHAPTER 4 Reproductive Biology 205

4.1 Introduction 206

4.2 Materials and methods 208

4.2.1 Glasshouse trials 209

4.2.2 Pollen-ovule ratios 211

4.2.3 Seed size and number 211

4.2.4 Seedling establishment 212

4.2.5 Longevity 212

4.3 Results and observations 212

4.3.1 Direct and indirect evidence of breeding systems 212

4.3.2 Observations of floral biology 218

4.3.3 Pollen vectors 219

4.4 Discussion 220

4.4.1 Mode of reproduction 220

4.4.2 Breeding systems 220

4.4.3 Breeding systems and generation length 221

4.4.4 Seed size and number 223

4.4.5 Dispersal potential 225

4.4.6 Seedling establishment 226

4.4.7 Combinations of reproductive traits: r- and K-selection 228

4.5 Conclusions 235

CHAPTER 5 Recombination in *Senecio* 237

5.1 Introduction 238

5.2 Materials and methods 240

5.3 Results and discussion 241

5.3.1 Chromosome numbers 241

5.3.1.1 Ploidy distributions in *Senecio* 241

5.3.1.2 Polyploidy and recombination 249

5.3.1.3 Polyploidy and speciation 251

5.3.2 Effects of chiasma frequency and position 253

5.3.3 Effects of breeding systems 255

5.3.4 Effects of generation lengths 257

5.3.5 Pair-wise associations of regulatory factors 258

5.3.5.1 Breeding system and generation length 259

5.3.5.2 Breeding system and chromosome number 261

5.3.5.3 Breeding system and chiasma frequency 261

5.3.5.4 Generation length and chromosome numbers 264

5.3.5.5 Generation length and chiasma frequency 264

5.3.5.6 Chiasma frequency and chromosome number - the "Recombination Index" 265

5.3.6 Recombination systems in *Senecio* 267

5.4 Conclusions 272

CHAPTER 6 Nuclear DNA Amounts 277

6.1 Introduction 278

6.2 Materials and methods 280

6.2.1 Source of material 280

6.2.2 Feulgen stain and SO_2 water 281

6.2.3 Cultivation 281

6.2.4 Preparation of slides 281

6.2.5 Measurement of DNA amount 283

6.2.6 Analysis of results 284
6.2.7 Hydrolysis times and root tip sizes 284
6.2.8 Selection of a calibration standard 286
6.2.9 Cell volumes 287
6.3 Results and discussion 287
6.3.1 Terminology 287
6.3.2 Comparison of calibration standards 288
6.3.3 Reliability of DNA estimates and size of significant differences 290
6.3.4 Interspecific differences in DNA amounts 292
6.3.5 Intraspecific differences in DNA amounts 296
6.3.6 Nucleotypic effects 298
6.3.6.1 Size of structures 299
6.3.6.2 Cell cycle times 300
6.3.6.3 Minimum generation times 302
6.3.7 Nature of changes in DNA amounts 304
6.3.8 Speculations on the direction of changes in DNA amounts 306
6.4 Conclusions 312

CHAPTER 7 Karyotypes 314
7.1 Introduction 315
7.2 Materials and methods 316
7.2.1 Karyotype construction 316
7.2.2 Interspecific comparisons 318
7.3 Results and discussion 325
7.3.1 Illustration of karyotypes 325
7.3.2 Comparison of karyotypes 326
7.3.2.1 Size of significant differences 326
7.3.2.2 Interpretation of data 326

7.3.2.3 Relationships deduced from percentage similarities 327
7.3.2.4 Satellite chromosomes 331
7.3.3 Karyotype symmetry and evolutionary advancement 334
7.3.4 Changes in absolute chromosome size 339
7.3.5 The basic chromosome number of *Senecio* 340
7.3.5.1 Karyotype symmetry and absolute chromosome size 342
7.3.5.2 The number of satellite chromosomes 343
7.4 Conclusions 345

CHAPTER 8 Natural and Synthetic Hybrids 347
8.1 Introduction 348
8.2 Materials and methods 349
8.3 Results and discussion 350
8.3.1 Natural hybrids 350
8.3.1.1 Characteristics of hybrid and parent plants 350
8.3.1.2 Evidence used to determine parent species 361
8.3.1.3 Pollen and seed development 363
8.3.2 Crossing programs 365
8.3.2.1 Program 1 367
8.3.2.2 Program 2 370
8.3.3 Extended studies of *S. pterophorus* x *S. hypoleucus* 370
8.3.3.1 Frequency of natural hybridization 371
8.3.3.2 Likelihood of fertile hybrid formation 373
8.3.3.3 Evidence of additive gene effects 374

8.3.4 The rayed gene complex in *Senecio* 375
8.3.5 Origins of decaploid species 377
8.3.6 Hybridization and polyploidy in *Senecio* and Senecioneae 379

CHAPTER 9 General Conclusions 383
9.1 Systematics of Senecioneae in Australia 384
9.1.1 The generic status of species examined 384
9.1.2 Subdivisions of Australian species of *Senecio* 387
9.2 The application of current evolutionary theories 389
9.2.1 r- and K-selection 389
9.2.2 Recombination systems 390
9.2.3 The C-value paradox 392
9.2.4 Karyotype evolution 393
9.3 Polyploid evolution in Senecioneae and *Senecio* 394
9.4 The size of *Senecio* 398

REFERENCES 400

APPENDIX 1 420

APPENDIX 2 434

APPENDIX 3 446

CHAPTER 4

Reproductive Biology

4.1 Introduction

4.2 Materials and Methods

- 4.2.1 Glasshouse trials
- 4.2.2 Pollen-ovule ratios
- 4.2.3 Seed size and number
- 4.2.4 Seedling establishment
- 4.2.5 Longevity

4.3 Results and Observations

- 4.3.1 Direct and indirect evidence of breeding systems
- 4.3.2 Observations of floral biology
- 4.3.3 Pollen vectors

4.4 Discussion

- 4.4.1 Mode of reproduction
- 4.4.2 Breeding systems
- 4.4.3 Breeding systems and generation length
- 4.4.4 Seed size and number
- 4.4.5 Dispersal potential
- 4.4.6 Seedling establishment
- 4.4.7 Combinations of reproductive traits: r- and K-selection

4.5 Conclusions

4.1 Introduction

The term "reproductive biology" is often equated with breeding or mating systems, but I have adopted the more general interpretation of events from anthesis to the establishment of the next generation. Five characteristics are therefore considered: (1) breeding systems, or factors controlling the parentage of seeds, (2) seed size and number, (3) dispersal potential, (4) seedling establishment and (5) generation length. Chromosome numbers and chiasma frequencies are also of relevance, but as they are extensive topics I have discussed them in the next chapter. Interspecific hybridization is considered in Chapter 8 as meiotic configurations and karyotype morphologies are an integral part of the evidence.

The effects of differing modes of reproduction are diverse. Ornduff (1969) discussed the relationship between reproductive biology and systematics, commenting that "systematists should be fully aware of the morphological patterns that are associated with different reproductive systems." For example, convergent evolution in species adapted to the same pollinator or in species possessing similar breeding systems may lead to the erroneous grouping of distantly related taxa. Similarly, the evolution of autogamy in one of two closely related taxa may lead to rapid morphological divergence and subsequent misclassification. Errors of this kind are often avoided if a range of microcharacters, not influenced by selection for different reproductive strategies, are included in the study.

Differing reproductive systems affect not only the direction of morphological change, but also the patterns of variability found within and between populations. Obligate selfing will

increase the chances of genetic homozygosity and the phenotypic expression of locally different forms. Outcrossing, by promoting inter-population gene-flow, will reduce the chance of localized differentiation. As taxonomic delimitation of species relies on the presence of discontinuities in variation, breeding systems will strongly affect the final form of a classification.

Baker (1959) illustrated the effects of breeding systems using the orchid genus Epipactis as an example. Very local, more or less cleistogamous forms have been described as species on the basis of differences in floral characters while the smaller number of outbreeding species are acknowledged to be extremely variable.

Although morphological expression may be increased by homozygosity in autogamous populations, the overall genetic variation within such populations may be less. Hamrick et al. (1979) reviewed evidence of genetic variation in plants determined by studies of enzyme polymorphisms. They compared overall genetic variation with a range of life history characteristics and concluded that, in the case of breeding systems, genetic variation was generally less in primarily selfing species.

Thus breeding systems, and other aspects of reproductive biology, will affect both the extent of genotypic variation and its expression in the phenotype.

The idea that the amount of genetic variation within a population might be regulated by differing reproductive strategies was considered in detail by Grant (1958). Grant discussed the influence of a range of plant features - including longevity, breeding system, dispersal potential and population size - on the amount of genetic recombination expressed per unit of chronological time. Combinations of factors promoting recombination, such as short generations, cross pollination, wide dispersal

and large populations characterize "open" recombination systems whereas long generation times, autogamy, restricted dispersal and small populations characterize "restricted" recombination systems. "Closed" systems are associated with asexual reproduction. According to Grant (1958) and Stebbins (1958) different recombination systems are selected for when environmental conditions favour genetic uniformity or genetic diversity. Although the factors listed by Grant have not met with opposition, the concept of "group" selection of these factors has been strongly opposed by Maynard Smith (1964), Williams (1966) and Lloyd (1979). As Lloyd commented, the evolution of different reproductive strategies "must be sought in selective forces affecting individuals in each generation," not in their hypothetical long-term advantages. However, Lloyd also recognised that long-term advantages will be significant in determining the persistence and multiplication of populations. In my opinion it is therefore necessary to consider both the immediate and long-term advantages of reproductive characters if present patterns of population variation are to be understood.

4.2 Materials and Methods

All species included in this study were observed both in the field and under glasshouse conditions. Glasshouse specimens were usually raised from seed but perennial species (with the exception of *Bedfordia salicina*) could also be readily propagated from cuttings. Axillary shoots about 5 cm long removed with a short "heel" of tissue were most successful, and produced roots within two to three weeks when treated with a commercial preparation of

hormonal rooting powder.[1] Pots of fresh cuttings were placed in beds of moist vermiculite, but were left uncovered, as high levels of humidity seemed to promote fungal growth and to adversely affect herbaceous stems. Glasshouse space did not permit specimens to be maintained in pots larger than 13 cm in diameter, but this was not a serious problem as all perennial species responded well to heavy pruning. Plants were regularly sprayed with a pyrethrum-based "garden safety spray" as aphids were otherwise a problem, causing distortion or withering of developing capitula. Rare infestations by red spider mites were treated with Malathion.

4.2.1 Glasshouse Trials

The breeding systems of nine species (see Table 4.1) were tested by a series of four treatments of capitula:

1. Tagged but otherwise untreated to test for seed set under glasshouse conditions;
2. Bagged but otherwise untreated to test for self-compatibility;
3. Bagged after removal of bisexual florets to test for apomixis;
4. Bagged after removal of bisexual florets and later cross-pollinated with a plant of the same species to determine seed set in self-incompatible species.

Twenty capitula from each species were tested in each treatment - a total of 80 capitula per species. To achieve this number a minimum of 8 and a maximum of 12 plants were raised from seed collected from several plants in a population. Methods were as follows:

1. Capitula and treatments were identified by hardware

---

[1] "Serradix" 2. for semi hardwoods. May and Baker (M and B)Pty.Ltd.

labels attached to each peduncle.

2. Pollen was excluded by bags constructed from rectangles of glassine paper edged with double-sided adhesive (Fig 4.1A). When capitula were ready to be bagged, the adhesive backing was removed and the bag formed by folding the rectangle in half and sealing the edges (Fig. 4.1B). If necessary, bags were opened by inserting the tips of a pair of forceps into the bag next to the peduncle and gently prising the edges apart. This method, described to me by Dr. R. O. Belcher (personal communication), has the advantages of negligible bag weight and a bag size appropriately adjusted to capitulum size. It was therefore possible to treat many capitula without unduly weighting the inflorescence.

3. Capitula of radiate and erechthitoid species were effectively emasculated by removing all bisexual florets before any florets had opened (Fig. 4.1C, D, G and H). The remaining female florets were bagged for two to four days until styles had emerged (Fig. 4.1 E and I) and then pollinated if included in treatment 4. Capitula with all bisexual florets (_Senecio hypoleucus_, _S. odoratus_ and _S. vulgaris_) were not emasculated. Ornduff (1964) removed pollen from similar capitula by cutting off the upper portions of young flower heads with a razor blade. However, his technique relies on spatial separation of the style apex and anther collar which did not occur in _S. hypoleucus_ and _S. odoratus_. In both species the style apex remained just below the anther collar throughout development. Treatment 3 was therefore omitted for the above-mentioned capitula, and cross-pollination of entire capitula examined in treatment 4.

4. Capitula were harvested when the phyllaries spread apart (Fig. 4.1F and J) indicating full seed development. Bagged capitula and their identifying labels were harvested into

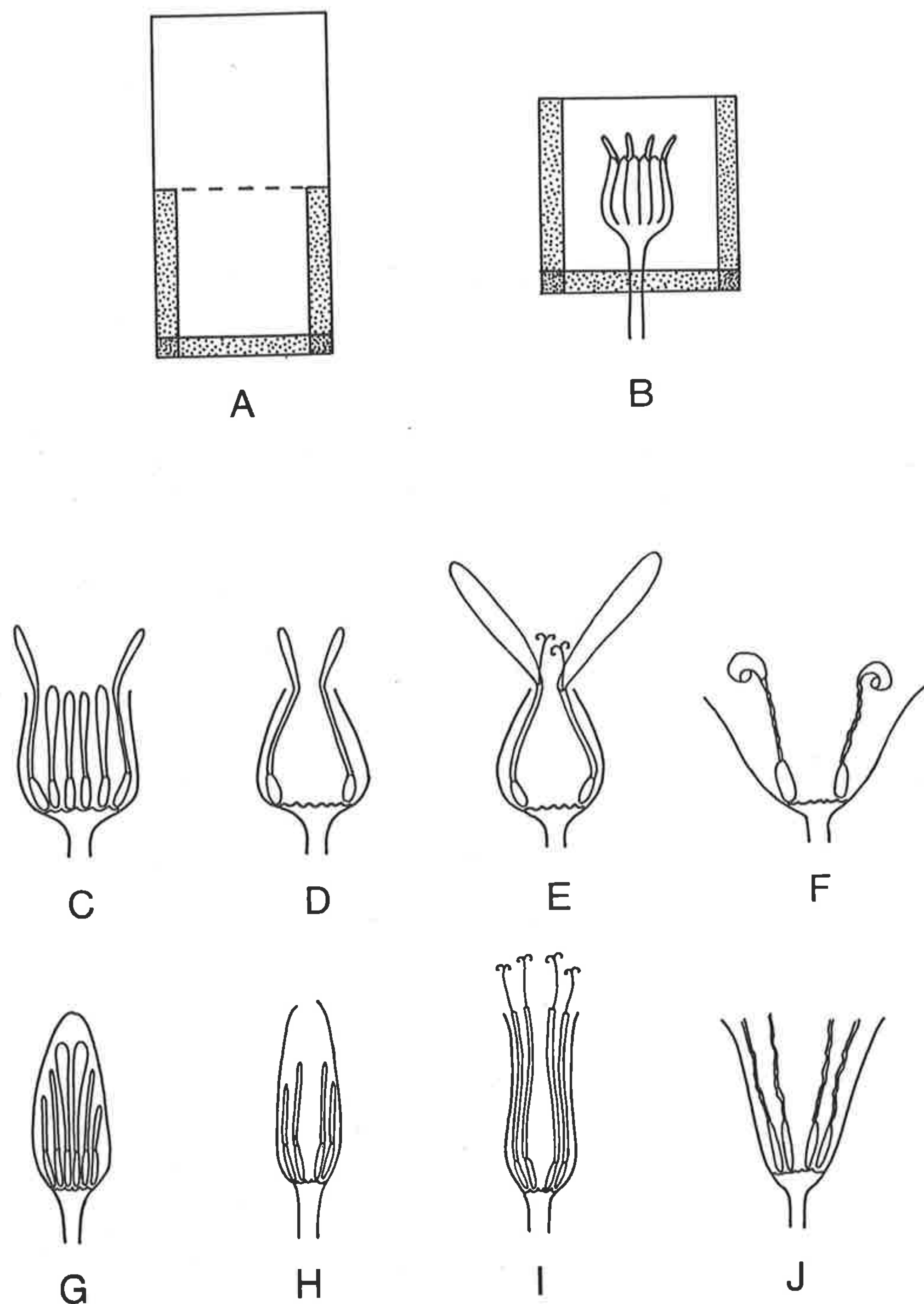


Fig. 4.1 Treatment of capitula during crossing programs.
A. Glassine bag edged with double-sided adhesive for pollen exclusion. B. Bag folded over immature capitulum. C-F Effective emasculation of a radiate capitulum by removal of bisexual florets, and subsequent development of female ray florets.
G-J. Similar sequence in an erechthitoid capitulum with female filiform florets.

individual envelopes, and total floret numbers and proportionate seed set scored in the laboratory. In these experiments, fertile seed was always plump and variously coloured whereas sterile seed was white and shrivelled. Intermediates were encountered only in the case of interspecific crosses described in Chapter 8.

4.2.2 Pollen-Ovule Ratios

These were determined by counting all pollen grains in one anther lobe from each of five plants. Whole anthers were mounted in water, squashed gently to spread the pollen grains in a single layer, and examined at 100X magnification. Pollen-ovule ratios are usually determined by dividing the number of pollen grains per flower by the number of ovules per flower (Cruden 1977). As Compositae florets mature only one ovule the calculation for bisexual florets is simple. However, some florets within radiate and erechthitoid capitula are female and produce no pollen. I therefore decided to calculate pollen-ovule ratios as "per capitulum" values, as parental resource allocation with respect to pollen must account for both female and bisexual floret numbers. Pollen-ovule ratios per capitulum were calculated using the following formula:

$$\frac{\text{pollen grains/lobe} \times \text{lobes/floret} \times \text{bisexual florets/capitulum}}{\text{total floret number/capitulum}}$$

4.2.3 Seed Size and Number

Seed weight was used as an indicator of seed size. Individual weights were calculated from the total weight of 50 seeds as many seeds were very small (about 0.0002 gms). Seed number per plant was estimated from the total floret number per plant - the product

of florets/capitulum, capitula/inflorescence and inflorescences/ plant.

4.2.4 Seedling Establishment

Percentage germination was recorded for fifty seeds of each taxon. A standard soil mix of two parts sterile garden loam and one part vermiculite was used in all cases. The following parameters were measured in 10 seedlings of each species: 1. hypocotyl length 2. cotyledon petiole length 3. cotyledon blade length and width. All measurements were made when the first true leaf had extended to one quarter the length of a cotyledon (about one week after germination) as structures expanded rapidly during the first few days.

4.2.5 Longevity

Field and glasshouse observations were combined to assess longevity. All species except *Bedfordia salicina* were maintained under glasshouse conditions for at least 18 months, or in the case of annuals, until natural senescence occurred. The longevity of four species is still in doubt as they behaved as annuals in the glasshouse but appeared, by size and branching patterns, to be short-lived perennials in the field. These species are indicated by a question mark in Table 4.2.

4.3 Results and Observations

4.3.1 Direct and Indirect Evidence of Breeding Systems

The breeding systems of nine species were directly assessed by glasshouse trials. The indirect evidence of capitulum "showiness", pollen-ovule ratios and seed set in untreated capitula were then used to predict the breeding systems of other species. Results of the four treatments used in glasshouse

trials are given in Table 4.1. Very low seed set (.6 to 1.9%) was recorded for S. lautus and S. pterophorus in treatment 3, in which all bisexual florets had been removed. These results could be evidence of a low level of apomixis but I believe that accidental contamination is a more likely explanation. Pollen exclusion by bagging in treatment 2 indicated that four species are self-compatible and five are self-incompatible. However, none of the self-compatible species are obligate selfers as high seed set was recorded for cross-pollinated female florets (treatment 4). Treatments 1 and 2 gave very similar results, so that seed set in untreated capitula (treatment 1) is indicative of breeding systems.

The indirect evidence of pollen-ovule ratios (Cruden 1977) and capitulum showiness (Stebbins 1958) are also indicative of breeding systems. The four autogamous species have a pollen-ovule ratio of less than 200 and inconspicuous capitula, whereas all self-compatible species have pollen-ovule ratios in excess of 2000 and showy capitula. Showy capitula have either large ray florets or densely clustered discoid capitula with well exserted florets. Inconspicuous capitula have very reduced ray florets or discoid capitula with florets scarcely exserted. On the basis of results presented in Table 4.1 the breeding systems of all other species were predicted from observations of seed set in untreated glasshouse plants, pollen-ovule ratios and capitulum morphology. Results are presented in Table 4.2 along with seed parameters and evidence of longevity. Data for subspecies and varieties have been pooled to give mean values for each species. New Zealand plants of S. lautus, however, are listed separately as their breeding system differs from that of mainland forms.

TABLE 4.1

Direct and Indirect Evidence of Breeding

System in Nine Species of Senecio

Species, collection numbers**		Percentage seed set* 1	2	3	4	Breeding System	P/O Ratio	Capitulum Type
S. lautus	644	0.3	0	0.6	80.5	I	3345	R/Sh
S. gregorii	1010	0	0	0	64.2	I	5066	R/Sh
S. pterophorus	647	0	0.6	1.9	63.0	I	2252	R/Sh
S. hypoleucus	646	0	0	-	62.7	I	2980	D/Sh
S. odoratus	657	0	0.2	-	73.3	I	2870	D/Sh
S. glossanthus	475	68.0	74.2	0	82.2	C	146	R/In
S. vulgaris	552	83.7	71.5	-	87.2	C	68	D/In
S. quadridentatus	790	47.6	41.0	0	61.3	C	68	E/In
S. glomeratus	648	61.2	58.4	0	73.4	C	135	E/In

* mean values of 20 capitula in each of four treatments described in 4.2.1.

** see population localities given after each species description in Chapter 3.

Symbols: I = self-incompatible; C = self-compatible; P/O = pollen-ovule; R = radiate;
D = discoid; E = erechthitoid; Sh = showy; In = inconspicuous

TABLE 4.2

Features of Reproductive Biology

	pollen-ovule ratio per capitulum	capitulum type	longevity and habit	mean seed weight gm x 10^5	seeds per plant	% seed germination
OUTBREEDING SPECIES						
Senecio lautus (Australia)	3345	R/S	PS	23	3	80
S. spathulatus	4426	R/S	PS	148*	2	95
S. gregorii	5066	R/S	E	590*	2	85
S. magnificus	7008	R/S	PS	385*	3	5
S. pectinatus	3522	R/S	PH	-	1	-
S. velleioides	4333	R/S	?A	54	3	35
S. amygdalifolius	4948	R/S	PH	185	2	-
S. macranthus	5890	R/S	PS	240	3	65
S. vagus	5234	R/S	?A	241	3	30
S. linearifolius	2315	R/S	PS	29	4	75
S. sp. A	2903	R/S	PS	26	3	-
S. hypoleucus	2980	D/S	PS	22	5	80
S. odoratus	2870	D/S	PS	25	4	85
S. cunninghamii	3200	D/S	PS	29	3	65
S. anethifolius	4090	D/S	PS	27	3	-
S. gawlerensis	4240	D/S	PS	57	4	95

Table 4.2 - Continued

	pollen-ovule ratio per capitulum	capitulum type	longevity and habit	mean seed weight gm x 10^5	seeds per plant	% seed germination
S. discifolius	4556	R/S	E	29	1	65
S. pterophorus	2552	R/S	PS	17	5	80
S. mikanioides	2847	D/S	PH	-	5	-
Bedfordia salicina	3030	D/S	T	36*	5	0
		INBREEDING SPECIES				
S. lautus (N. Zealand)	830	R/I	A	21	1	85
S. glossanthus	146	R/I	E	(49 / 24)	2	95
S. quadridentatus	68	E/I	PH	15	5	95
S. gunnii	70	E/I	PH	18	5	85
S. aff. apargiaefolius	64	E/I	?PH	18	2	90
S. runcinifolius	93	E/I	PH	20	3	100
S. biserratus	108	E/I	A	17	3	70
S. sp B	102	E/I	A	24	4	100
S. squarrosus	129	E/I	A	31	1	55
S. bipinnatisectus	103	E/I	A	22	3	70
S. minimus	43	E/I	A	18	3	90
S. picridioides	127	E/I	A	20	3	85
S. glomeratus	135	E/I	A	10	3	95
S. hispidulus	237	E/I	A	15	3	90
S. sp. C	117	E/I	A	27	2	85
S. vulgaris	512	D/I	A	22	3	90
Arrhenechtites mixta	296	R/I	?A	162	1	65
Erechtites valerianaefolia	302	E/I	A	39	5	95

Table 4.2 - continued

Symbols:

Capitulum type: R = radiate, D = discoid, E = erechthitoid,
S = showy, I = inconspicuous

Longevity: E = ephemeral, A = annual, PH = perennial herb,
PS = perennial shrub, T = tree

Seeds per plant: 1 = $<$1000, 2 = 1001-2000, 3 = 3001-10,000,
4 = 10,000-20,000, 5 = $>$20,000

* = achenes with a persistent pappus

4.3.2 Observations of Floral Biology

Stages of anthesis observed in *Senecio* are typical of the family Compositae (Carlquist 1976). The inflorescence is determinate with central or apical capitula maturing first (Fig. 4.2A), but the sequence of anthesis within capitula is indeterminate as marginal florets mature first (Fig. 4.2 B to D). All bisexual florets are protandrous but capitula with marginal female florets are functionally protogynous. As Carlquist (1976) observed, the opportunities for various degrees of outbreeding and inbreeding are therefore numerous on morphological grounds alone.

In all species examined, anthers dehisced when the florets were closed, but self-pollination at this stage is unlikely as the style branches are closely adpressed and are held together by the narrow corolla. Pollination occurs only after the style branches have extended beyond the collar of anthers, at which point the branches spread apart and expose the stigmatic surface (Fig. 4.2 H). In *Senecio* (and probably in many other Compositae) I found that presentation of pollen appeared to be dependent on drying of the anthers. Immediately before anthesis the style apex is positioned at the base of the anthers, but the latter forms only a loose collar as tissues are moist and expanded (Fig. 4.2 E). When the corolla opens, the anther collar dries and contracts and is exserted above the corolla as the style elongates (Fig. 4.2 F). In most species the staminal filaments are coiled in bud, presumably to allow for the elevation of anthers. When the style eventually passes between the anthers, the collar has narrowed so that most pollen is pushed ahead of the apical sweeping hairs on the style (Fig. 4.2 G). Possible functions of two staminal structures deserve comment. Firstly, the sterile distal appendages on the anthers appear to serve as

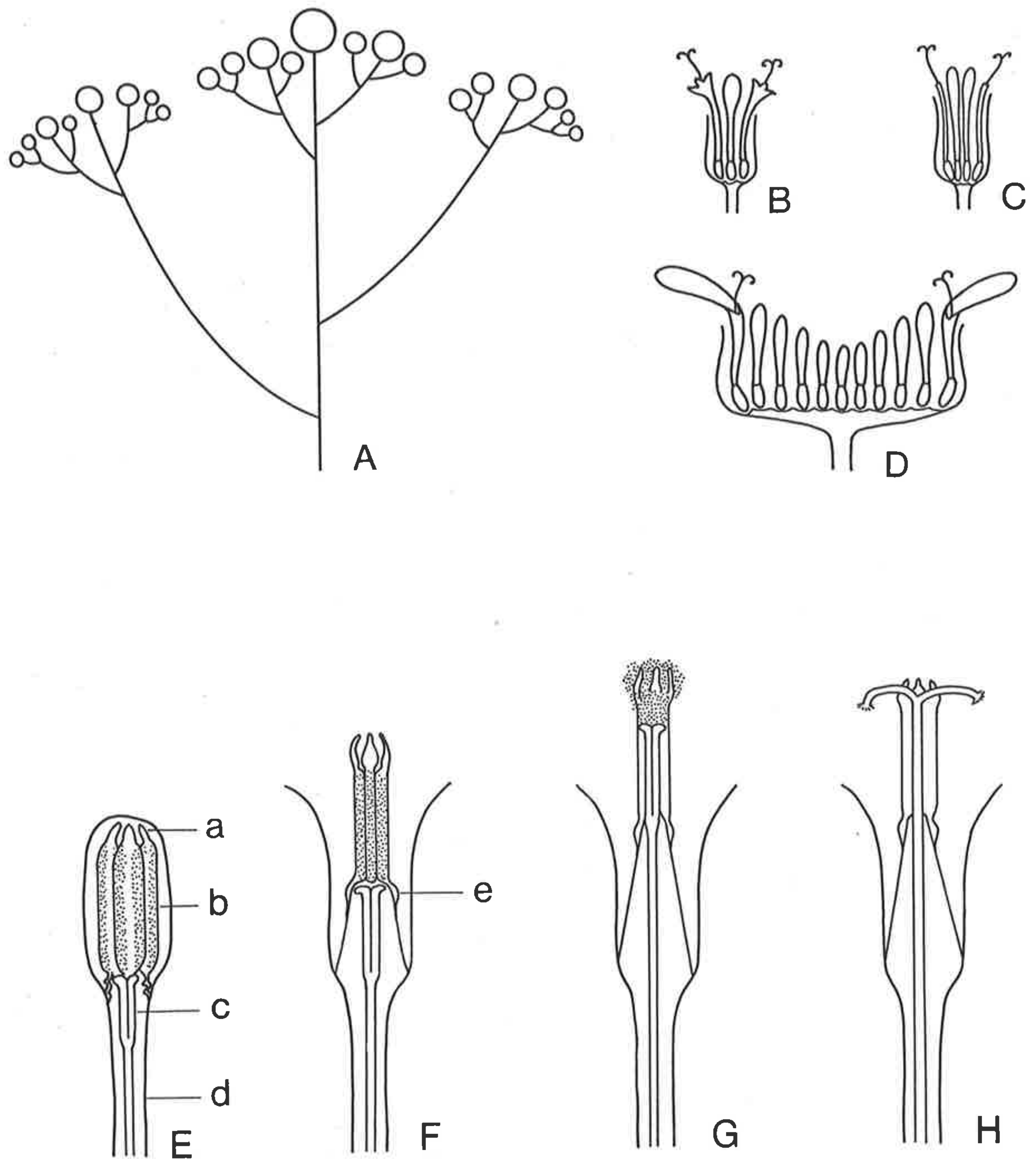


Fig. 4.2 Sequence of anthesis in Australian species of Senecio. A. Determinate flowering of capitula. B-D. Indeterminate flowering of florets within capitula. E-H. Movement of style and stamens during anthesis (see text for explanation). a-sterile appendages, b-anther collar, c-style, d-corolla, e-filament collar.

a cup in which pollen is retained before the style emerges. As Carlquist (1976) observed, this arrangement may lend itself to precision in pollination by minimising the scattering of pollen by insect visitors. Secondly, it is possible that the filament collar of thickened cells positioned below the anthers may prevent premature opening of the style branches. Shortly after anthesis the style extends beyond the narrowed basal portion of the corolla (Fig. 4.2 F). At this point the branches could conceivably open before passing through the anther collar. The thickened filament collar may prevent this occurring by forming a more resilient barrier at the style apex.

4.3.3 Pollen Vectors

Field observations indicated that native bees (family Halictidae) are the most important pollinators of outbreeding species of *Senecio*. In agricultural areas the introduced honey bee (genus *Apis*) is also a regular visitor. Hoverflies (family Syrphidae) were observed visiting a number of capitula but with less regularity than bees. Michener (1970) estimated that there may be as many as 3000 bee species in Australia. In view of the number of species, many of which are undescribed, I did not attempt to classify to species the insect visitors of *Senecio*.

Species of thrips (family Thripidae) occur in large numbers in the capitula of all species collected. Thrips normally feed on soft recent growth (Reed 1970) but do not appear to damage *Senecio*, and may instead be feeding on nectar in the corolla tube. I believe thrips may be significant but passive pollen vectors of inbreeding species of *Senecio*. Although normally found in the corolla tube, thrips were also observed moving about on the capitulum surface and may therefore transport pollen from bisexual to female florets.

4.4 Discussion

4.4.1 Mode of Reproduction

All species examined reproduce sexually. No conclusive evidence of apomixis or obligate selfing was detected, and although vegetative reproduction can occur in *Senecio pectinatus*, *S. spathulatus* and *S. cunninghamii*, it is not the predominant mode of reproduction. One advantage of sexual reproduction over asexual reproduction is the production of variable genotypes by segregation and recombination (Williams 1975, Maynard Smith 1977). The maintenance of sexual reproduction in Australian species of *Senecio* may therefore be due to their frequent occurrence in temporary or disturbed environments (see Section 4.4.7) such as regions of irregular rainfall, unstable soil types or forest clearings. Apomixis or obligate selfing might restrict populations to one type of environment and reduce survival chances in temporary or changing conditions.

4.4.2 Breeding Systems

Of the 37 species examined (Table 4.2) 20 are self-incompatible and 17 are self-compatible but capable of outcrossing. Actual amounts of cross-pollination are therefore most important. Species with large showy inflorescences and high pollen-ovule ratios are apparently obligate outcrossers. I found no instances of seed set in situations that might favour or reveal a low potential for selfing - for example, isolated capitula flowering out of season, solitary plants in marginal field conditions and solitary plants in glasshouse or garden situations. I do not exclude the possibility of occasional selfing in these species, but believe such events must be extremely rare. By comparison, the functionally protogynous capitula of all Australian autogamous species enables

them to be cross pollinated. Values from 1% (Hull 1974) to 22% (Campbell and Abbott 1976) cross pollination were recorded for the autogamous Senecio vulgaris. Australian autogamous species differ from S. vulgaris in having one to several rows of marginal female florets. As female florets mature before bisexual florets, levels of cross pollination in Australian autogamous species might be even higher than those of S. vulgaris. The occurrence of occasional interspecific hybrids (see Chapter 8) is evidence that at least some cross pollination occurs naturally in autogamous species.

4.4.3 Breeding Systems and Generation Length

There is a strong correlation between breeding system and generation length in the species listed in Table 4.2. I have summarized the evidence in Table 4.3. Most of the self-incompatible species (80%) are perennial and most self-compatible species are annual (76%). Stebbins (1958) observed a similar trend in the tribe Cichorieae (Lactuceae) of the family Compositae, and considered that stability of the environment was an important causal factor. Thus an unstable environment would favour the establishment of inbreeding annuals capable of mass reproduction, whereas stable environments would favour outbreeding perennials. A number of general observations can be made of Australian species considered in this study. 1. Six outbreeding perennials - Senecio macranthus, S. gawlerensis, S. anethifolius, S. sp. A, S. pectinatus and Bedfordia salicina - are restricted to diverse but nonetheless stable environments. 2. None of the inbreeding species are restricted to stable environments. 3. Of the species more or less confined to unstable environments, S. glossanthus and Erechtites valerianaefolia are inbreeding annuals, S. gregorii and S. discifolius are outbreeding annuals and S. pterophorus and

TABLE 4.3

Comparison of Breeding System and Longevity

Longevity (habit)	self-incompatible	self-compatible
2-4 months (ephemerals)	2	2
5-12 months (annual herbs)	2	9(2)*
2-5 years (perennial herbs)	3	4
2-?10 years (shrubs)	12	-
>10 years (trees)	(1)*	-

* Numbers in parentheses apply to genera other than *Senecio*.

S. mikanioides are outbreeding perennials. A possible explanation is that although both annual and perennial species occur, all species of *Senecio* are capable of flowering within one year of germination. Perennial species can therefore persist in unstable environments provided that (in the case of outcrossing taxa) populations are large enough to ensure fertilization. The relationship between longevity and breeding system observed in *Senecio* may represent alternative strategies for producing similar amounts of recombinant genotypes. Factors enhancing recombination, such as short generations and outcrossing, are therefore

combined with factors restricting recombination such as long generations and inbreeding (Grant 1958, Stebbins 1958). The long-term advantage of such balanced combinations is evident in the wide and diverse distributions of both outcrossing and inbreeding populations. The short term advantage of assured seed set associated with autogamy may account for the evolution of this breeding system in Australia.

4.4.4 Seed Size and Number

Compromises between size and number have been observed both for pollen grains and for seeds (Salisbury 1942, Harper et al. 1970, Stebbins 1971b and Lloyd 1979) with the general conclusion that size and numbers are alternative strategies in parental resource allocation. Thus selection for increased seed size is often offset by a decrease in number, and vice versa. Increased seed size may be advantageous in situations requiring rapid initial growth such as high seedling density or very short growing periods. However, large seeds may have difficulty obtaining sufficient soil-water for germination in marginal conditions.

In Australian species of *Senecio* seed size varies from 0.1 mg in *S. glomeratus* to 5.9 mg in *S. gregorii* (Table 4.2) but the majority of species have seeds weighing between 0.1 and 0.6 mg. Seed numbers were roughly estimated by calculating the number of florets per plant. Unlike seed size, seed number is subject to great phenotypic plasticity. Coded figures given in Table 4.2 therefore represent very crude estimates. Amounts vary from less than 1000 (code 1) to in excess of 20,000 (code 5) seeds per plant, but there is no apparent balance between seed size and number. Instead, seed number appears to be a function of plant size. For example, small numbers of seeds are produced by *S. pectinatus*, *S. discifolius*, *S. lautus* (New Zealand population)

and Arrhenechtites mixta, all of which are little-branched herbs with comparatively few capitula. Larger seed numbers are produced by well-branched annual herbs and shrubs of greater stature.

A more significant relationship exists between seed size, floret number and involucre size. I calculated the area occupied by seeds within a capitulum assuming all florets were fertilized, and then compared the area with capitulum size. Assuming full seed set, the capitula of most species could physically accomodate between 85% and 100% of the seeds. The lower values might be increased to 100% by slight expansion of the involucre observed in most species. However, four species with very large seeds could not apparently accomodate full seed set. The values calculated were 55% for S. magnificus, 50% for S. vagus, 44% for S. macranthus and 18% for S. gregorii. S. magnificus and S. macranthus are perennial, so that the disadvantage of reduced seed number per capitulum may be offset by repeated production of seeds and increased seed size. Similar arguments may apply to S. vagus but the longevity of this species is less certain. S. gregorii, however, is a widespread ephemeral of arid inland areas, so that reproductive effort and productivity should be maximized. S. gregorii has overcome the potential reduction in seed number accompanying increased seed size in a novel way - by allowing for extensive expansion of the involucre. The involucre of S. gregorii consists of fused, comparatively thin and membranous bracts so that expansion to at least twice the size at anthesis is possible. All other species have free and interlocking bracts so that involucral expansion is possible but limited. I therefore consider that compromises between seed size and number are most critical within capitula in Senecio, and are not strongly correlated with seed number per plant.

4.4.5 Dispersal Potential

The amount of gene exchange within a population is largely controlled by breeding systems, but gene exchange between spatially separated populations will be determined by dispersal of seeds and pollen. Studies have shown that most dispersal units, whether they be seeds or pollen grains, travel comparatively short distances from the parent plant (Colwell 1951, Stephens and Finkner 1953, Salisbury 1961, Sheldon and Burrows 1973, Stergios 1974) and that dispersal over distances of more than a few meters is a rare event. Furthermore, interpopulation dispersal of a seed will be more important than the dispersal of one pollen grain, as successful establishment of one seed will be equivalent to the dispersal of thousands of pollen grains. I therefore consider that seed and pollen dispersal together are most important within a population but that seed dispersal alone is most likely to effect interpopulation gene exchange.

The seeds of all species examined in this study possess a pappus and are dispersed by wind, but differences in pappus persistence and the ratio between pappus and seed size both affect dispersal distance. Sheldon and Burrows (1973) examined the effectiveness of the achene-pappus unit and found that pappus complexity, plant height and environmental factors were also significant. In *Senecio* the most "complex" pappus occurs in species with slender caducous bristles. When open, this type of pappus is three-dimensional and offers greater wind resistance than a pappus of stout persistent bristles. Bristles of the latter pappus type often occupy only one plane when the pappus has expanded. Only three species of *Senecio* have a persistent pappus - *S. gregorii*, *S. magnificus* and *S. spathulatus*. Each has large seeds and occurs in a comparatively open environment (deserts, arid shrublands and sandy beaches, respectively).

I believe the achene-pappus unit of these species may be best adapted for being blown along the ground. Species with caducous bristles rapidly lose their pappus if seeds move along the ground. A persistent pappus may have evolved as an adaptation to move heavy seeds greater distances. Two species, S. macranthus and S. vagus have large seeds and a caducous pappus, but both are comparatively uncommon and are restricted to wet-sclerophyll forests.

Variation in seed size also occurs within some capitula. Most species with large capitula produce slightly shorter and fatter marginal seeds, but this variation may be a function of the capitulum shape. In the self-compatible S. glossanthus, marginal seeds produced by female florets are longer and twice as heavy as those produced by bisexual florets. Furthermore, the marginal seeds have a pappus of few bristles and are retained in the expanded involucre for longer periods. S. glossanthus therefore ensures that seeds of female florets fall close to the parent plant while those of bisexual florets may be dispersed over longer distances. This form of seed distribution would be advantageous, as S. glossanthus frequently occurs in favourable microenvironments in otherwise harsh conditions, such as soil pockets or moss patches on rocky outcrops or in the shade of trees and shrubs. An additional benefit is that seeds produced by occasional outcrossing of female florets will fall in areas of known success whereas population replacement is ensured by general dispersal of many selfed progeny.

4.4.6 Seedling Establishment

Germination percentages and early seedling morphology were recorded to determine some of the factors affecting seedling establishment. Germination percentages were generally high

(Table 4.2) and not affected by dormancy requirements. However, comparatively low percentages were recorded for S. magnificus, S. velleoides and S. vagus. Germination in these species may be controlled by physiological factors such as the action of growth substances or the breakage of dormancy. Germination control may explain why each of these species is restricted to one environment - arid shrublands in the case of S. magnificus and wet-sclerophyll forests in the cases of S. velleioides and S. vagus. The reproduction of Bedfordia salicina requires further investigation. Natural seed set is very low and as yet, attempts to germinate seeds have been unsuccessful.

Seedling size will affect establishment when competition is high or time for establishment is very brief. The shape and size of seedlings varies considerably among species examined (Fig.4.3) but is largely dependent on seed size and shape. Large seedlings occur in arid zones when rapid establishment after heavy rains would be advantageous (e.g. S. gregorii, S. magnificus) and in wet-sclerophyll forests where plant density or low light levels would favour large seedlings (e.g. S. macranthus, S. vagus, Erechtites valerianaefolia and Arrhenechtites mixta). S. spathulatus has large seeds, but seed reserves are mainly used to produce an exceptionally long hypocotyl. As S. spathulatus occurs on drifting beach sand, the long hypocotyl may be an adaptation to prevent seedlings being buried during early development.

A further adaptation in many species is the secretion of a mucilaginous coat by achenal hairs. Harper et al. (1970) examined the relationship between water uptake and seed size. They found that large seeds required much higher water tensions for water uptake because of the changing shapes of menisci. The effect of a mucilaginous coat is to increase the area of seed-water contact. S. gregorii, an arid-zone ephemeral has the

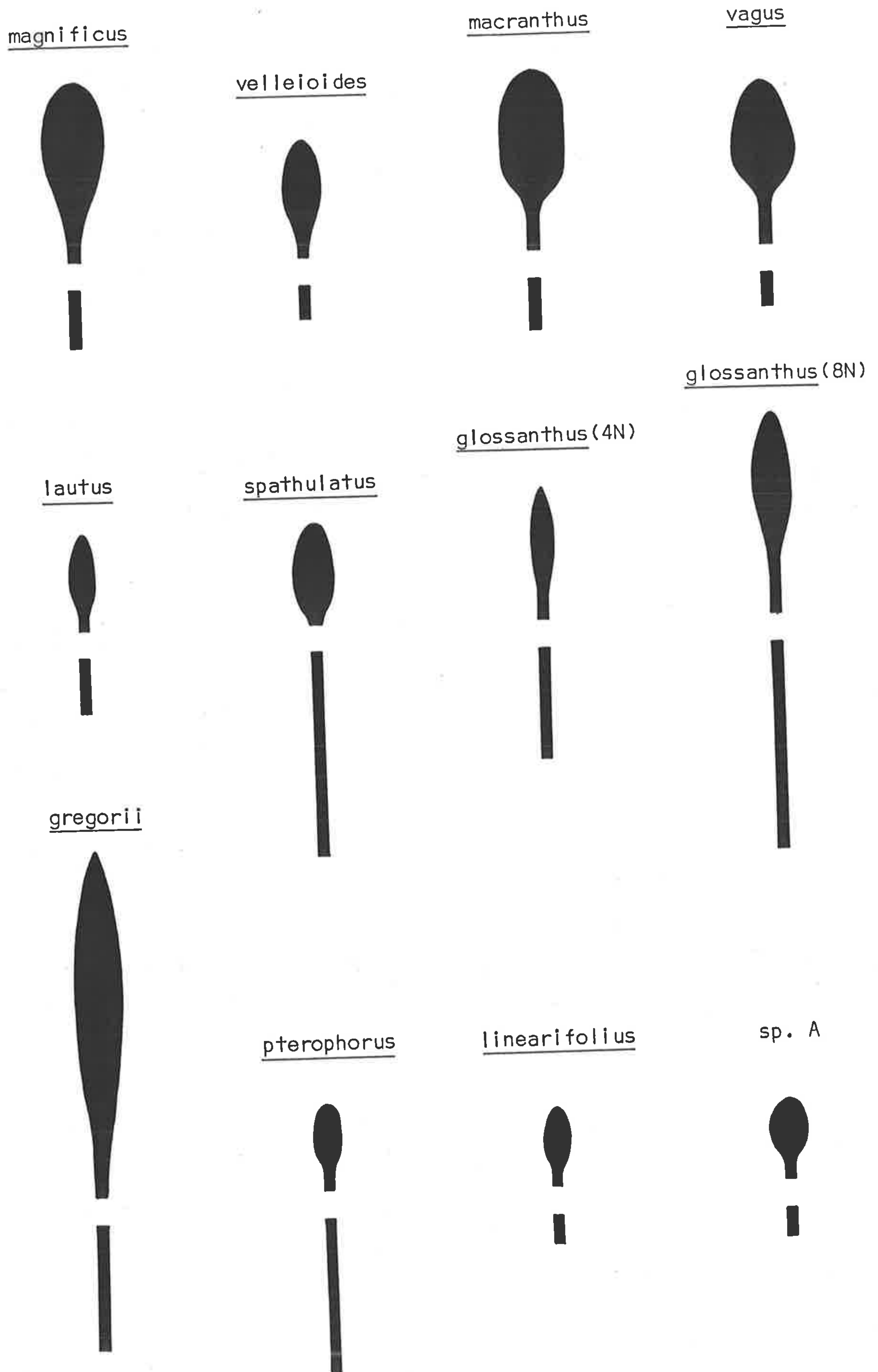


Fig. 4.3 Cotyledon shape and hypocotyl length of 28 species of <u>Senecio</u>. All figures 2x actual size.

hypoleucus

odoratus

cunninghamii

gawlerensis

vulgaris

quadridentatus

gunnii

aff. apargiaefolius

sp. B

squarrosus

minimus

picridioides

glomeratus

hispidulus

sp. C

biserratus

largest seeds of species examined, but also has the longest and most dense covering of achenal hairs. The extensive mucilaginous coat produced by *S. gregorii* would therefore greatly assist germination. It is significant that glabrous seeds occur only in species found in areas of high rainfall, for example *S. macranthus*, *S. vagus* and *S. spathulatus*.

4.4.7 Associations of Reproductive Traits - The Predictions of r- and K-selection

The evolution of different reproductive strategies can be considered from two viewpoints - either as selection for genetic systems that optimise the rate of expression of genetic variability (Grant 1958, Stebbins 1958), or as selection for reproductive systems that ensure success in a variety of environments (Stearns 1976, Moore 1976). Although the two ideas are interrelated I will consider only the latter at this point and will discuss genetic systems in the next chapter.

In his review of ideas concerning life-history tactics, Stearns (1976) compared two models - "bet-hedging" and "r- and K-selection" - that seek to explain the association of reproductive traits in differing situations. The bet-hedging model (a term coined by Stearns) is based on fluctuations in adult or juvenile mortalities, and is not necessarily dependent on environmental stability. Theoretically, if adult survival rates are unpredictable, then short-lived organisms with increased reproductive effort will be favoured. Variable juvenile survival rates will favour long-lived organisms with reduced reproductive effort. Fluctuations in mortality rates are not considered by the model of r- and K-selection. MacArthur and Wilson (1967) coined the term "r-selection" for selection in density-independent

environments favouring rapid population growth (increased growth rate r) and "K-selection" for selection in saturated density-dependent environments favouring ability to compete and to avoid predators (increased carrying capacity K). The models of bet-hedging and r- and K-selection can lead to conflicting predictions. For example, if juvenile mortalities are variable in a stable environment then bet-hedging will predict short generation times and large reproductive efforts whereas K-selection will favour long generations and smaller reproductive efforts. However, fluctuations in mortality rates are likely to be due to fluctuations in environmental conditions so that both models should generally give identical predictions. As I did not make detailed observations of mortality rates I have largely confined the remaining discussion to the predictions of r- and K-selection. In most instances I believe the predictions of bet-hedging would be identical.

An assessment of environmental stability is necessary to test predictions of the r- and K-selection model, but stability can be viewed in terms of a number of independent parameters such as regularity of rainfall, physical disturbances or successional stages of vegetation. Absolute comparisons of stability levels are therefore difficult. Senecio spathulatus, for example, occurs in areas of high and regular rainfall but on unstable beach sand whereas S. magnificus occurs in areas of low and irregular rainfall but often among climax vegetation types. Environmental stability must obviously be considered carefully for each species. I have presented three parameters in Table 4.4 - occurrence in environments disturbed by man, regularity of rainfall and natural habitat. A stable environment is then one which is not disturbed by man, has a regular rainfall (or water supply in the case of arid zone perennials) and consists of climax vegetation in the

TABLE 4.4

Comparison of Habit and Aspects of Environmental Stability

Species	roadside weed	agricultural weed	rainfall zone*	natural habitat	general stability**
			Outbreeding Trees		
Bedfordia salicina	-	-	4	closed forests, often in gullies	S
			Outbreeding Shrubs		
Senecio lautus (Australia)	+	+	1-4	extremely variable	U
S. spathulatus	-	-	4	sandy foreshores, uncommon	U
S. magnificus	+	-	1-2	open shrublands often near watercourses	U
S. amygdalifolius	+	-	4	closed forests often in clearings	U
S. macranthus	-	-	4	closed forests usually in gullies, rare	S
S. linearifolius	+	+	(3-)4	in or near clearings in open and closed forests	U
S. sp. A	-	-	2	near rocky summits of hills	S
S. hypoleucus	+	-	3	rocky hillslopes in open forests and woodlands	U
S. odoratus	+	-	3(-4)	coastal shrublands and woodlands	U
S. cunninghamii	+	+	1-2	shrublands and woodlands	U
S. anethifolius	-	-	(1-)2	rocky outcrops or gorges, sometimes near creekbeds	S

Table 4.4 - continued

Species	roadside weed	agricultural weed	rainfall zone*	natural habitat	general stability**
S. gawlerensis	-	-	2	near rocky summits of hills	S
S. pterophorus	+	+	3	clearings in woodlands (introduced weed)	U
S. mikanioides	+	+	3-4	woodlands or forest margins (introduced liana)	U
Outbreeding Perennial Herbs					
S. pectinatus	-	-	4	alpine meadows	S
Outbreeding Annuals (?)					
S. velleioides	+	-	4	closed forests often in or near clearings	U
S. vagus	+	-	4	closed forests often in or near clearings	U
Outbreeding Ephemerals					
S. gregorii	+	-	1(-2)	open shrublands and deserts	U
S. discifolius	+	+	3	largely unknown (occurs only in South Africa)	U
Inbreeding Perennial Herbs					
S. quadridentatus	+	+	1-3	extremely variable	U
S. gunnii	+	-	4	alpine woodlands and meadows	U
S. aff. apargiaefolius	+	-	3-4	woodlands and open forests	U
S. runcinifolius	-	-	1-2	inland riparian habitats, rare	U

Table 4.4 - continued

Species	roadside weed	agricultural weed	rainfall zone*	natural habitat	general stability**
				Inbreeding Annuals	
S. lautus (New Zealand)	+	-	4	coastal dunes and cliffs	U
S. biserratus	+	-	3-4	coastal woodlands and and forest clearings	U
S. sp. B	+	-	(3-)4	open forests and woodlands	U
S. squarrosus	+	-	3	open grassy woodlands, rare	U
S. bipinnatisectus	+	-	4	forest margins and clearings	U
S. minimus	+	-	3-4	woodlands, forest margins and clearings	U
S. picridioides	+	+	3	coastal and inland shrub-lands and woodlands	U
S. glomeratus	+	+	3(-4)	meadows, woodlands, forest margins	U
S. hispidulus	+	-	3-4	woodlands, forest margins and clearings	U
S. sp. C	-	-	3	clearings in open forests, rare	U
Arrhenechtites mixta	+	-	4	open forests and clearings in closed forests	U
Erechtites valerianaefolia	+	+	4	forest margins (introduced weed)	U
				Inbreeding Ephemerals	
Senecio glossanthus	-	-	1-2(-3)	temporarily wet micro-environments	U
S. vulgaris	+	+	3-4	gardens and nurseries (introduced weed)	U

* 1=<250mm, 2=250-300mm, 3=500-750mm, 4=>750mm
** S = stable, U = variously unstable

successional sense. Environments that do not fulfill these requirements are classified variously as disturbed. The r- and K-selection model predicts that in stable environments late maturity, few large young, a long life and small reproductive effort will be selected for (K-selection) and in unstable environments early maturity, many small young, a short life and large reproductive effort will be selected for (r-selection).

Considering each factor in turn, age at sexual maturity was comparatively uniform among both annual and perennial species of *Senecio* raised in glasshouse conditions. Most produced flowers within 4 to 6 months of germination. Shorter times were recorded for two inbreeding ephemerals, *S. glossanthus* and *S. vulgaris*, both of which produced flowers within two months of germination. As all species of *Senecio* are capable of flowering in the first season, and most occur in variously unstable environments, the general predictions of r-selection are supported. However, different responses by the same species growing in optimal and marginal conditions maximise the chance of reproductive success.

Size and number of young (seeds) discussed in section 4.4.4 again support the general prodictions of r-selection, as most species of *Senecio* produce a large number of small seeds. However, the few species producing large seeds do not necessarily produce fewer seeds or occur in stable environments (the predictions of K-selection). Longevity also shows only a partial correlation with environmental stability. All short lived species (annuals and ephemerals) occur in variously unstable environments but perennial species occur in both stable and unstable environments. Significantly, the few species occurring only in stable environments - *Senecio pectinatus*, *S. macranthus*, *S.* sp. A, *S. anethifolius*, *S. gawlerensis* and *Bedfordia salicina* are all perennials.

Reproductive effort refers to the proportion of parental resources allocated to reproduction. Although I have not compared weights of vegetative and reproductive structures in *Senecio* I believe ratios would be similar for both annual and perennial species. Perennial species either regenerate annually or shed leaves from older stems so that large resources need not be used to maintain older growth. Furthermore, increased vegetative growth is apparently accompanied by increased reproductive effort, as the number of capitula per plant is most closely correlated with plant size (see section 4.4.4). Reduced reproductive effort occurs only when conditions are unusually harsh after germination, but this is apparently a short-term strategy to ensure at least some reproductive success. I therefore consider that reproductive effort is generally high in both annual and perennial species of *Senecio*.

When viewed as a whole Australian species of *Senecio* appear to be the products of r-selection. Most combine the traits of early reproductive maturity, many small seeds, a short life and a large reproductive effort, and most occur in variously unstable environments. A small number of species do occur only in stable environments, and in these, the predictions of K-selection are partially supported. It is possible that all Australian species of *Senecio* evolved in situations favouring r-selection, and the few now inhabiting stable environments are able to compete effectively without all of the traits predicted by K-selection. However, in natural populations r- and K-selection will be operating together (Demetrius 1975) so that observed reproductive strategies need not represent alternative selective products, but instead, some integral of combined r- and K-selection.

4.5 Conclusions

Ornduff (1969) suggested that an understanding of reproductive methods will "strengthen the foundations upon which taxonomic judgements are made." His comment is particularly valid in the case of Australian species of *Senecio*. The classification of species as "radiate", "discoid" or "erechthitoid" is largely indicative of breeding systems. All erechthitoid species are autogamous and have very reduced capitula whereas the large and showy inflorescences of radiate and discoid species reflect their reliance upon cross-pollination. The only exception, *S. glossanthus*, belongs to the radiate group but is inbreeding. However, the identification of the breeding system of *S. glossanthus* explained why this species has much smaller capitula than other radiate species. *S. gregorii* has been cited as "anomalous" in its present position in *Senecio* (Nordenstam 1977, Jeffrey et al. 1977) presumably because of its fused involucral bracts, coarse and persistent pappus, and large and hairy seeds. I believe all of these characters may be adaptations to maintain self-incompatibility in an arid-zone ephemeral - a combination which might normally lead to reduced seed set. The fusion of involucral bracts allows extensive expansion of the involucre and thus the maturation of a high number of large seeds. Seed germination is assisted by a mucilaginous coat so that rapid germination can occur in favourable conditions. The coarse persistent pappus and heavy seeds would favour seeds being blown along the ground, with aggregates forming in low-lying areas or among other vegetation. This method of dispersal would increase the chance of germination in the vicinity of other individuals, an essential factor in the survival of an outcrossing species. The unusual morphology of *S. gregorii* may therefore be due to unusual reproductive

strategies, rather than to distant relationships with other Australian species of Senecio.

All species examined are similar in that they reproduce sexually and are capable of at least some outcrossing. However, different breeding systems might be expected to lead to different levels of variability within populations. For example, Hamrick et al. (1979) concluded that high outcrossing rates generally lead to the maintenance of higher levels of genetic variation, although they acknowledged exceptions to the rule. Australian species of Senecio are almost equally divided between inbreeders and outbreeders. Species of each breeding type are geographically widespread, occur in localized populations and are apparently adapted to disturbed or temporary conditions. I believe under these conditions comparatively high levels of genetic variation may be maintained in both inbreeding and outcrossing populations. In the case of inbreeding species, population variability may be maintained by the rare dispersal of new genotypes (via seeds) from other populations followed by a low level of outcrossing within the recipient population. As inbreeding species of Senecio are predominantly annuals, rapid population replacement and low outcrossing levels may lead to levels of variability similar to those found in outcrossing perennial populations.

Although two different breeding systems and a variety of generation lengths occur in Australian species of Senecio, most possess a combination of reproductive traits predicted by r-selection (Stearns 1976). These are early reproductive maturity (in perennial species), many small seeds, a short life and a large reproductive effort. The diversity of reproductive traits observed in this study may therefore represent alternative strategies to meet the same demands - that is, reproductive success in a variety of unstable environments.

CHAPTER 5

Recombination in *Senecio*

5.1 Introduction

5.2 Materials and Methods

5.3 Results and Discussion

5.3.1 Chromosome numbers

5.3.1.1 Ploidy distributions in *Senecio*

5.3.1.2 Polyploidy and recombination

5.3.1.3 Polyploidy and speciation

5.3.2 Effects of chiasma frequency and position

5.3.3 Effects of breeding systems

5.3.4 Effects of generation lengths

5.3.5 Pair-wise associations of regulatory factors

5.3.5.1 Breeding system and generation length

5.3.5.2 Breeding system and chromosome number

5.3.5.3 Breeding system and chiasma frequency

5.3.5.4 Generation length and chromosome numbers

5.3.5.5 Generation length and chiasma frequency

5.3.5.6 Chiasma frequency and chromosome number - the "Recombination Index"

5.3.6 Recombination systems in *Senecio*

5.4 Conclusions

5.1 Introduction

In Chapter 4, reproductive traits observed in Senecio were discussed in terms of r- and K-selection. These models predict the age of sexual maturity, size and number of young (seeds), generation length and reproductive effort in a range of environmental conditions. Associations of reproductive traits including chromosome number and chiasma frequency have also been explained in terms of the regulation of genetic recombination (Grant 1958). In any sexually reproducing organism, both existing (parental) and new (recombinant) genotypes are produced by segregation and recombination of genes. However, the number of recombinant genotypes produced per unit of time will vary. Grant (1958, 1975) listed nine factors thought to regulate recombination in plants. These are as follows:

1. chromosome number
2. frequency and position of chiasmata
3. sterility barriers
4. breeding system
5. pollination system
6. dispersal range
7. population size
8. isolating mechanisms
9. generation length

Collectively, the regulatory factors are known as the recombination system, the main function of which, according to Grant, is to achieve an optimum balance in the amount of genetic variability released for selection. The balance, it is argued, is between reproductive constancy favoured in the existing parental environment, and reproductive variability thought to be necessary for long-term flexibility (Mather 1943, Grant 1958,

Stebbins 1958). As Grant (1975) commented, some restriction on recombination is universal, but it is the fraction of attainable recombination which varies in different species and groups.

More recently, arguments have been advanced in favour of selection of regulatory factors that are of immediate benefit to the individual, rather than selection for long-term population benefits (Arroyo 1973, Rees and Dale 1974, Lloyd 1979). In order to compare this literature with the models proposed by Grant (1958) and Stebbins (1958), I have restricted this chapter to discussions of observed and hypothetical relationships between chromosome number, chiasma frequency and position, breeding system and longevity. Aspects of pollination systems and dispersal range were considered in Chapter 4 and observations of sterility barriers and isolating mechanisms will be considered in Chapter 8. The effects of changes in chromosome numbers on recombination rates are considered at length, as numerical variants in Senecio are mainly due to polyploidy. In his discussion of the regulation of recombination, Grant (1858, 1975) considered the effects of changes in basic chromosome number, but did not comment on the effects of polyploidy.[1] As one third of all species of flowering plants originated by polyploidy (Stebbins 1971), the effects of polyploidy on recombination rates are a basic issue.

---

[1]Grant described the altered segregation of polyploids in Chapter 13 of his 1975 publication, but does not comment on the subject in Chapter 23 - "The regulation of recombination."

5.2 Materials and Methods

The determination of breeding systems and generation lengths is described in Chapter 4. Techniques used in the preparation of both mitotic and meiotic material are documented in Lawrence (1980; see copy bound with thesis). Whenever possible, chromosome numbers were determined from a number of populations. However, because of limited time, chiasma frequencies were determined for only one population of each taxon. Collection numbers of each population are listed in Table 5.2 and localities are given after each species description in Chapter 4. Chiasma frequencies were calculated as the mean value of ten cells in all cases. Although higher cell numbers could be scored for outcrossing taxa, this number was chosen as inbreeding taxa proved difficult, often producing very few bisexual florets and as few as ten pollen mother cells per anther.

5.3 Results and Discussion

5.3.1 Chromosome numbers

Chromosome numbers for most species were previously reported in Lawrence (1980) but determinations for two species, *Senecio amygdalifolius* and *S. runcinifolius*, have since been made. Population records of these and of three species from other genera are given in Table 5.1. All chromosome numbers are included in Table 5.2 and are summarized in Table 5.3.

5.3.1.1 Ploidy distribution in *Senecio*.

Patterns of polyploidy in *Senecio* were discussed in Lawrence (1980) and are briefly reviewed here. The basic chromosome number of *Senecio* is effectively x = 10 found in 9% of recorded counts. Three African species do have N = 5, and arguments both for (Turner and Lewis 1965) and against (Ornduff et al. 1963, 1967) a basic number of x = 5 have been advanced. I have considered the topic again in the light of karyotype evidence (Chapter 7), but as species with N = 5 represent less than 1% of over 400 counts recorded in the literature, x = 10 is the effective basic chromosome number of the genus.

Africa would appear to be the place of origin of both *Senecio* and Senecioneae as the greater percentage of *Senecio* species with N = 10 and all but one of the 14 genera of Senecioneae that have x = 5 or 10 are of African origins. However, diversification of *Senecio* on most other continents has occurred mainly at the tetraploid level (41% of all taxa in the literature) and in Australia, at the tetraploid and hexaploid level. Stebbins (1971) described a similar situation in the grass genus *Danthonia*. The basic number of x = 6 occurs in South African diploids,

TABLE 5.1

Chromosome Numbers Not Reported in Lawrence (1980)

Species and Locality	Chromosome No. N	2N	Figure
**Senecio amygdalifolius* F.v.Muell.			
Tallawudjah Creek, N.S.W.	19	38	5.1A
**S. runcinifolius* Willis			
Chowilla Creek, S.A.	20	40	5.1C
Arrhenechtites mixta (A.Rich.) Belcher			
3 km W. Katoomba city center, N.S.W.	50	50	5.1D
5 km S. Jenolan Caves, N.S.W.	50		
Erechtites valerianaefolia (Wolf)DC.			
1 km SW. Berkley Vale, N.S.W.	20	40	
29 km N. Wingham, N.S.W.	20	40	5.1B
Bedfordia salicina (Labill.)DC.			
9 km N. Cape Otway lighthouse, Vic.	30		

*Taxa not previously reported

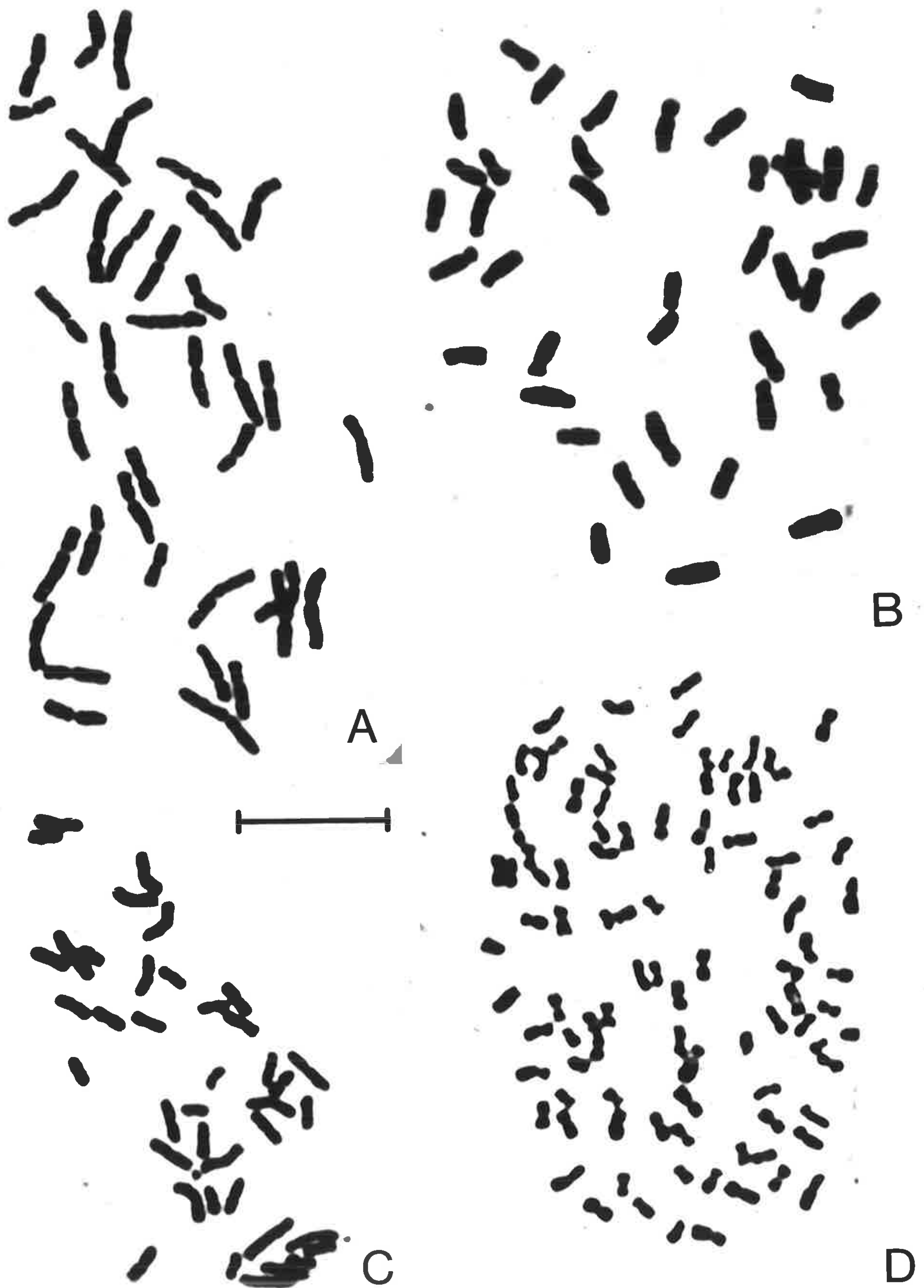


Fig. 5.1 A. Senecio amygdalifolius, 2N=38. B. Erechtites valerianaefolia, 2N=40. C. Senecio runcinifolius, 2N=40. D. Arrhenechtites mixta, 2N=100. All figures at same magnification. Scale 10 μm.

TABLE 5.2

Associations of Factors Regulating Recombination in Senecio

	collection number	chiasma positions**	$\bar{x}$ chiasmata per cell	$\bar{x}$ chiasmata per bivalent	chromosome number (N)	recombination index
Outbreeding trees (>10 years)						
Bedfordia salicina	1132	T+I	36.3	1.21	30	66.3
Outbreeding shrubs (2-?10 years)						
Senecio lautus ssp. dissectifolius	644	T+I	25.3	1.27	20	45.3
ssp. lanceolatus	1080	T+I	23.9	1.20	20	43.9
ssp. maritimus	615	T+I	28.6	1.43	20	48.6
ssp. alpinus	1442	T+I	26.2	1.31	20	46.2
S. spathulatus	1305	T+I	26.7	1.34	20	46.7
S. magnificus	801	T	32.1	1.64	20	52.7
S. amygdalifolius	1477	T	34.4	1.81	19	43.4
S. macranthus	1393	T	40.7	2.04	20	60.7
S. linearifolius						
var. linearifolius	1302	T+I	41.3	1.38	30	71.3
var. A	1237	T+I	33.2	1.11	30	63.2
var. B	1191	T+I	41.0	1.37	30	71.0
S. sp. A	1445	T+I	38.0	1.27	30	68.0
S. hypoleucus	646	T+I	45.8	1.53	30	75.8
S. odoratus var. odoratus	657	T+I	35.2	1.17	30	65.2
var. obtusifolius	609	T+I	44.9	1.50	30	74.9

TABLE 5.2 (continued)

	collection number	chiasma positions	x̄ chiasmata per cell	x̄ chiasmata per bivalent	chromosome number	recombination index
S. cunninghamii var. cunninghamii	775	T+I	43.4	1.45	30	73.4
var. A	764	T+I	39.7	1.32	30	69.7
S. anethifolius	1004	T+I	44.0	1.47	30	74.0
S. gawlerensis	856	T+I	35.7	1.19	30	65.7
*S. pterophorus	647	T+I	10.1	1.01	10	20.1
*S. mikanioides	1007	T+I	12.7	1.27	10	22.7
Outbreeding perennial herbs (2-5 years)						
S. pectinatus	1397	T+I	57.0	1.43	40	97.0
Outbreeding annuals ?(5-12 months)						
S. velleioides	1099	T	30.6	1.61	19	49.6
S. vagus ssp. vagus	1276	T	82.3	1.65	49	132.3
ssp. eglandulosus	1349	T	89.4	1.79	49	139.4
Outbreeding ephemerals (2-4 months)						
S. gregorii	1010	T+I	34.4	1.72	20	54.4
*S. discifolius	1080	T+I	9.0	1.80	5	14.0
Inbreeding perennial herbs (2-5 years)						
S. quadridentatus	790	T+I	39.4	1.97	20	59.4
S. gunnii	1402	T+I	36.7	1.83	20	56.7
S. aff. apargiaefolius	1249	T+I	33.0	1.65	20	53.0
S. runcinifolius	1065	T+I	37.2	1.86	20	57.2

TABLE 5.2 (continued)

	collection number	chiasma positions	x̄ chiasmata per cell	x̄ chiasmata per bivalent	chromosome number	recombination index
			Inbreeding annuals (5-12 months)			
*S. lautus ssp. lautus	-	T+I	30.6	1.53	20	50.6
S. biserratus	1149	T+I	90.9	1.80	50	140.0
S. sp. B	1324	T+I	58.0	1.93	30	88.0
S. squarrosus	1178	T+I	55.4	1.85	30	85.4
S. bipinnatisectus	1348	T+I	53.7	1.79	30	83.7
S. minimus	1143	T+I	45.1	1.50	30	75.1
S. picridioides	897	T+I	58.0	1.93	30	88.0
S. glomeratus	648	T+I	57.5	1.92	30	87.5
S. hispidulus var. hispidulus	1083	T+I	54.4	1.81	30	84.4
var. dissectus	1215	T+I	59.4	1.98	30	89.4
S. sp. C	1162	T+I	55.1	1.84	30	85.1
Arrhenechtites mixta	1361	T+I	103.3	2.06	50	153.3
*Erechtites valerianaefolia	1321	T	30.7	1.53	20	50.7
			Inbreeding ephemerals (2-4 months)			
Senecio glossanthus (tetraploid)	475	T+I	33.3	1.67	20	53.3
(octoploid)	476	T+I	54.8	1.37	40	94.8
*S. vulgaris	552	T+I	33.4	1.67	20	53.4

* Species (or subspecies) not native in Australia

** T = terminal, I = interstitial

but in Australia, where the genus is most diversified, the principal numbers are N = 12 (tetraploid) and N = 24 (octoploid). Like *Danthonia*, *Senecio* is therefore characterized by a secondary cycle of polyploidy, which exists where the basic chromosome number of a genus is poorly represented in diploids and diversification has occurred at a higher ploidy level and often on a different continent (Stebbins 1971).

Ploidy distributions among Australian species of *Senecio* are summarized in Table 5.3. Significant ploidy differences occur between the three major species groups based on capitulum morphology. All discoid species are hexaploid (N = 30) and most radiate species are tetraploid (N = 20). Two radiate species are hexaploid but both are otherwise closer to discoid species although they do have ray florets. The majority of erechthitoid species are also hexaploid but a significant proportion (four species) are tetraploid. In total, most Australian species are either tetraploid or hexaploid, with hexaploids being slightly more abundant. In Lawrence (1980) I commented that the abundance of hexaploids in Australia might be a taxonomic artifact, as species with N = 30 have in the past been split off as separate genera (e.g. the New Zealand *Brachyglottis*, *Dolichoglottis* and *Urostemon*). However, I now agree with Nordenstam's (1977) view that most genera of Senecioneae based on N = 30 represent a divergent evolutionary line, and that N = 30 is truly rare in *Senecio*. Nordenstam observed that many genera of *Senecio* with N = 30 also have a continuous stigmatic surface, polarized endothecal tissue and a cylindrical filament collar (Chapter 2.12). *Senecio* and allied genera are characterized by N = 20, marginal stigmatic lines, radial endothecal tissue and variously swollen filament collars. Nordenstam termed the first group of genera "cacalioid" and the second group "senecioid".

TABLE 5.3

Distribution of Gametic Chromosome Numbers of Species of <u>Senecio</u> in Australia*

	5	10	19	20	30	40	49	50	
Radiate	(1)	(1)	2	6	2**	1	1		
Discoid		(1)		(1)	5				
Erechthitoid				4	8			1	
TOTALS	(1)	(2)	2	10(1)	15	1	1	1	30(4)

* Species not native are shown in parentheses.

** <u>S. linearifolius</u> and <u>S</u>. sp A have ray florets but are otherwise morphologically closer to discoid species.

(AAaa) will produce five progeny types (AAAA, AAAa, AAaa, Aaaa, aaaa) in the ratios of 1:8:18:8:1. If dominance is complete then 1/4 of the diploid progeny and 1/36 of the autotetraploid progeny will differ phenotypically from the parent. Alternatively, if dominance is incomplete then the autotetraploid will produce a greater proportion of intermediate progeny. The effect on recombination is illustrated in Figure 5.2 (graphs plotted from values given by Sybenga (1972)). Starting with a heterozygous diploid (Aa) in case A and a heterozygous tetraploid (AAaa) in case B, the proportion of the population homozygous for one gene locus are shown in successive generations of selfing. Although both populations approach complete homozygosity, the autotetraploids do so at a much slower rate. The effect of autopolyploidy is therefore to buffer intermediate genotypes and retard evolution when selection is for homozygous or extreme phenotypes.

As two or more genomes are combined in an allopolyploid, the behaviour of an allopolyploid will depend on the degree of differentiation between parental genotypes. Harlan and deWet (1975) surveyed a wide range of polyploids and concluded that the most likely pathway to a successful polyploid in nature involves neither wide crosses nor strict autopolyploidy but crosses between races, ecotypes and cytotypes within a biological species. Wide crosses do occur in nature, but Harlan and deWet believe this is a comparatively uncommon mode of polyploid evolution. However, polyploids are more likely to hybridize successfully among themselves than diploids so that "true" allopolyploids may form by this method.

Recombination of allopolyploid genes will still be affected by substantial gene duplication. If one or more chromosomes or chromosome segments are homologous, then random reassortment will give ratios like those of autopolyploids. However, if

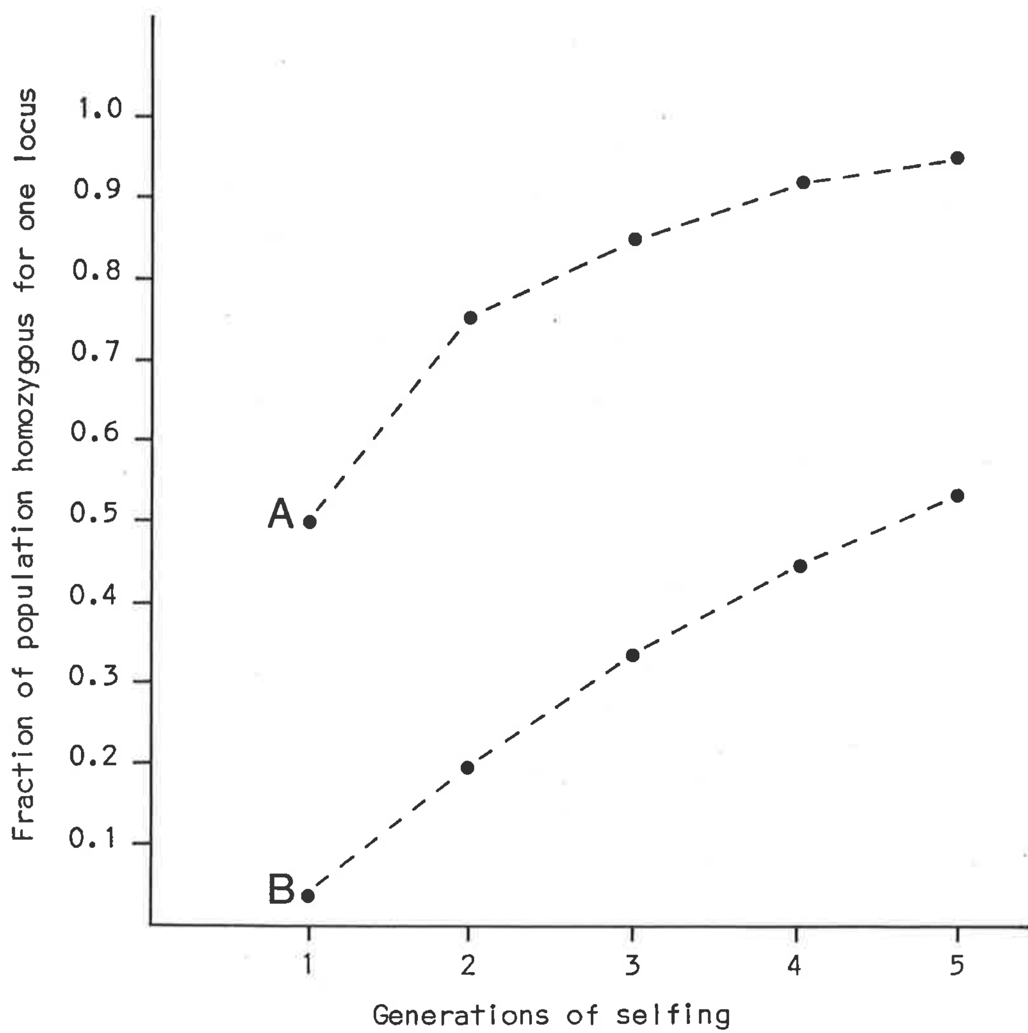


Fig. 5.2 Fraction of population homozygous at one gene locus starting with a diploid heterozygote (A) and its autotetraploid derivative (B). Values from Sybenga (1972).

Since Lawrence (1980) I have examined typical "cacalioid" features in Bedfordia salicina. Apart from a chromosome number of N = 30, Australian hexaploid species of Senecio are "senecioid" in all other respects. Senecio in Australia is therefore truly atypical as more than half of the species examined are hexaploid. Outside Australia the highest proportion of hexaploids in Senecio is 16% (9 species) found in South America (Lawrence 1980).

5.3.1.2 Polyploidy and recombination rates. Grant (1958,1975) listed chromosome numbers as an important factor in the regulation of recombination, stating that an organism with the haploid chromosome number of N and heterozygous for one gene pair on each chromosome can produce 2^N genetically different gametes. Although Grant's observation is quite valid, it assumes a level of heterozygosity that may not occur in many plant groups - as polyploidy is a widespread phenomenon in the plant kingdom. A second point is that selection will act on the progeny of an individual, so that the ratios in which progeny are formed are equally important. As Grant (1975, in a separate chapter) and many others have indicated, the proportion of recombinant progeny produced by polyploids is very different from that of diploids. All Australian species of Senecio examined in this study are polyploids; 11 are tetraploid, 15 are hexaploid, 1 is octoploid and 2 are decaploids. Aneuploidy has occurred following polyploidy in only two species, so that the effects of polyploidy, and not changes in basic chromosome numbers are of fundamental significance in Senecio.

Autopolyploidy will have the greatest effects on recombination rates as the duplicated genomes are identical. A diploid heterozygous at locus A will produce three progeny types (AA, Aa, aa) in ratios of 1:2:1, whereas its autotetraploid derivative

differentiation results in preferential pairing then segregation will depend on the distribution of genes at each locus. If each chromosome pair is homozygous but the genotype is heterozygous (AAaa) then no recombination will occur and the heterozygosity will be fixed. However, if each pair is heterozygous (AaAa) then 1/16 of the progeny will be recessive. If the latter situation is extended to an allohexaploid, then only 1/64 of the progeny will be recessive. Recombination of duplicate genes will therefore be reduced in allopolyploids as well as in autopolyploids. Effects will be more pronounced at higher ploidy levels so that the decaploid species of Senecio might be expected to respond extremely slowly to selective pressures, even though genotype variation may be considerable.

Concerning population variability in polyploid species, Hamrick et al. (1979) found that the percentage of polymorphic loci detected by electrophoresis increased with increasing chromosome numbers. Categories compared covered diploid chromosome numbers of 10-20, 22-30 and greater than 30. Hamrick et al. commented that "species that produce a large variety of recombinant progeny (those with high chromosome numbers) might be expected to maintain more genetic variation. The results bear out this expectation." However, if chromosome increases are due to polyploidy, which is more than likely with $2N > 30$, then genetic variation could be maintained by fixed heterozygosity in which case the number of recombinant progeny will be zero. Although this is an extreme effect of polyploidy on recombination, it exemplifies the buffering effect of polyploidy on heterozygotes and the restricted formation of homozygous or extreme phenotypes.

As all Australian species of Senecio are polyploids, the relationship between chromosome number and recombination rates described by Grant (1958, 1975) does not apply, as Grant

UNIVERSITY OF ADELAIDE LIBRARY

considers only changes in basic chromosome numbers. I believe it is unlikely that any extant Australian species of Senecio is a strict autopolyploid for two reasons. Firstly, diploid species of Senecio are more or less confined to Africa and Europe (see 5.3.1.1) and polyploids reaching Australia may have diversified during their extensive migration. Secondly, I found no evidence of multivalents even at the highest ploidy levels (decaploids). Multivalent formation is by no means conclusive evidence of ploidy origins (Stebbins 1971), but the absence of multivalents in all species of Senecio is one indication of hybrid origins. If Australian species of Senecio are allopolyploids, then the limiting effects of polyploidy on recombination will not be as severe as in autopolyploids. Furthermore, mutation at duplicate gene loci may have produced effectively diploid loci (diploidization). However, it is most unlikely that all gene loci will behave as in diploids, so that the buffering effects of polyploidy should influence recmobination in Senecio, particularly at the hexaploid and decaploid levels.

5.3.1.3 Polyploidy and speciation. In the previous section the buffering effect of polyploidy on segregation and recombination was discussed. Such an effect might be expected to retard evolution and restrict speciation except at the diploid level. However, speciation in Senecio has occurred mainly at the tetraploid level (Lawrence, 1980) and in Australia, also at the hexaploid level. The short term as well as the long term consequences of polyploidy need therefore to be considered.

Autopolyploids are generally thought to be adaptively inferior in the first instance (Stebbins 1971, Harlan and deWet 1975, Jackson 1976) although Jackson (1976) reviewed some evidence to suggest that autopolyploids may be more resistant to low

temperatures and therefore able to colonize areas outside their progenitors range. In the case of allopolyploids, there is considerable evidence to suggest that these polyploids possess novel characteristics when first formed. Gottlieb (1976) reviewed evidence of enzyme multiplicity in allopolyploids, and presented data from allopolyploid species of *Tragopogon* (Compositae) known to be of recent origin. Gottlieb and others workers cited by him, found that when duplicated genes specify different polypeptide subunits of a multimeric enzyme, the allohexaploids produce both the homomeric parental enzymes as well as novel heteromeric enzymes not produced by either parent. If the allopolyploid is a "fixed heterozygote" for that gene duplication (e.g. AAaa) then a heterozygous phenotype is reconstituted at fertilization even though each individual's genes are homozygous. As Gottlieb commented, "enzyme multiplicity provides a reasonable hypothesis to account for the wider distribution of tetraploid species relative to the diploid progenitors."

In *Senecio*, the initial success of allotetraploids compared with diploids may have been due to enzyme multiplicity. Further evolution may then have occurred both by hybridization between polyploids and by gene mutation leading to partial diploidization of duplicate genes. However, I believe the long term buffering effects of polyploidy may be illustrated in some Australian species of *Senecio*. A number of species have what appear to be relict distributions. *S. anethifolius* is found in the Flinders Ranges of South Australia and in hills north of Griffith in New South Wales, *S. gawlerensis* occurs only on rocky hilltops in the Gawler Ranges in South Australia, *S. hypoleucus* occurs only in the Mount Lofty Ranges in South Australia and on Mt. Arapiles in Victoria and *S.* sp. A is found in a few isolated rocky areas around Wagga Wagga in New South Wales. All of these species

are hexaploid. On the other hand, the most widespread species occupying a diversity of habitats are S. lautus and S. quadridentatus, both of which are tetraploid. Ploidy levels may offer one explanation for these distributions. Inland areas of the south-eastern states do not offer the diversity of environments found in eastern montane regions. As conditions became progressively drier in Australia, species would have therefore had to adapt genetically or be restricted to isolated refuges. The present distribution of hexaploids, and to a lesser extent, of tetraploid species of Senecio may reflect their inability to evolve rapidly because of the buffering effect of polyploidy. Significantly, the few widespread species are tetraploids - the lowest ploidy level found in Australia. The initial success and spread of tetraploid species of Senecio may have been due to advantageous enzyme multiplicity, but the persistance of such species will depend upon their ability to respond to changing environmental conditions. The presence of seven subspecies in S. lautus suggests that in this species effective diploidization of duplicate genes may have allowed for selection of extreme phenotypes. However, the relict distribution of some hexaploids suggests that these species were unable to respond to changing conditions.

5.3.2 Effects of chiasma frequency and position on recombination rates.

Genes on different chromosomes are recombined by random reassortment at meiosis, but genes on the same chromosome can only be recombined by chiasma formation between homologous chromosomes. Increased chiasma frequencies will most effectively increase recombination if the chiasmata are randomly distributed

(Grant 1958). In some species, the location of chiasmata observed at diplotene differs from that at diakinesis as repulsion of chromosomes leads to terminalization of chiasmata. Terminalization effects can also apparently lead to misinterpretations of chiasma numbers. Grant (1975) reinvestigated chiasma frequencies in *Gilia* and found that previously reported (Brown 1961) differences between inbreeding and outbreeding species "tend to disappear as one goes back from metaphase I to diplotene." In this study, all chiasma frequencies were scored either at diakinesis or metaphase I as high chromosome numbers (N = 20 to 50) made clearly separated preparations difficult to obtain at earlier stages of meiosis. However, in species with chiasmata restricted to chromosome ends (see Table 5.2) partially separated diplotene stages were always examined to see if chiasmata were truly terminal, or if instead, they had undergone terminalization. I found no significant evidence of terminalization of chiasmata in *Senecio*, and concluded that metaphase I configurations are indicative of both chiasma frequency and position. Structural rearrangements of chromosomes are also known to reduce recombination by restricting chiasma formation. However, I found no evidence in the form of bridge-fragment configurations at anaphase I or multivalents at metaphase I to suggest that large structural rearrangements are common in *Senecio*.

Chiasma frequencies may be affected by seasonal environmental conditions such as temperature extremes and low water content (Sybenga 1972). High temperatures, for example, may lead to reduced chiasma frequencies, or in extreme cases, complete failure of pairing. Ideally, plants should therefore be raised in controlled glasshouse conditions. Australian species of *Senecio*, however, grow in a diversity of conditions ranging from inland deserts (*S. gregorii*) to alpine herbfields (*S. pectinatus*).

As optimum glasshouse conditions could not be simulated for all species, I determined chiasma frequencies from apparently healthy and unstressed field plants. Small differences in chiasma frequency per bivalent shown in Table 5.2 might therefore be due to environmental effects, but I believe larger differences truly reflect genetic differences.

Chiasma frequencies in *Senecio* range from 1.01 per bivalent in *S. pterophorus* to 2.04 per bivalent in *S. macranthus*. Relationships between chiasma frequency and other factors regulating recombination are discussed later in this chapter, but one point deserves comment here. Darlington (1965) suggested that chiasma frequency is partly dependent upon chromosome size, with larger chromosomes having more chiasmata. Five native species of *Senecio* have larger than average chromosomes (see karyotypes in Chapter 7). These are *S. magnificus*, *S. amygdalifolius*, *S. macranthus*, *S. velleioides* and *S. vagus*. In each, the chiasma frequency is significantly higher than in species with similar breeding systems, generation lengths and ploidy levels (Table 5.2) but smaller chromosomes. However, frequencies are increased by chiasmata localized at chromosome ends (Figs. 7 and 10 in Lawrence 1980) so that it is not obvious that the greater chiasma frequencies of these species will have an effect on recombination rates.

5.3.3 Effects of breeding systems on recombination rates

According to Grant (1958, 1975) a truly closed recombination system exists where no sexual reproduction occurs, that is, when reproduction is entirely vegetative or occurs only by apomixis. Among sexually reproducing organisms recombination rates will be affected by the degree of outcrossing. Grant (1958) and

Stebbins (1958) observed that cross-fertilization results in a large number of genetic recombinants among progeny and therefore promotes population variability, whereas self-fertilization results in fewer recombinant progeny and decreased variability within populations. Population variability has since been compared for inbreeding and outbreeding species, but with some mixed results (see review by Jain, 1976). However, Hamrick et al. (1979) compared published data of electrophoretically detectable genetic variation in 110 species of higher plants, and found that on average, outbreeding does lead to higher levels of genetic variation than inbreeding.

The evolution of different breeding systems has been considered at length. The general opinion is that self-fertilization is a derived condition (Lewis 1954, Fryxell 1957, Stebbins 1957), but that self fertilization often leads to decreased vigor or seed production (Lloyd 1965, Antonovics 1968). Two general hypotheses have arisen to explain the evolution of inbreeding when the consequences are apparently disadvantageous. The first suggests that inbreeding evolves in situations favouring genetic uniformity of populations rather than genetic flexibility (Mather 1943, Stebbins 1950, 1957, Grant 1975), whereas the second considers that inbreeding evolves in situations favouring greater fertilization assurance for the individual (Baker 1955, Arroyo 1973, Lloyd 1979, 1980). In the latter case, Arroyo (1973) concluded that inbreeding depression is easily surmounted as survival of populations is often entirely dependent upon the evolution of inbreeding. In support of this view, Lloyd (1979) commented that advocates of the regulation of recombination systems "have exaggerated the importance of selection for recombination in controlling the components of breeding patterns" and that "the evolution of self- and cross-fertilization ... must

be sought in forces affecting individuals in each generation." Although I agree that in many instances the selective advantage of inbreeding is likely to be a greater assurance of fertilization an exception may arise in cases of possible hybridization between previously allopatric species. If the species are self-incompatible and prezygotic isolating mechanisms are poorly developed, hybridization could lead to the production of many maladapted genotypes because of interspecific recombination. In this case, inbreeding would be selectively advantageous because it would ensure the production of well adapted genotypes; not because of a greater assurance of fertilization. However, the persistence and multiplication of populations with different characteristics will depend on the long-term advantage of each combination of characteristics. This, I believe, is what the theory of recombination regulation seeks to explain, rather than selective forces affecting the "short-term" appearance of individual characteristics

5.3.4 Effects of generation lengths on recombination rates

Grant (1958, 1975) included generation length among the nine factors listed as regulators of recombination per unit of time, but he provided very little direct discussion of this factor. Grant compared a bacterium and an oak tree to illustrate the effects of generation length, but this example is unrealistic, and does not consider two factors of importance in any study. These are the time required toreach sexual maturity and the stability of the population under consideration. In a density-independent situation, such as expansion following colonization, age at sexual maturity will determine expression of recombinants per unit of time. However, in a density-dependent situation, longevity (or generation length) will be critical as it will

regulate the frequency with which individuals are replaced.

In the case of a bacterium and an oak tree, age at sexual maturity and generation length are positively correlated, but in *Senecio* both annual and perennial species are capable of reaching sexual maturity in one year. If both produce the same proportion of recombinant genotypes, then generation length will only affect the expression of recombinants in a stable density-dependent situation. In a density-independent situation annual and perennial species will have similar potentials for expression of recombinants as both reach sexual maturity within one year. As few species of *Senecio* form truly stable populations (see Chapter 4) differences in longevity are by themselves comparatively unimportant. Differences in generation length in a density-independent situation will be important if sexual maturity is delayed in the perennial species, as for example, in long-lived trees. As few genera contain both annuals and long-lived trees, the inherent problems of comparing generation length in a density-independent situation should apply to genera other than *Senecio*.

5.3.5 Associations of regulatory factors

In previous sections of this chapter the effects of chromosome number, chiasma frequency, breeding system and generation length on the regulation of recombination per unit of time have been considered individually. Grant (1975) suggested that associations of factors that both promote and restrict recombination permit a fine adjustment of the rate at which recombination occurs. Although this is undoubtedly true, alternative hypotheses have in some cases been advanced to explain pair-wise associations of life history traits. Although *Senecio* shows some variation

in each factor examined in this chapter, I have discussed associations in a pair-wise fashion to facilitate comparisons with other cases in the literature. The four factors are considered together in my concluding remarks.

5.3.5.1 Breeding system and generation length. Correlations between breeding system and generation length in *Senecio* have already been mentioned (Chapter 4). Most Australian species of *Senecio* are either annual and inbreeding or perennial and outcrossing (Fig. 5.3). This is precisely the relationship observed by Grant (1958) and used by him as an example of combined restrictive and expansive regulatory factors. Stebbins (1958) suggested that the association of short generations and inbreeding is advantageous in situations requiring a rapid buildup of well adapted genotypes whereas long generations and outcrossing are advantageous in stable populations requiring a small proportion of highly fit individuals for replacement. However, I do not believe that Stebbins' suggestion or Grant's idea of balanced regulation necessarily apply in the case of breeding system and generation length associations in *Senecio*. Firstly, all Australian species of *Senecio* reach sexual maturity within one year irrespective of generation length, so that in unstable environments both annual and perennial species are equally capable of rapid population expansion and rapid expression of parental or recombinant genotypes. Secondly, both annual and perennial species occur in unstable environments (Chapter 4) which by Stebbins' (1958) argument would favour inbreeding annuals. I therefore believe that associations of breeding systems and generation lengths observed in *Senecio* may represent alternative strategies to ensure fertilization.

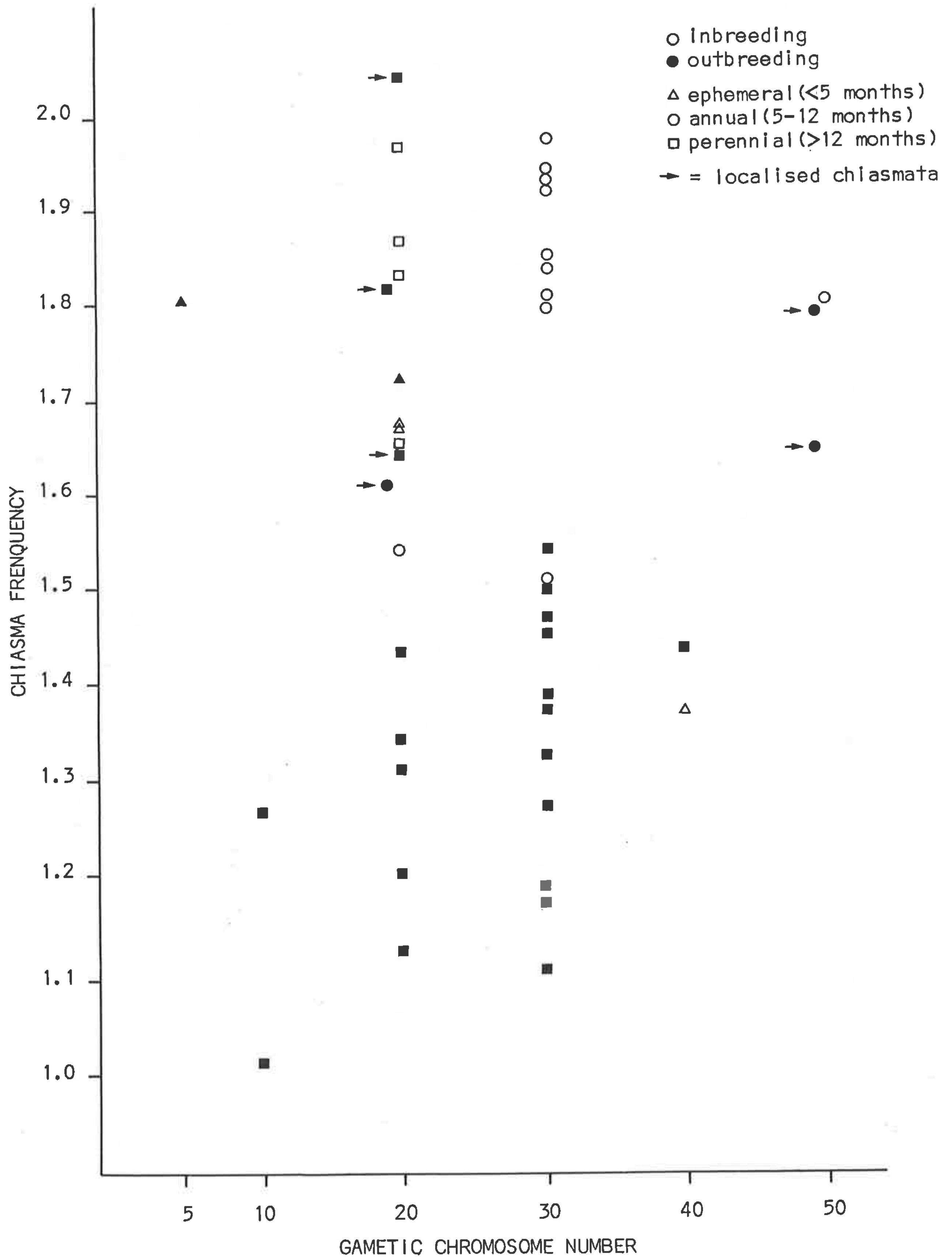


Fig. 5.3 Relationship between chiasma frequency, chromosome number, breeding system and longevity of 34 species and 11 varieties of Senecio.

The primitive condition in Compositae is apparently outcrossing (Stebbins 1958) and shrubiness or perennial growth forms (Carlquist 1976, Cronquist 1977). A perennial and outcrossing species would be disadvantaged in a colonizing or unstable situation, if age at sexual maturity was delayed. However, by reducing the age at sexual maturity to a level comparable with that of annuals, an outcrossing perennial would be just as effective (with the exception of single introductions) as a colonizing species. With the evolution of inbreeding, fertilization would be assured irrespective of longevity so that annual species could evolve without the risk of reduced seed set. The four species of Senecio that are inbreeding and perennial (Table 5.2) regenerate annually from a perennial rootstock, and may represent a transitional stage between outcrossing perennials and inbreeding annuals. One native species, Senecio gregorii, is an outcrossing ephemeral but the specialized mode of seed development, dispersal and germination in this species may account for the maintenance of outcrossing and a short generation. Although the association of outcrossing with long generations and inbreeding with short generations in Senecio would at first appear to support the views of Grant (1958) and Stebbins (1958), the occurrence of both combinations in unstable environments and unstable populations is contrary to the predictions of these authors. Alternative strategies to assure fertilization is, I believe, a more likely explanation of the short-term advantages leading to the selection of these associations in Senecio. However, when chiasma frequency and chromosome number are also considered, the long-term persistence of such associations may well be explained by the advantages of restricting recombination suggested by Grant (1958, 1975) and Stebbins (1958).

5.3.5.2 Breeding system and chromosome numbers.

Grant (1958) observed that lower chromosome numbers and outcrossing were combined in some species whereas high chromosome numbers and inbreeding were combined in others. In *Senecio* there is no general relationship between chromosome numbers and breeding systems (Fig. 5.3), presumably because variation in chromosome numbers is due to polyploidy and not to changes in basic numbers.

In 1975, Grant added a discussion of relationships between low basic chromosome numbers and breeding systems. Grant suggested that reduced basic numbers should be prevalent in outcrossing and heterozygous plants which place an emphasis on restricted recombination. Predominantly self-fertilizing and homozygous plants should display a variety of high and low chromosome numbers. After surveying the Compositae, Grant found that all of the nine species with haploid chromosome numbers less than six are outcrossing. Only one species included in this study, *Senecio discifolius* from Africa, has a chromosome number in the range specified by Grant. *S. discifolius* may have evolved by aneuploid reductions in chromosome number (Chapter 6) and supports Grant's hypothesis as it has N = 5 and is outcrossing.

5.3.5.3 Breeding system and chiasma frequency.

The relationship between breeding system and chiasma frequency is fairly well established. Associations of high chiasma frequencies with inbreeding and low chiasma frequencies with outcrossing have been observed in *Sorghum* (Garber 1950), *Lolium* (Rees and Thompson 1956), *Crepis* (Stebbins 1958), *Limnanthes* (Arroyo 1973) and *Senecio* (Gibbs et al. 1975). The same relationship occurs in Australian species of *Senecio* (Fig. 5.3).

A few outcrossing species do have high chiasma frequencies, but in these the chiasmata are localized and probably do not effectively increase recombination.

Although the relationship is established, hypotheses advanced to explain the relationship vary considerably. Stebbins (1950) suggested that high chiasma frequencies may evolve after the evolution of inbreeding, to allow for the rapid recombination of heterozygotes following occasional outcrossing. Inbreeding populations, initially highly adapted to a particular environment could therefore maintain some variation as insurance against long-term changes in environments. Arroyo (1973) found that high chiasma frequencies evolved at the same time as inbreeding. As increases in chiasma frequency may disrupt adaptive gene combinations, the evidence presented by Arroyo contradicts Stebbins' (1950) hypothesis that inbreeding evolves when highly adapted genotypes are favored. Arroyo considered that inbreeding evolved in *Limnanthes* to ensure fertilization, and that increased chiasma frequencies arose at the same time to maintain genetic variability. Reduced genetic variability among inbreeding populations is therefore the effect of the evolutionary change and not its cause. A more recent hypothesis by Rees and Dale (1974) suggests that increased chiasma frequencies are selected for, "albeit unconsciously," whenever disruptive selection for extreme phenotypes occurs. Rees and Dale present their own evidence of increased chiama frequencies in *Lolium* and *Festuca* populations subjected to artificial selection pressures, and the results of Harinayarana and Murty (1973) in which disruptive selection for early and late flowering in *Brassica* is accompanied by increases in chiasma frequencies. Rees and Dale suggest that the high chiasma frequencies observed in inbreeding populations and annual species are in all probability "relics of the cytological

prerequisites upon which their evolution was founded," and that compensatory roles such as rapid recombination of heterozygotes are a "fortuitous bonus."

Most species of Senecio examined in this study are either self-compatible with a high chiasma frequency or self-incompatible with a low chiasma frequency. No intermediate cases were found that would help to distinguish between the three above-mentioned hypotheses. However, I believe the exceptional combination of traits in Senecio gregorii lends support to the model proposed by Rees and Dale (1974). S. gregorii is a highly specialized arid zone ephemeral that combines outcrossing with a high chiasma frequency. Stebbins (1958) found that outcrossing and short generations were sometimes combined in populations fluctuating greatly in size (e.g. in some species of Crepis and the tribe Madinae) but in these, temporary genetic constancy was achieved by a low basic chromosome number and a low chiasma frequency. However, S. gregorii is a tetraploid with 2N = 40 and has a high chiasma frequency. I believe the evolution of a high chiasma frequency in S. gregorii may have accompanied selection for an extremely specialized reproductive strategy (as the model of Rees and Dale (1974) predicts). The specialized strategy would increase the chance of fertilization (see Chapter 4.5), but the very open recombination system of S. gregorii might also have led to reduced population variability. This suggestion is supported by my preliminary observations of field populations. Individuals show great developmental plasticity in size at flowering in different environments, but very little variation in leaf or capitulum morphology. If my predictions concerning the evolution of S. gregorii are correct, then uniform and well-adapted progeny may be produced because of very low population variation, rather than by restrictions imposed by the

recombination system. Although genotypically less variable, the progeny of S. gregorii may have greater developmental plasticity than the progeny of an inbreeding diploid as, being tetraploid, gene loci may be fixed in a heterozygous state. I believe the unusual combination of traits in S. gregorii warrant further study, particularly by electrophoretic techniques, as these may reveal reduced population variation in an outcrossing species.

5.3.5.4 Generation length and chromosome number.

Grant (1958) observed that among dicotyledons, the herbaceous members have a modal chromosome number of N = 7 while the woody members of the same subclass have a modal number of N = 14. Grant listed the two factors as another example of a balance between restricted and expansive regulatory factors. In Senecio there is no correlation at all, as both annual and perennial species occur at each ploidy level (Fig 5.3). However, Grant described changes in basic chromosome numbers, whereas the changes in Senecio are due to polyploidy. As polyploidy will in many instances retard the expression of recombinants, the relationship between breeding system and chromosome number observed by Grant does not apply to Senecio.

5.3.5.5 Generation length and chiasma frequency.

In Senecio, high chiasma frequencies are found in annual species and low chiasma frequencies in perennials (Fig. 5.3). Grant (1958, 1975) did not compare these factors, but in view of the relationship, might have added them to his list of combinations of restrictive and expansive regulatory factors. Using the model proposed by Rees and Dale (1974) it could also be argued that the association of high chiasma frequencies with

short generations is due to the specialized nature of the latter (see part 5.4.5.4). Alternatively, the relationship may be of a secondary nature, as most annual species of Senecio are inbreeders and most perennials are outcrossers. The significance of this pairwise association therefore depends upon whether inbreeding or short generations evolved first. The intermediate growth form of four perennial but inbreeding species of Senecio (Table 5.3) could be evidence that inbreeding did, in fact, evolve first. If this is the case, then the correlations between generation length and chiasma frequency may be secondary.

5.3.5.6 Chiasma frequency and chromosome number - The Recombination Index.

Darlington (1939) combined the two quantifiable features of chromosomes in a recombination index - which is the haploid number of chromosomes plus the average number of chiasmata per cell. The recombination index has not been widely used although it is mentioned in a number of comparatively recent texts (Stebbins 1971, Mather 1973, Grant 1975). However, none of the authors discuss situations in which the recombination index cannot be applied. Its recent use by Gibbs et al. (1975) falls into such a category.

Gibbs et al. discussed correlations between the breeding system and recombination index of five species of Senecio. However, the three outcrossing species examined were diploid (N = 10) and the two inbreeding species were tetraploid (N = 20). As the recombination index of inbreeding species was doubled by chromosome number alone, Gibbs et al. were surprised to find that the inbreeding species also had increased chiasma frequencies. In view of the buffering effect of polyploidy (part 5.3.1.3) it is most unlikely that doubling of chromosome numbers will double the

recombination potential of these species. Increased chiasma frequencies among inbreeding species will increase recombination of occasional outcrosses, but as Rees and Dale (1974) suggested, this effect may be a "bonus" for otherwise specialized populations with low levels of variability. I calculated the recombination index of each species included in this study (Table 5.3) and believe that the values are very misleading. For example, *S. velleioides* and *S. vagus* occur in similar habitats in wet sclerophyll forests, are both outcrossing and are annual or short lived perennial herbs. Both have similar chiasma frequencies but very different chromosome numbers. Because of the latter, the recombination indexes are 50 for *S. velleioides* and 132 and 139 for the two subspecies of *S. vagus* - an almost threefold difference. Similar comparisons can be made between species of inbreeding annuals. I therefore believe that Darlington's (1939) recombination index should only be used when (1) basic chromosome numbers have been altered and (2) when chiasma frequencies are altered by randomly positioned chiasmata. As chromosome number increases in *Senecio* are due to polyploidy, and chiasma frequency increases are caused in some cases by localized chiasmata, the recombination index as proposed by Darlington (1939) is of little value in this genus. However, if the recombination index could be modified to account for autopolyploidy and allopolyploidy (which would require a second index of the degree of allopolyploidy) then the recombination index might be found to correlate more accurately when polyploid series are involved.

5.3.6 Recombination systems in *Senecio*

In the previous section, factors regulating recombination were considered in a pair-wise fashion, as this is the form in which they most frequently occur in the literature. Pair-wise comparisons have led to considerable speculation concerning the evolution both of individual factors and of commonly observed associations of particular pairs. However, such comparisons are only truly valid if other regulatory factors do not vary, and all factors considered in this chapter show some variation in *Senecio*. Pair-wise comparisons of factors in this genus may be useful in determining short-term evolutionary pathways, but do not contribute greatly to considerations of long-term persistence of populations. The latter is best viewed in the light of recombination systems.

Recombination systems of *Senecio* (in terms of the four factors considered in this chapter) are listed in Table 5.4. I have provided a very simple index of the "degree of openness" of each system by scoring each of the four factors as "zero" if they restrict recombination and "two" if they promote recombination (see note below Table 5.4). The scoring of generation length and chromosome number have each been divided into three categories. A compromise was made with generation length. If effects on recombination are viewed in terms of the age at sexual maturity, then ephemerals would score two and annuals and perennials would both score 0. However, in terms of longevity ephemerals, annuals and perennials would score 2, 1, and 0, respectively. Neither system is entirely satisfactory in the case of *Senecio*. I adopted the latter scores as they account for species that do occur in stable environments. Chromosome numbers in *Senecio* increase by polyploidy, and the effects on recombination

TABLE 5.4

Recombination Systems Found in Native Australian Species of *Senecio*

System and Species	No. of Species	Index*
1. ephemeral, outcrossing, high C.F.**, tetraploid		
S. gregorii	1	8
2. ephemeral, inbreeding, high C.F., tetraploid		
(octaploid) *S. glossanthus*	1	6(4)
3. annual, outcrossing, low effective C.F.,		
tetraploid *S. velleioides*	1	5
4. annual, inbreeding, high C.F., hexaploid		
S. sp. B, *S. squarrosus*, *S. bipinnatisectus*, *S. minimus*, *S. picridioides*, *S. glomeratus*, *S. hispidulus*, *S.* sp C	8	4
5. perennial, outcrossing, low C.F., tetraploid		
S. lautus, *S. spathulatus*, *S. magnificus* *S. amygdalifolius*, *S. macranthus*	5	4
6. perennial, inbreeding, high C.F., tetraploid		
S. quadridentatus, *S. gunnii*, *S.* aff. *apargiaefolius*, *S. runcinifolius*	4	4

Table 5.4 - continued

System and Species	No. of Species	Index*
7. perennial, outcrossing, low C.F., hexaploid S. *linearifolius*, S. sp. A, S. *hypoleucus*, S. *odoratus*, S. *cunninghamii*, S. *anethifolius*, S. *gawlerensis*	7	3
8. annual, inbreeding, high C.F., decaploid S. *biserratus*	1	3
9. annual, outcrossing, low effective C.F., decaploid S. *vagus*	1	3
10. perennial, outcrossing, low C.F., octoploid S. *pectinatus*	1	2

*Index of "degree of openness" in four factors considered for each recombination system

Scores:					
ephemeral	2	outcrossing	2	tetraploid	2
annual	1	inbreeding	0	hexaploid	1
perennial	0			octoploid/ decaploid	0
high C.F.	2				
low C.F.	0				

**C.F. = chiasma frequency

will be opposite to those predicted by Grant (1958, 1975). As tetraploids represent the lowest ploidy level in Australia, I have scored tetraploids and hexaploids as 2 and 1, and octoploids and decaploids both as 0 (the latter category is for convenience as few species are very high polyploids).

Only one species, Senecio gregorii, has a completely open recombination system (index of 8) within the range of variables examined. Grant (1958, 1975) suggested that comparatively open systems predominate in stable and closed habitats as replacement of individuals is limited and genotypic constancy will be brought about by centripetal selection. However, S. gregorii occurs in perhaps the most unstable environment considered in this study - temporary habitats of inland deserts. Both Grant (1958) and Stebbins (1958) predicted that in such environments recombination should be very restricted. Although contradictory in terms of its recombination system, the situation in S. gregorii may be comparable in terms of production of well-adapted genotypes. Grant (1958) commented that some restriction on recombination is universal, yet the system of S. gregorii is completely open in terms of the four factors considered. It is possible that this "excessively" open recombination has led to very reduced population variability, and that selection has produced populations of uniformly well-adapted genotypes. S. gregorii may therefore consist of locally very uniform populations producing generally well-adapted progeny, but nonetheless capable of rapid recombination if dispersal or migration leads to interactions between populations. If this is the case, then population studies of S. gregorii using electrophoretic techniques may yield an unexpected relationship between outcrossing and population variability.

At the opposite extreme, *Senecio pectinatus* occupies a comparatively stable environment yet has the least open recombination system - again contradicting the predictions of Grant (1958). Although stable (in the sense of predictability and vegetation type) the alpine environment of *S. pectinatus* is extremely harsh. In such conditions, successful species would need to be very specialized in growth form and flowering times, and might not benefit from the production of a large number of recombinant genotypes. The maintenance of a restricted recombination system by *S. pectinatus* may therefore be related to the stable yet highly specialized nature of its environment.

Grant (1958) suggested that recombination systems may be open or closed, but intermediate and restricted systems will usually be advantageous as they combine immediate fitness with long-term flexibility. If the advantages of particular systems are reflected in the survivorship of those systems, then Grant's suggestion is generally true of *Senecio*. The majority of species (27 out of 30) have recombination systems that contain a balance of restrictive and expansive regulator factors (index values of 3, 4, and 5). Although the components of these "intermediate" systems differ greatly, the occurrence of each type in similar unstable environments suggests that their end effects on recombination rates are comparable. It is perhaps because of the wide variation of regulatory factors in *Senecio* that individual factors do not behave as in less variable genera. For example, inbreeding has been associated with unstable environments and outcrossing with stable environments (Stebbins, 1958), yet in *Senecio* both breeding systems occur in unstable environments. A possible explanation is that the effects of breeding systems on recombination are balanced by different combinations of generation length and chromosome number, and that very different systems

therefore produce the same end effect. In the previous chapter I concluded that the diversity of reproductive traits found in *Senecio* probably represent alternative strategies to achieve reproductive success in a variety of unstable environments. I believe a similar observation can be made of recombination systems in *Senecio*. Although very different in individual composition, the majority of recombination systems consist of a balance between restrictive and expansive regulatory factors. The combination, for example, of either an annual growth form, inbreeding, a high chiasma frequency and a hexaploid chromosome number or a perennial growth form, outcrossing, a low chiasma frequency and a tetraploid chromosome number may produce similar effects with respect to recombination. As both systems occur among species capable of rapid colonization one can conclude that both systems are equally successful in terms of the persistence and multiplication of populations.

5.4 Conclusions

The evolution of recombination systems has in the past been discussed from two viewpoints; the selective advantages of individual regulatory factors and the selective advantages of combinations of regulatory factors. Lloyd (1979) considers that in the first case, advantages are immediate and therefore affect individuals most strongly whereas in the second case, advantages are long-term and determine the relative persistence and multiplication of populations. It would appear that disagreements have arisen because of misinterpretations of the two situations. Arroyo (1973) and Lloyd (1979), for example, argued against short-term selection of individual regulatory factors such as breeding systems and chiasma frequencies in order to optimize the rate of recombination in a population, citing Grant (1958) and others as

supporters of this idea. Yet as I understand it, the theory of recombination regulation proposed by Grant does not attempt to explain short-term selective advantages of individual factors, but rather, the advantages of combinations of factors in a variety of environmental conditions.

Comparisons of pairs of regulatory factors (as in Section 5.3) have been utilized to deduce the selective advantages both of pairs and of individual regulatory factors. Presumably the greater proportion of literature deals with pair-wise comparisons as these provide a simple experimental system. Relationships between breeding system and chiasma frequency, for example, are best deduced from species that have the same chromosome number and generation length. As I have not followed evolutionary changes in consecutive generations, my conclusions as to why individual characters or pair-wise associations have arisen are speculative, and are based on the "best fit" of my data with a number of hypotheses. My conclusions are as follows.

The primitive breeding system in Compositae is apparently outcrossing (Stebbins 1958) so that inbreeding is a derived condition. I believe the most likely advantages leading to the selection of inbreeding in _Senecio_ are a greater assurance of fertilization and the retrieval of reproductive effort. Both would be important for species of unstable environments, and in either stable or unstable environments retrieval of reproductive effort might become a causal factor if previously allopatric populations come together. Inbreeding plants would then be favoured as they would produce less hybrid and potentially mal-adapted progeny. In the case of _Senecio_, I believe it is un-likely that inbreeding evolved in unstable environments so that uniform and well-adapted genotypes would be produced - as both inbreeding and outcrossing species are equally successful in these conditions.

High chiasma frequencies in *Senecio* are apparently related to two quite different factors. Firstly, Darlington (1965) suggested that chiasma frequency is partly related to chromosome size, as higher numbers of chiasmata may be needed to orient large chromosomes during meiosis. A number of outcrossing species of *Senecio* with very large chromosomes were found to have high numbers of localized chiasmata whereas outcrossing species with small chromosomes had fewer chiasmata. Secondly, Rees and Dale (1974) suggested that a greater number of randomly positioned chiasmata are selected for, "albeit unconsciously", whenever selection favours an extreme or specialized phenotype. This model explains both the commonly observed association of high chiasma frequencies and inbreeding (a derived condition) in *Senecio* as well as the high chiasma frequency of the highly specialized but outcrossing ephemeral, *S. gregorii*.

Carlquist (1976) and Cronquist (1977) suggest that shrubbiness is primitive in the Compositae. In *Senecio*, shrubby (or perennial) and annual species are equally successful in unstable environments. Although most perennial species are also outbreeders, I believe their success may be partly due to an ability to flower in the first season. The evolution of annual and predominantly inbreeding species may therefore represent an alternative rather than a more advantageous strategy in an unstable environment.

All Australian species of *Senecio* examined and the majority of species from other parts of the world are polyploids. The complete absence of multivalents at meiosis in any of the species examined suggests (but does not prove) that Australian species of *Senecio* are allopolyploids. If this is the case, then a likely explanation for the initial success and spread of polyploid species of *Senecio* is the production of both parental and

novel multimeric enzymes. Novel characteristics would allow species to spread outside the range of their progenitors.

The persistence of populations with any of the above-mentioned characteristics will depend on long-term effects rather than short-term advantages. Inbreeding, for example, has been found to lead to reduced population variability. Increased chiasma frequencies will initially promote recombination but the action of selection on recombinant progeny may also reduce population variability. Polyploidy will have a buffering effect on evolutionary changes as fewer homozygous progeny are produced in any one generation. If a sudden environmental change favours extreme genotypes, then polyploidy may be disadvantageous as it retards the process of selection. However, that same process may be advantageous for fluctuating populations in an unstable environment, as it would reduce the chance fixation of undesirable genes in a homozygous condition. I therefore consider that selective advantages that act either over a short period or a long period of time need to be considered in a study of recombination systems. The immediate or short term effects of a change in regulatory factors will contribute to the immediate fitness of a population whereas the long-term consequences of combinations of factors will influence the flexibility and persistence of that population.

In terms of persistence, Grant (1958, 1975) observed that colonizing species or species of unstable environments most frequently have restricted recombination systems and species of stable and closed communities are often characterized by more open systems. Most Australian species of _Senecio_ occur in variously unstable environments and, as predicted by Grant, most do have restricted recombination systems (neither fully "open" nor fully "closed"). However, the components of each

recombination system are very different. Outcrossing and inbreeding species, for example, are equally successful in unstable environments probably because these regulatory factors are balanced by other restrictive and expansive factors. A departure from the predictions of Grant (1958, 1975) and Stebbins (1958) is found in *Senecio gregorii*. This species has a very open recombination system yet occurs in unstable inland deserts. Both authors suggest that in such environments, recombination is usually very restricted so uniformly well-adapted genotypes are produced. However, if the "excessively" open recombination system of *S. gregorii* has led to reduced within-population variability, then this species may also produce uniformly well-adapted genotypes.

The most unusual aspect of this study of recombination systems is that chromosome numbers vary by polyploidy. To my knowledge, the only previous study including polyploid species in an investigation of recombination systems was by Gibbs et al., (1975), but I believe the results were misinterpreted as increases by polyploidy were treated as changes in basic chromosome numbers. Recombination will be restricted both by increases in ploidy levels and by decreases in basic chromosome numbers. The predominance of polyploidy among Australian species of *Senecio* might therefore be equated with the predominance of aneuploid reductions among annual species of Cichorieae (Stebbins 1958). Both groups occur in unstable environments and both have restricted recombination systems.

CHAPTER 6

Nuclear DNA Amounts

6.1 Introduction

6.2 Materials and Methods

6.2.1 Source of Material

6.2.2 Feulgen Stain and SO_2 Water

6.2.3 Cultivation

6.2.4 Preparation of Slides

6.2.5 Measurement of DNA Amount

6.2.6 Analysis of Results

6.2.7 Hydrolysis Times and Root Tip Sizes

6.2.8 Selection of a Calibration Standard

6.2.9 Cell Volumes

6.3 Results and Discussion

6.3.1 Terminology

6.3.2 Comparison of Calibration Standards

6.3.3 Reliability of DNA Estimates and Size of Significant Differences

6.3.4 Interspecific Differences in DNA Amounts

6.3.5 Intraspecific Differences in DNA Amounts

6.3.6 Nucleotypic Effects

6.3.6.1 Size of structures

6.3.6.2 Cell cycle times

6.3.6.3 Minimum generation times

6.3.7 Nature of Changes in DNA Amounts

6.3.8 Speculations on the Direction of Changes in DNA Amounts

6.4 Conclusions

6.1 Introduction

Among eukaryotes, the haploid DNA content per nucleus (C-value) varies from 0.005 picograms (pg) in yeast (Sokurova 1973) to 200 pg in the dinoflagellate Gonyaulax (Holm-Hansen 1969) - a 40,000-fold range. Sparrow et al. (1972) found only a very general positive correlation between DNA amount and evolutionary advancement. For example, prokaryotes generally have less DNA than eukaryotes. However, there are many instances in which less advanced organisms have as much if not more DNA than advanced organisms (Price 1976). It is therefore evident that DNA content is not necessarily correlated with evolutionary advancement or structural complexity, a phenomenon termed the "C-value paradox" (Thomas 1971).

Estimates of the number of structural genes expressed in different eukaryotes vary from 4000 to 50,000 (Hereford and Rosbash 1977, Ohata and Kimura 1971, Kiper et al. 1979). If an average gene length is taken to be 1,400 nucleotides (Kiper et al. 1979) and 1 picogram is equivalent to 0.965×10^9 nucleotides (Bennett and Smith 1976) then an organism with 1 picogram of DNA contains 700,000 gene-sized units. It would therefore appear that at most only 10% of 1 picogram of DNA is expressed, and that the bulk of nuclear DNA does not code for proteins. Similar considerations have led to suggestions that most of the eukaryotic genome contains "junk" (Ohno 1972), has "no function" (Gierer 1974), is "selfish" (Doolittle and Sapienza 1980) or is "parasitic" (Orgel and Crick 1980), and that natural selection acting on phenotypic characteristics is relatively unimportant in determining C-values. Instead, it is argued that there is a tendency to acquire DNA that is independent of natural selection.

According to Cavalier-Smith (1977) and Doolittle and Sapienza (1980) the genetic unit involved is a transposon or translocatable genetic unit that contains inverted repeat sequences separated by a spacer. The entire unit is transposable and can apparently be inserted anywhere in the genome. Doolittle and Sapienza (1980) suggest that "transposability itself ensures the survival of the transposable element, regardless of effects on organismal phenotype or evolutionary adaptability" and that "a sequence which spawns copies of itself elsewhere in the genome can only be eradicated by simultaneous multiple deletions." Orgel and Crick (1980) considered that the selective disadvantage of one sequence of 1000 base pairs would be only 10^{-6} and would therefore require 10^6 to 10^8 years to be eliminated by competition. The concept of "selfish" or "parasitic" DNA is therefore thought to explain the C-value paradox in terms of a universal tendency for DNA amounts to increase and selection against such increases, particularly in rapidly growing organisms.

An alternative hypothesis proposed by Cavalier-Smith (1978, 1980) is based on observations that DNA amounts affect cell size, cell cycle times and minimum generation length - collectively called nucleotypic effects by Bennett (1972). Cavalier-Smith suggests that large cells actually require more DNA than do smaller ones. By his theory it is the "extra replicon origins not the large nucleus, or larger genome as such, which increases cell size: what the C-value controls more directly is the nuclear volume." The importance of "selfish" DNA is that it provides the variation upon which nucleotypic selection acts. Cavalier-Smith suggests that selection for different cell sizes and growth rates (as in r- and K-selection) are accompanied by selection for different numbers of replicon origins and nuclear volumes. Natural selection therefore controls DNA amount through the effects of the latter on phenotypic characteristics.

The above-mentioned hypotheses are similar in a number of respects. Both suggest that selection within the genome (intragenomic) for transposable DNA provides the basis for changes in DNA amounts. Both also suggest that natural selection acting on the phenotype will ultimately determine DNA amounts. However, the theories differ substantially in their emphasis. The theory of "selfish" or "parasitic" DNA suggests there is a universal tendency for DNA amounts to increase by non-phenotypic selection whereas Cavalier-Smith (1978) suggests that increases and decreases in DNA amounts are both the product of natural selection acting on the phenotype. Furthermore, the first theory supposes that selfish or non-specific DNA has no function, whereas the second suggests that non-specific DNA has a nucleotypic function - that is, the control of cell size and cycle time.

In this chapter I intend to examine DNA amounts in *Senecio* with respect to nucleotypic effects and environments, and to see if it is possible to distinguish between the two hypotheses.

6.2 Materials and Methods

6.2.1 Source of Material

Only one population of each taxon was included in this investigation. Populations correspond to collection numbers listed in Table 5.2. A single seed batch of *Pisum sativum* cv. Massey Gem was used as a standard in all DNA determinations. *Hordeum vulgare* labelled "Cape barley" was later included to compare standards. Seeds of both were obtained from M.F. Hodge and Sons, Pty. Ltd., Adelaide, South Australia.

6.2.2 Feulgen Stain and SO_2 Water

Leuco-basic fuchsin gives a purple colouration when it complexes with the aldehyde groups of DNA and RNA, but the latter is removed by acid hydrolysis (Bennett and Smith 1976). The method, first described by Feulgen and Rossenbeck (1924), is known as "Feulgen-staining" or "Feulgen microdensitometry". Leuco-basic fuchsin stain and sulphur dioxide water (SO_2 water) were both prepared by methods described by Darlington and LaCour (1976, p. 115). Basic fuchsin from British Drug Houses Ltd., England, was used in all stain preparation. The stain was stored in a tightly stoppered bottle in the dark at 4°C, and was reused for a maximum of four weeks. SO_2 water was mixed freshly for each DNA determination (set of 6 slides).

6.2.3 Cultivation

Primary roots from germinating *Senecio* seeds were in most cases extremely small whereas larger roots were produced by plants 4-8 weeks old. Several plants of each taxon were therefore raised in 10-cm pots set in trays of moist vermiculite. Collections were made when roots began to emerge from the drainage holes. Seeds of *Pisum sativum* were soaked overnight in water and sown in 15 cm pots of moist vermiculite. Collections were made 8-10 days after germination when primary roots were approximately 6 cm long. All material was maintained in a glasshouse at a temperature of 22°C.

6.2.4 Preparation of Slides

The schedule for slide preparation and staining is summarized in Table 6.1. The method (adapted from Martin and Hayman (1965) and Martin (1974)) aims to minimise experimental errors by including an internal standard on each slide, and to minimise

TABLE 6.1

Schedule for Preparation of Slides

for Feulgen Microdensitometry

Root Tip Pairs	Time
Collected into 3:1 alcohol:acetic acid	8 hours
70% alcohol at 4°C	16 hours
50% alcohol	2 minutes
30% alcohol	2 "
Distilled water	2 "
1N HCl at 20-25°C (room temperature)	45 "
Distilled water at 4 C	1 "
Squashed in distilled water	3 "
Coverslips removed after freezing with liquid CO_2	

Slides	
Absolute alcohol	2 minutes
90% alcohol	2 "
70% alcohol	2 "
50% alcohol	2 "
30% alcohol	2 "
Distilled water	2 "
Leuco-basic fuchsin (in dark)	120 minutes
SO_2 1	10 "
SO_2 2	10 "
SO_2 3	10 "
Distilled water 1	2 "
Distilled water 2	2 "
30% alcohol	2 "
50% alcohol	2 "
70% alcohol	2 "
90% alcohol	2 "
Absolute alcohol 1	2 "
Absolute alcohol 2	2 "
Xylene 1	2 "
Xylene 2	2 "
Mounted in XAM Neutral Mounting Medium	

position errors by alternating the position of standard and test cells on each slide. Root tips from the standard and test species, sufficient for one slide, were fixed simultaneously in one vial and were then treated together throughout the schedule. Root tips were identified by cutting them to different lengths. As six slides were prepared for each determination, six duplicate vials of root tips were collected.

Slides were marked with a diamond pencil so that standard and test preparations alternated in position as follows:

slide 1	S_1 T_1	slide 3	S_3 T_3	slide 5	S_5 T_5
slide 2	T_2 S_2	slide 4	T_4 S_4	slide 6	T_6 S_6

Root tip squashes were completed in the order indicated (i.e. S_1 T_1 T_2 S_2 S_3 T_3 etc.) and slides were processed in sequence throughout the treatment. Contents of each vial were processed at 3-minute intervals to allow for the time required for root-tip squashes. At completion, slides were stored in the dark for a minimum of three days and a maximum of two weeks before DNA amounts were measured.

6.2.5 Measurement of DNA Amounts

Relative DNA amounts of prophase nuclei (4C stage) were measured using a Barr and Stroud integrating microdensitometer GN 2 (Deeley 1955) set to a wavelength of 5480 Å. Identical control settings were used throughout the study to minimise experimental error (first field stop -10X, absorption range -20, extinction coefficient - 0.5). The relative DNA amount of each cell was determined by firstly measuring the cell and then an adjacent blank background region three times in rapid succession. Cell values are therefore the average difference between three pairs of readings. Fifteen cells were measured for each coverslip,

a total of 90 cells for both the standard and test species. Bennett and Smith (1976) observed that DNA amounts tend to be underestimated by Feulgen microdensitometry as DNA density increases. For this reason mid-prophase cells were always chosen and later prophase or metaphase cells avoided. Cells with most of the nuclear material clumped in one region, with broken cell walls or with apparently foreign inclusions were also avoided.

6.2.6 Analysis of Results

A Fortran computer program written by N. G. Martin (Martin 1974) was used to complete an analysis of variance for each set of six slides and to calculate the ratio and standard error of the species measured. Absolute DNA amounts for each test species were determined by multiplying the ratio and its standard error (always relative to *Pisum sativum*) by 19.46 - the 4C DNA amount in picograms calculated for *Pisum sativum* cv. Minerva Maple by Bennett and Smith (1976). An example of the program output is shown in Table 6.2.

The analysis partitions variation between species (*Pisum sativum* and the test species) between replicate slides and within root tips. A significant difference was often recorded between slides indicating real variation in staining procedure. However, this was not important as the interaction mean square (between slides and species) was generally negligible ($P>.05$).

6.2.7 Hydrolysis Times and Root Tip Size

Bennett and Smith (1976) observed that reduced staining intensity can be caused either by insufficient or by excessive hydrolysis of material. An optimum hydrolysis time was therefore determined using *Senecio quadridentatus*. Slides were prepared by

TABLE 6.2

Sample Output of Program DNA; An Analysis of Variance of Original Measurements

SENECIO BIPINNATISECTUS VERSUS STANDARD (PISUM SATIVUM)

MICRODENSITOMETER READINGS

SLIDE	1		2		3		4		5		6	
SPECIES	A	B	A	B	A	B	A	B	A	B	A	B
	23.2	30.7	18.8	29.2	20.5	34.3	19.3	27.7	23.2	29.2	20.8	28.5
	21.7	30.8	19.0	32.0	20.2	31.3	18.3	26.2	22.7	28.2	20.3	30.3
	19.8	28.2	21.2	29.5	24.7	30.2	18.7	26.8	22.7	30.3	23.0	30.7
	21.3	29.7	23.0	31.5	23.2	29.0	20.2	30.3	21.7	25.3	21.7	26.8
	20.2	31.7	22.3	31.0	23.7	30.3	19.7	26.3	20.7	26.7	21.2	30.3
	18.7	28.3	22.0	31.8	20.7	30.3	17.5	25.3	20.8	26.7	20.8	30.8
	21.5	30.5	22.7	32.5	22.7	31.0	18.0	30.5	21.2	29.8	20.7	30.0
	20.0	31.2	18.8	30.0	21.8	29.0	17.7	25.2	19.8	27.7	21.3	30.5
	20.8	27.5	25.2	31.0	24.0	31.3	18.8	26.7	22.0	26.7	22.7	29.3
	20.7	28.7	24.7	30.2	22.8	30.8	17.8	29.7	22.5	28.5	22.0	28.8
	19.3	31.0	23.3	30.8	20.8	30.2	18.2	28.8	24.0	28.2	20.5	28.7
	20.8	29.7	29.7	28.5	22.8	31.0	17.7	29.2	19.0	27.8	20.7	31.7
	20.7	27.7	24.2	30.5	22.7	31.7	17.7	26.8	19.8	29.7	22.3	29.0
	20.2	29.0	22.5	30.8	22.7	33.2	21.2	25.8	19.7	30.3	19.5	30.7
	20.2	28.8	21.7	28.2	22.7	31.7	17.7	25.3	20.5	28.5	21.7	29.5
MEAN	20.6	29.6	22.6	30.5	22.4	31.0	18.6	27.4	21.4	28.2	21.3	29.7
C.V.	.052	.046	.124	.041	.060	.045	.059	.068	.069	.052	.045	.041
NUMBER	15	15	15	15	15	15	15	15	15	15	15	15
RATIO	.6970		.7412		.7221		.6783		.7561		.7163	

MEAN RATIO (A/B) = .7184 +- .0063

	D.F.	SUMS OF SQUARES	MEAN SQUARE	F VALUE	PROB	F (NO INTERACTION)	PROB
BETWEEN SLIDES	5	279.5418	55.9084	24.3228	.0000	23.6822	-.0000
BETWEEN SPECIES	1	3074.3734	3074.3734	1337.4964	.0000	1302.2723	-.0000
INTERACTION	5	22.2489	4.4498	1.9359	.0909		
WITHIN SAMPLES	168	386.1653	2.2986				
TOTAL	179	3762.3295					

MEAN OF SPECIES 1 = 21.14
NUMBER = 90

MEAN OF SPECIES 2 = 29.40
NUMBER = 90

the schedule given in Table 6.1, but hydrolysis times were varied from 10 to 120 minutes. The effect of root tip size was examined at the same time by using one large root tip (1 mm diameter) as the standard and three smaller root tips (0.3-0.5 mm diameter) as the test. Measurements of absorbance in arbitrary units indicated that maximum absorbance was reached after 35 minutes, and was maintained until 80 minutes after which abosrbance decreased. No significant difference was found in the absorbance properties of cells from different sized root tips. Although a broad range of hydrolysis times could be used, an hydrolysis time of 45 minutes (at 22°C) was finally chosen as maceration and squashing of material treated for this period produced fewer damaged cells.

6.2.8 Selection of a Calibration Standard

Bennett and Smith (1976) listed eight species with 4C DNA amounts ranging from 5.88 to 69.27 picograms as calibration standards. To determine the size of DNA amounts in *Senecio*, *Pisum sativum* was arbitrarily selected as a standard and compared with three morphologically diverse species - *S. hypoleucus*, *S. quadridentatus* and *S. pterophorus* - known to differ in chromosome number. DNA amounts were found to be 17.92, 12.75 and 4.22 respectively, relative to the 19.46 picograms of *Pisum sativum*. In descending order of DNA amounts *Pisum sativum* is seventh in the list of Bennett and Smith (1976) and *Senecio vulgaris* (5.88 picograms) is eighth. *S. vulgaris* occurs as a garden weed in Australia, and from preliminary results appeared equally suitable as a calibration standard. However, I found scanning of cells with less than 10 picograms of DNA per 4C nucleus very time consuming as the nuclei are only faintly coloured. In view of the number of slides and cells to be measured *Pisum sativum* was therefore selected as the calibration standard.

6.2.9 Cell Volumes

Time did not permit critical estimates of cell volumes by sectioning techniques. Instead, volumes of root tip cells at mid prophase were examined on slides used for DNA estimates. Volume was crudely estimated as length x width ^{2} for 10 cells from each of a selection of species varying in DNA amounts per nucleus and per genome. Values are likely to be overestimated as slight squashing would have increased cell dimensions. However, it was hoped that the error factor would be proportional in cells of different sizes.

Pollen grain volume was estimated from cell diameters, excluding the exine so as to avoid errors due to surface sculpturing.

6.3 Results and Discussion

6.3.1 Terminology

DNA amounts estimated by Feulgen microdensitometry are usually expressed as "C" values, a concept introduced by Swift (1950) to avoid confusion with chromosome numbers. The 1C DNA amount of a species is the DNA content of the unreplicated haploid chromosome complement. Cells at mitotic telophase or early interphase have 2C DNA amounts whereas prophase cells (containing duplicated chromosome complements) have 4C DNA amounts. DNA amounts obtained by chemical extraction are generally expressed as amounts per cell, and usually correspond most closely with 3C DNA amounts (Van't Hof 1965, Bennett 1972). I have avoided the use of "DNA amount per cell", and instead, have discussed DNA amounts in terms of their 4C value as all measurements were made of prophase cells. DNA amounts per genome are also discussed, and correspond to the DNA amount of the basic chromosome complement. In a diploid species the DNA amount per genome is equivalent to the 1C

DNA amount, but the latter must be divided by 2 for a tetraploid by 3 for a hexaploid and by 4 for an octoploid. As an example, the basic chromosome number of *Senecio* is 10. A tetraploid (2N = 40) with a 4C DNA amount of 12 pg has a 1C DNA amount of 3 pg and a DNA amount per genome of 1.5 pg.

6.3.2 Comparisons of Calibration Standards

Although *Pisum sativum* was used as a standard throughout this study, one of the test species, *Senecio vulgaris*, is also listed as a calibration standard by Bennett and Smith (1976). It was therefore possible to compare an observed and an expected ratio of means. Bennett and Smith (1976) calculated 4C DNA amounts of *Pisum sativum* and *Senecio vulgaris* relative to *Allium cepa* and obtained values of 19.46 ± 0.30 pg and 5.88 ± 0.22 pg, respectively. The expected ratio of *Senecio vulgaris* relative to *Pisum sativum* was therefore 0.3022 but the observed ratio was 0.4020. The 4C DNA amount calculated for *Senecio vulgaris* in this study was 0.4020 x 19.46 = 7.82 ± 0.09 pg, which differs significantly ($P < .001$) from the 5.88 pg calculated by Bennett and Smith. Results indicated that either the DNA amount of *Pisum sativum* or of *Senecio vulgaris* differed from listed values.

Hordeum vulgare was therefore introduced as a third calibration standard and compared on the same slide first with *Pisum sativum* and then with *Senecio vulgaris*. A direct comparison of *Pisum sativum* and *Senecio vulgaris* was also repeated. Forms and varieties compared in this study are compared with those used by Bennett and Smith (1976) in Table 6.3. As none of the varieties correspond, it was necessary to assume that DNA amounts did not differ significantly between varieties. Table 6.4 gives the DNA amount of each species calculated relative to the other two standards. DNA amounts calculated for *Pisum sativum* and *Hordeum*

TABLE 6.3

Forms and Varieties Used as Calibration Standards

Species	Bennett and Smith (1976)	Present Study
Senecio vulgaris	PBI population	Adelaide Hills no.ML566
Pisum sativum	cv. Minerva Maple	cv. Massey Gem
Hordeum vulgare	cv. Sultan	"Cape barley"

TABLE 6.4

DNA Amount of Each Calibration Standard

Relative to the Other Two

Standard	Standard 4C DNA Value*	DNA amount relative to standard *S. vulgaris*	*P. sativum*	*H. vulgare*
S. vulgaris	5.88 ± 0.22	-	14.89 ± 0.23	16.30 ± 0.12
P. sativum	19.46 ± 0.30	7.76 ± 0.12	-	21.54 ± 0.26
H. vulgare	22.24 ± 0.57	8.02 ± 0.09	20.20 ± 0.28	-

* from Bennett and Smith (1976)

vulgare using Senecio vulgaris as the standard both differed significantly ($P<.001$) from amounts listed by Bennett and Smith (1976). However, when Pisum sativum and Hordeum vulgare were compared directly, the mean ratio and therefore the calculated DNA amounts did not differ significantly ($P>.05$). Results therefore indicated that the DNA amount of S. vulgaris calculated in this study differed significantly from the listed value. There are three possible reasons for the discrepancy.

1. The use of a calibration standard with a very different DNA amount (Allium cepa, 4C DNA = 67.00 pg) led to underestimation of the DNA amount of Senecio vulgaris by Bennett and Smith (1976).

2. The use of "hot" hydrolysis (10 minutes in 1M HCl at $60^{\circ}C$) by Bennett and Smith (1976) may have caused underestimation of the DNA amount of Senecio vulgaris. Decosse and Aiello (1966) and Fox (1969) have shown that cold hydrolysis (45-60 minutes in 1N HCl at $22^{\circ}C$ used in this study) is more reliable and has less critical hydrolysis times than hot hydrolysis.

3. The population os S. vulgaris examined in this study has a different DNA amount to the population tested by Bennett and Smith (1976). Direct comparisons on the same slide of the two populations of S. vulgaris would indicate if the different DNA amounts are evidence of intraspecific variation or instead, of different experimental methods.

6.3.3 Reliability of DNA Estimates and Size of Significant Differences

In most instances only one estimate of DNA amount was made because of limited time. An indication of the reliability of estimates is therefore useful. During comparisons of calibration standards (Sec. 6.3.2) the 4C DNA amount of Senecio vulgaris was estimated in three independent trials (Table 6.5). There are no significant differences between the estimated values ($P>.05$).

TABLE 6.5

Independent Estimates of the 4C DNA Amount of *Senecio vulgaris*

Standard	Replicate	4C DNA amount relative to standard
Pisum sativum	1	7.76 ± 0.12
	2	7.80 ± 0.09
Hordeum vulgare	1	8.02 ± 0.09

Estimates of DNA amounts were also repeated for *S. magnificus* and *S. velleioides*. Although superficially similar, *S. magnificus* has a chromosome number of 2N = 40, whereas *S. velleioides* has 2N = 38. If *S. velleioides* is an aneuploid derivative of *S. magnificus* then DNA amounts should be about equal if chromosomes fused or less in *S. velleioides* if a chromosome pair was lost. However, the 4C DNA amount of *S. velleioides* was calculated to be 33.05 ± 0.23 pg - 1.35 pg greater than that of *S. magnificus* (31.70 ±0.15 pg). As the result appeared to be unusual, a second estimate was obtained by comparing *S. magnificus* and *S. velleioides* directly (i.e. not relative to *Pisum sativum*). The mean ratio of *S. magnificus* relative to *S. velleioides* obtained by direct comparison was 1.0525 ± 0.0083, and the ratio obtained by comparing each with *Pisum sativum* was 1.0630 ± 0.0070. The two are not significantly different ($P>0.05$).

The two instances of reproducible results mentioned above are indicative that other estimates of DNA amounts are reliable. Reliability is also suggested by the very similar DNA amounts calculated for the closely related native discoid species and for

annual species of the erechthitoid group (Table 6.6).

Bennett and Smith (1976) estimated that DNA of species with amounts of 0.5 to 2.0 times that of the standard species are probably accurate to within 5-10%. However, their method did not include an internal standard. A large proportion of species of Senecio have 4C DNA amounts of about 18 pg and standard errors of 0.1 to 0.2. Using a Students t test differences of about 0.5pg are therefore significant at the 5% level, which suggests that differences as low as 3% can be detected by the method used in this study. Similar percentage differences can generally be detected for species with lower and higher DNA amounts.

6.3.4 Interspecific Differences in DNA Amounts

Nuclear DNA amounts of 34 species of Senecio, Arrhenechtites mixta and Erechtites valerianaefolia are given in Table 6.6. Variation is also illustrated by chromosome complements shown in Figures 6.1 and 6.2. There is a 10.2-fold difference in DNA amounts per 4C nucleus, from 4.22 pg in Senecio pterophorus to 42.90 pg in S. vagus. As chromosome numbers vary from 2N = 10 in S. discifolius to 2N = 100 in S. biserratus a 10-fold difference might be expected by polyploidy alone. However, DNA amounts per set of 10 chromosomes vary from 0.84 pg in S. glossanthus to 7.13pg in S. discifolius - an 8.5-fold difference. In nearly all species, 10 chromosomes represent one genome, but S. discifolius with 2N = 10 has a genome of 5 chromosomes. DNA amounts per genome therefore vary from 0.84 pg in S. glossanthus to 4.69 pg. in S. macranthus - a 5.6-fold difference. It is therefore apparent that variation is caused both by polyploidy and by changes within genomes.

DNA amounts per 4C nucleus have been reported which indicate a five-fold difference in Anemone (Rothfels et al. 1966), 3-fold

TABLE 6.6

DNA Amounts per 4C Nucleus and per Genome, Haploid Chromosome Number, Longevity, Breeding System and Morphological Grouping of 34 Species of *Senecio*, *Arrhenechtites mixta* and *Erechtites valerianaefolia* (collection numbers as in Table 5.2)

Species	4C DNA amount ± standard error (picograms)	DNA per genome (x=10 or *x=5)	2N	Longevity
GROUP 1A - Radiate species with continuous stigmatic surfaces				
Senecio magnificus	31.70 ± 0.15	3.96	40	P
S. velleioides	33.05 ± 0.23	4.24	38	A?
S. pectinatus	31.09 ± 0.14	1.95	80	P
S. amygdalifolius	26.87 ± 0.21	3.36	38	P
S. macranthus	37.48 ± 0.24	4.69	40	P
S. vagus subsp. *eglandulosus*	42.90 ± 0.46	2.19	98	A?
GROUP 1B - Radiate species with discrete stigmatic surfaces				
S. lautus subsp. *lautus*	10.81 ± 0.12	1.35	40	A
subsp. *dissectifolius*	10.63± 0.11	1.33	40	P
subsp. *maritimus*	10.19 ± 0.07	1.27	40	P
subsp. *alpinus*	10.74 ± 0.09	1.34	40	P
subsp. *lanceolatus*	9.79 ± 0.09	1.23	40	P
S. spathulatus	12.28 ± 0.11	1.54	40	P
S. glossanthus (tetraploid)	6.71 ± 0.06	0.84	40	E
(octoploid)	14.95 ± 0.13	0.93	80	E
S. gregorii	12.55 ± 0.12	1.57	40	E
*S. pterophorus***	4.22 ± 0.05	1.06	20	E
*S. discifolius***	14.27 ± 0.12	*3.57	10	E

Table 6.6 - continued

Species	4C DNA amount ± standard error (picograms)	DNA per genome (x=10 or *x=5)	2N	Longevity
GROUP 2A - Discoid species without marginal ray florets				
S. hypoleucus	17.92 ± 0.15	1.50	60	P
S. odoratus				
var. odoratus	18.14 ± 0.09	1.51	60	P
var. obtusifolius	18.18 ± 0.10	1.52	60	P
S. cunninghamii				
var. cunninghamii	18.90 ± 0.11	1.58	60	P
var. A	18.61 ± 0.18	1.55	60	P
S. anethifolius	17.35 ± 0.10	1.45	60	P
S. gawlerensis	20.39 ± 0.15	1.70	60	P
S. vulgaris**	7.82 ± 0.07	0.98	40	E
S. mikanioides**	11.78 ± 0.08	2.95	20	P
GROUP 2B - Discoid species with marginal ray florets				
S. linearifolius				
var. linearifolius	18.02 ± 0.15	1.50	60	P
var. A (alpine)	16.62 ± 0.10	1.38	60	P
var. B (Grampians)	18.74 ± 0.14	1.56	60	P
S. sp A	18.03 ± 0.12	1.51	60	P
GROUP 3A - Erechthitoid species, perennials, achenes slender				
S. quadridentatus	12.75 ± 0.07	1.59	40	P
S. gunnii	14.04 ± 0.18	1.76	40	P
S. aff. apargiaefolius	14.10 ±0.11	1.77	40	P
S. runcinifolius	16.15 ± 0.09	2.02	40	P

Table 6.6 - continued

Species	4C DNA amount ± standard error (picograms)	DNA per genome (x=10 or *x=5)	2N	Longevity
GROUP 3B - Erechthitoid species, annuals, achenes plump				
S. sp. B	19.94 ± 0.11	1.66	60	A
S. squarrosus	19.81 ± 0.21	1.65	60	A
S. pibinnatisectus	13.98 ± 0.14	1.17	60	A
S. minimus	19.82 ± 0.14	1.65	60	A
S. picridioides	19.68 ± 0.10	1.64	60	A
S. glomeratus	19.18 ± 0.09	1.60	60	A
S. hispidulus				
var. *hispidulus*	19.11 ± 0.13	1.60	60	A
var. *dissectus*	19.41 ± 0.11	1.62	60	A
S. sp. C	20.12 ± 0.14	1.68	60	A
S. biserratus	25.27 ± 0.15	1.27	100	A
Other Genera				
Arrhenechtites mixta	35.08 ± 0.23	1.76	100	A
*Erechtites valerianaefolia***	25.02 ± 0.16	3.13	40	A

**Species not native in Australia

Symbols: E = ephemeral, A = annual, P = perennial.

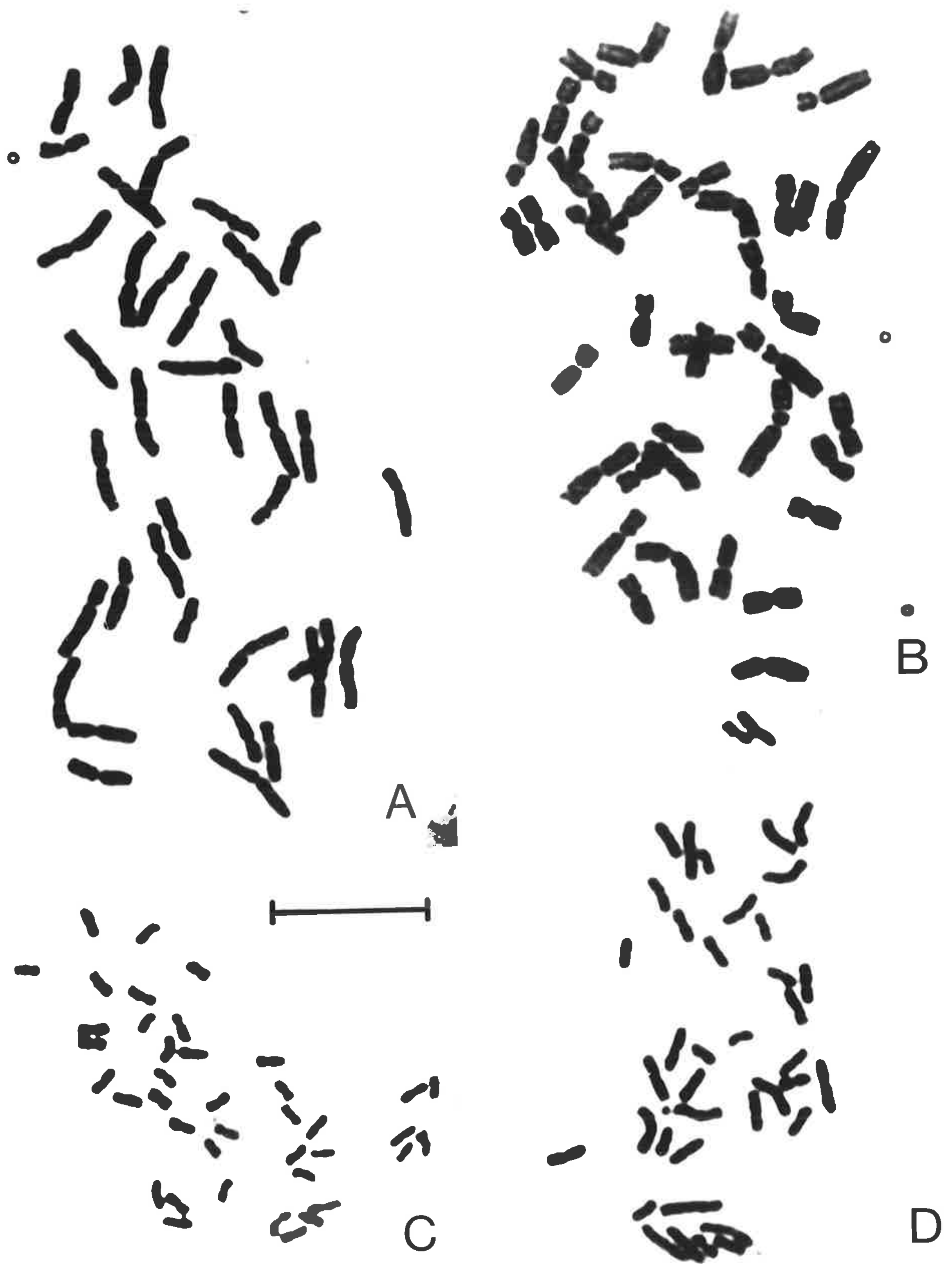


Fig. 6.1 Senecio species with the same chromosome number (2N=40) but different mean DNA amounts per chromosome (given in parentheses). A. S. amygdalifolius (0.336 pg). B. S. macranthus (0.468 pg). C. S. lautus subsp. lanceolatus (0.123 pg). D. S. runcinifolius (0.202 pg). All figures at same magnification. Scale 10 μm.

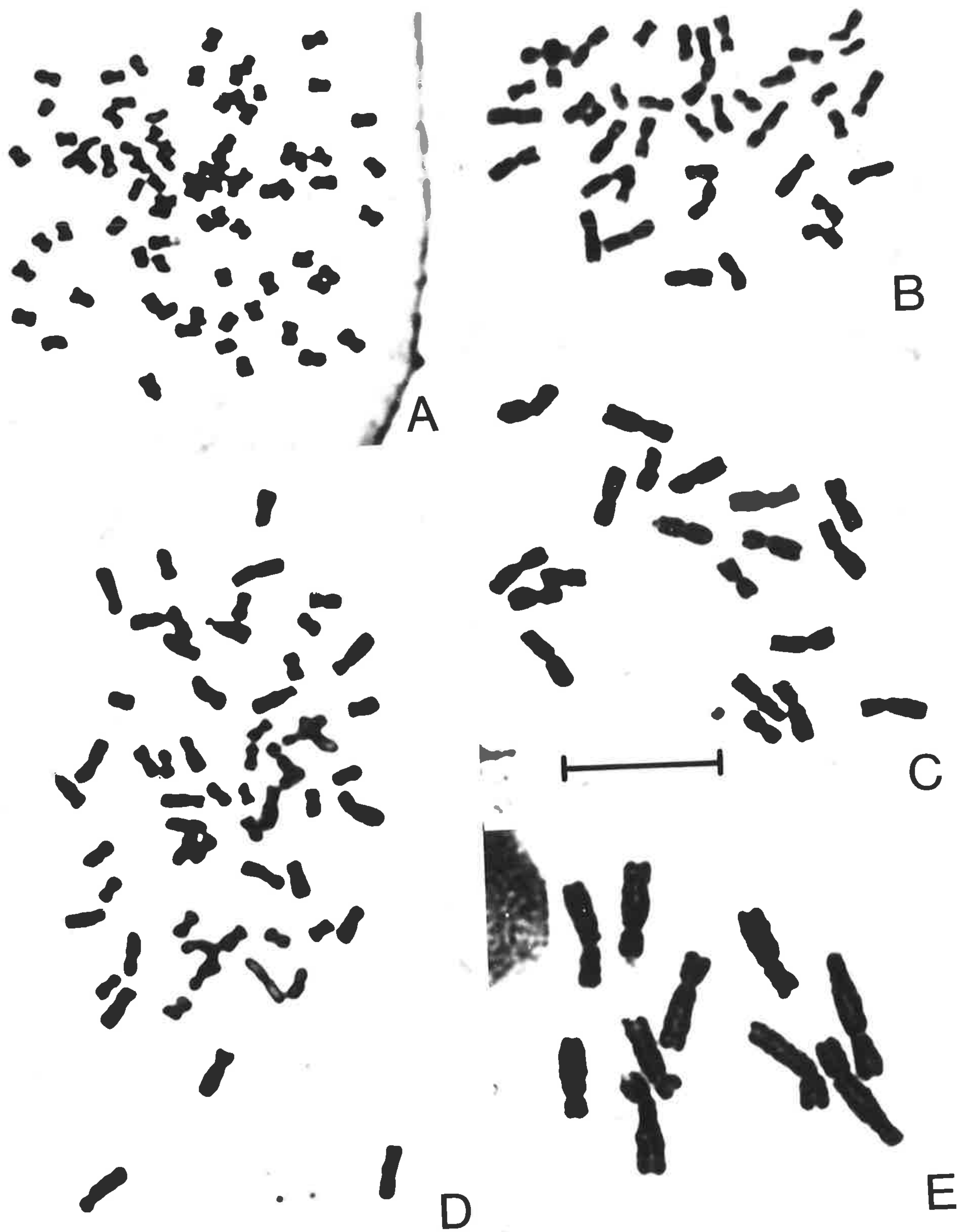


Fig. 6.2 Species of Senecio with different chromosome numbers but similar DNA amounts (11.78 - 14.95 pg / 4C nucleus).
A. S. glossanthus (2N=80). B. S. aff. apargiaefolius (2N=40).
C. S. mikanioides (2N=20). D. S. bipinnatisectus (2N=60).
E. S. discifolius (2N=10). All figures at same magnification.
Scale 10μ m.

in *Allium* (Jones and Rees 1968) and in *Lathyrus* (Rees and Hazarika 1969), 6-fold in *Aegilops* (Furuta 1970) and in *Vicia* (Chooi 1971), 13-fold in *Ranunculus* (Goepfert 1974), 9-fold in *Bromus* (Bennett and Smith 1976) and 10-fold in *Crepis* (Jones and Brown 1976). Genomic differences could not always be deduced from values given in the papers listed above, but average DNA amounts per chromosome could be calculated. Average DNA amounts per chromosome in *Senecio* vary from 0.084 pg to 0.713 pg, an 8.5-fold difference exceeded only by a 17-fold difference in *Crepis*. When compared with other surveyed genera the variation in DNA amounts of *Senecio* is therefore comparatively large.

6.3.5 Intraspecific Variation in DNA Amounts

Although large differences in DNA amounts have been reported between related species, it is generally considered that DNA amounts within a species are constant (Boivin et al. 1948, Mirsky and Ris 1949, Swift 1950). However, provided that some changes in DNA amount are gradual, then it is intuitively obvious that differences between species must have their beginnings in intraspecific variation. Exceptions are changes due to polyploidy and large structural changes within the chromosome complement, which may lead to quantum or saltational speciation. Improved techniques allowing detection of smaller differences may account for reports of intraspecific DNA variation in more recent years. Intraspecific variation has been reported in species of *Picea* (Dhir and Miksche 1974, Miksche 1968, Miksche 1971), in flax (Evans 1968, Durrant 1962) and in *Microseris* (Price et al. 1980). Miksche (1968) suggested that differences between provenances of *Picea glauca* are a reflection of adaptations to different environments. However, Teoh and Rees (1976) re-examined *Picea glauca* DNA amounts using improved techniques and could find no

significant differences. In the case of flax, heritable changes in DNA amounts have been induced by applications of particular nutrient combinations. As the experiment has been repeated by different workers it is difficult to dispute. DNA amounts have also been found to vary during tissue differentiation of an individual (see review by Nagl (1979)). For example, Nagl et al. (1979) found that DNA amounts of floral buds from three different species were consistently higher than DNA amounts of vegetative buds. It is therefore apparent that to detect intraspecific variation between individuals, experimental design must aim to minimise error due to methods and to avoid differences that might be developmental in origin.

In this study the same tissue (root tips) was collected from plants of approximately the same age grown in identical conditions. Furthermore, cases in which replicate experiments were performed gave identical results and the experimental method generally allows for detection of differences as small as 3%. Although only one estimate of DNA amount was made for most species, a number of species consist of two or more varieties (Table 6.6). No significant differences were found between varieties of *S. odoratus*, *S. cunninghamii* and *S. hispidulus*, but significant differences (P<0.001) were detected among subspecies of *S. lautus* and varieties of *S. linearifolius*. In the case of *S. lautus*, the DNA amounts of subsp. *lautus*, *dissectifolius* and *alpinus* are not significantly different but subsp. *maritimus* and *lanceolatus* differ significantly from each other and from the other subspecies. The 4C DNA amounts vary from 9.79 pg to 10.81 pg and represent a 10% difference. In the case of *S. linearifolius* the typical and Grampians (var. B) varieties are not significantly different whereas the alpine variety (var. A) differs significantly from both. Values range from 16.62 pg to 18.74 pg and

represent a 13% difference.

The results obtained for *S. lautus* and *S. linearifolius* suggest there may be intraspecific variation in DNA amounts. However, much larger samples compared directly rather than with *Pisum sativum* as a standard would be necessary to confirm such an event.

6.3.6 Nucleotypic Effects

Bennett (1971) used the term "nucleotype" to describe those conditions of the nucleus that affect the phenotype independently of the genotype or information content of the DNA. The DNA content of an organism has been found to correlate with chromosome volume, nuclear volume, cell size, nucleolar and nuclear dry mass, seed dry mass, minimum cell cycle time, meiosis duration, pollen maturation time and minimum generation time (Bennett 1972). It can therefore be said that the phenotype is a product of not only the genotype and the environment but also of the nucleotype. Cavalier-Smith (1978) extended the concept of nucleotypic effects by comparing the predictions of r- and K-selection (see Chapter 4.4.7) with respect to cell size, developmental rate and organism size, and the distribution of DNA amounts in a wide variety of organisms. His conclusions were as follows:

> "Though it could be argued that C-values vary for some mysterious unknown reason and that high c-value organisms simply happen to be pre-adapted to K-selected niches and low c-value ones to r-selected niches, it is more straightforward to postulate that the variation in c-values is simply the result of varying r- and K-selection: this solves the c-value paradox very simply."

Although Cavalier-Smith may be correct, relationships between DNA amounts and selective pressures are not quite so "straight forward" in the case of *Senecio* as variation in DNA amounts is

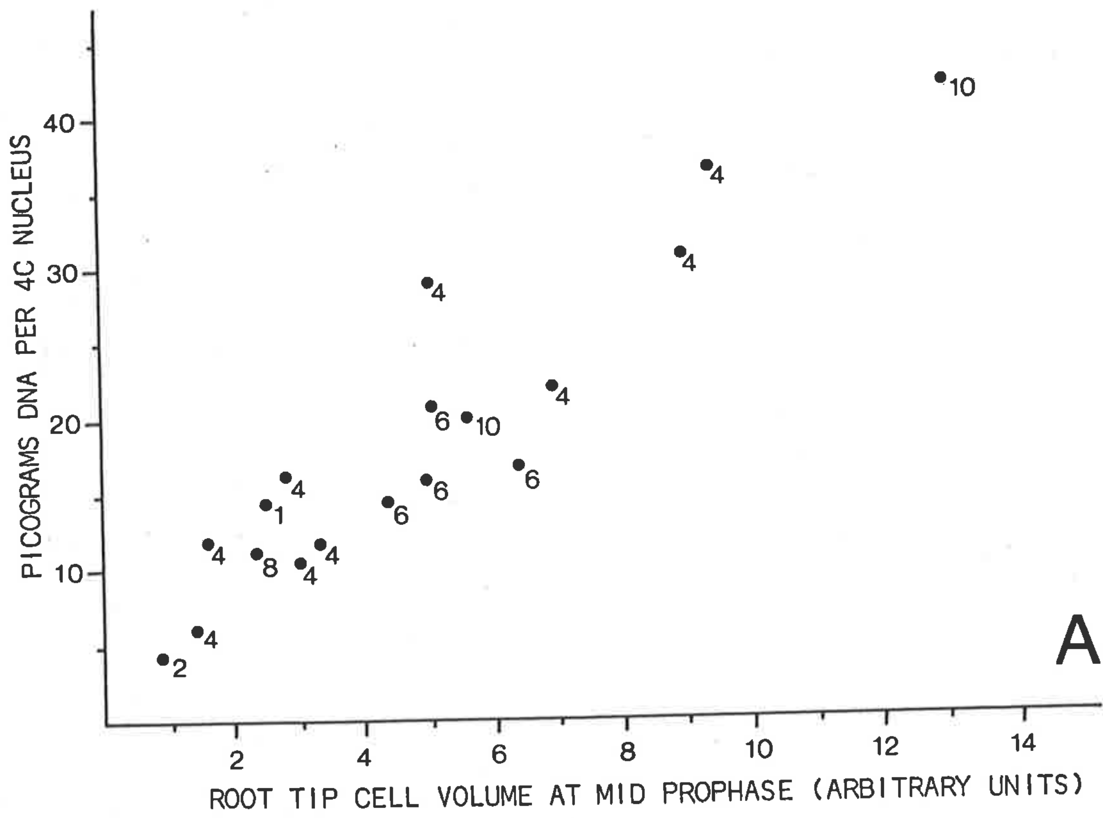
due both to polyploidy and to changes within genomes. As will be discussed in the appropriate portions of the text, polyploidy and genomic changes do not always produce the same nucleotypic effect.

6.3.6.1 Size of structures.

Holm-Hansen (1969), Gunge and Nakatomi (1972), Bennett (1972), Price et al. (1973) and Price and Bachmann (1976) found a direct relationship between nuclear DNA amount, nuclear volume and cell volume. It appears that doubling of DNA amount doubles cell volume irrespective of whether the increase involves polyploidy or genomic DNA amounts. A number of developmental structures were therefore examined in *Senecio* to see if a similar relationship exists between cell size and DNA amount, and to see if the relationship extends to multicellular structures.

1. Cell volumes. Eighteen taxa were selected to cover the maximum variation in DNA amounts per 4C nucleus and per genome. Volumes of mature pollen grains and of root tip cells at prophase are shown plotted against 4C DNA amounts in Figure 6.3A and B. Although species differ greatly in ploidy level and genome size there is a linear relationship between DNA amount per nucleus and cell volume. The results suggest that a 100% increase in DNA amount leads to a 100% increase in cell volume (or vice versa) irrespective of how the increase was achieved.

2. Multicellular structures. Changes in cell size will be important in unicellular organisms and unicellular structures such as gametes, and will also affect processes dependent upon the ratio of surface area: volume. However, it is possible for cell size and structure size to vary independently in multicellular organisms. Relationships between DNA amounts and structure sizes may therefore be obscured or absent altogether.



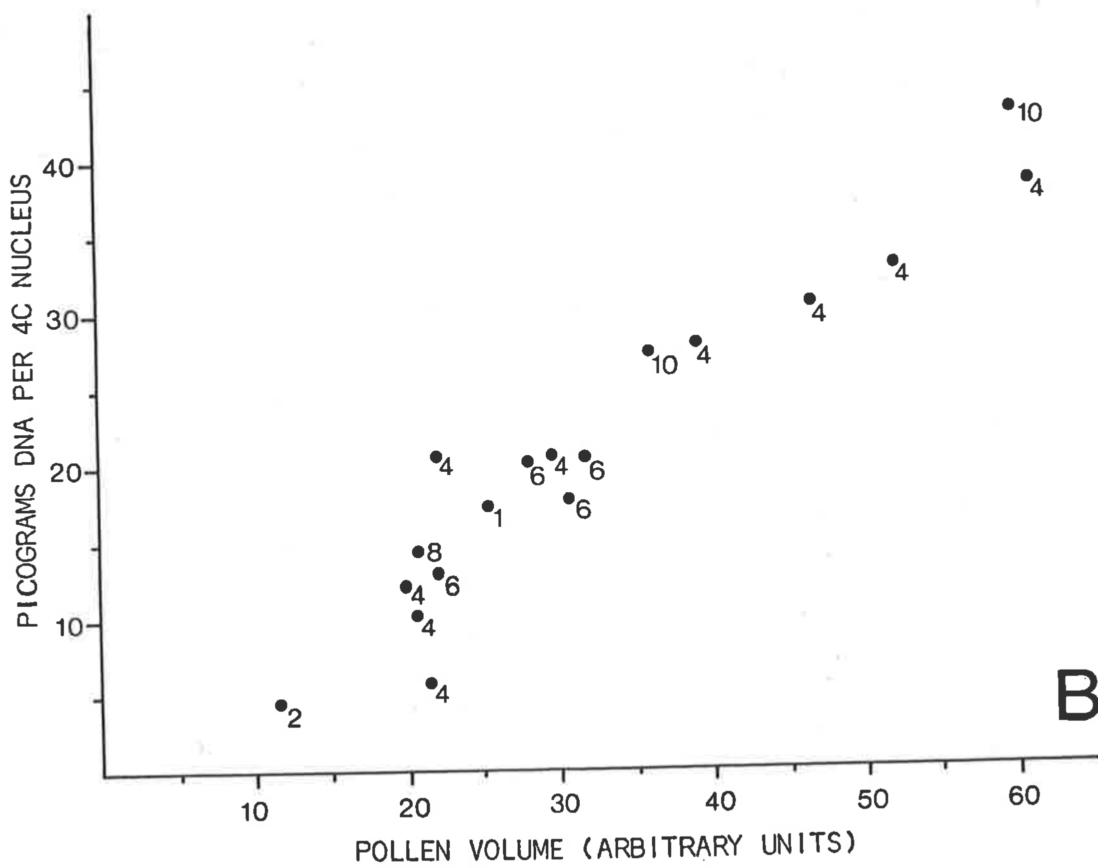


Fig. 6.3 Relationships between cell volumes and DNA amounts of 18 Senecio species. (number indicate ploidy levels)

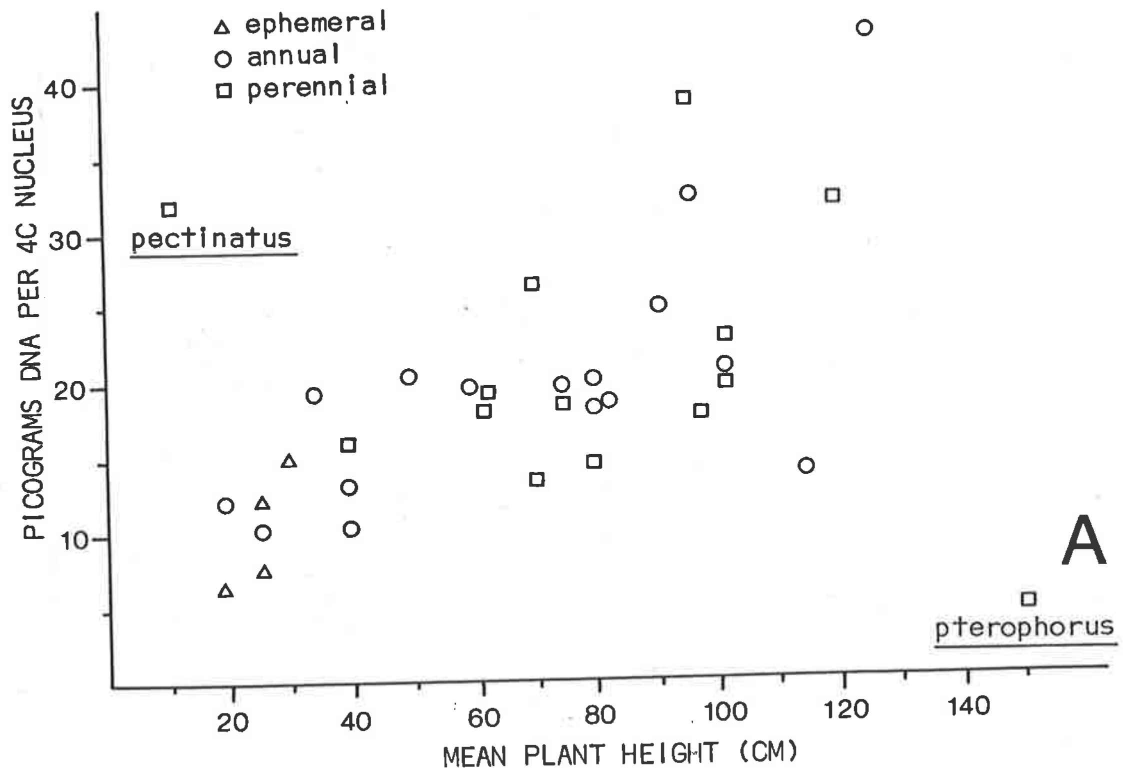
Data from species descriptions in Chapter 3 were used to compare structure sizes and 4C DNA amounts. There is a very general but positive relationship between mean plant height and 4C DNA amount (Fig. 6.4A). However, notable exceptions are *Senecio pterophorus* with the lowest DNA amount and the greatest mean height, and *Senecio pectinatus* with a very high DNA amount and the lowest mean height.

A similar comparison using values of seed mass gives widely scattered points showing little correlation with DNA amount (Fig. 6.4B). In contrast, significant positive correlations were found between seed mass and DNA amounts of species of *Crepis* (Jones and Brown 1976), *Allium* and *Vicia* (Bennett 1972). A possible reason for the lack of correlation in *Senecio* is that the basic structure of seeds varies greatly - from glabrous to very hairy and from smooth to deeply ribbed. When DNA amounts were compared with bisexual floret length a general but positive relationship was again apparent (Fig. 6.5A). Unlike seeds, bisexual florets of *Senecio* show little variation in morphology. Similarly the capitula of outcrossing radiate species show little variation in basic structure, and their total diameter (including rays) is positively correlated with DNA amount (Fig. 6.5B).

It would therefore appear that DNA amounts per nucleus may also affect the size of multicellular structures, although the relationship is not as precise as for single cells and may be absent when structural designs differ greatly.

6.3.6.2 Cell cycle times.

Although a positive correlation between cell cycle time and DNA amount is a general phenomenon among diploids (Bennett 1972) the effect of DNA increases by polyploidy is less clear. In different genera reports suggest that the cell cycle times of



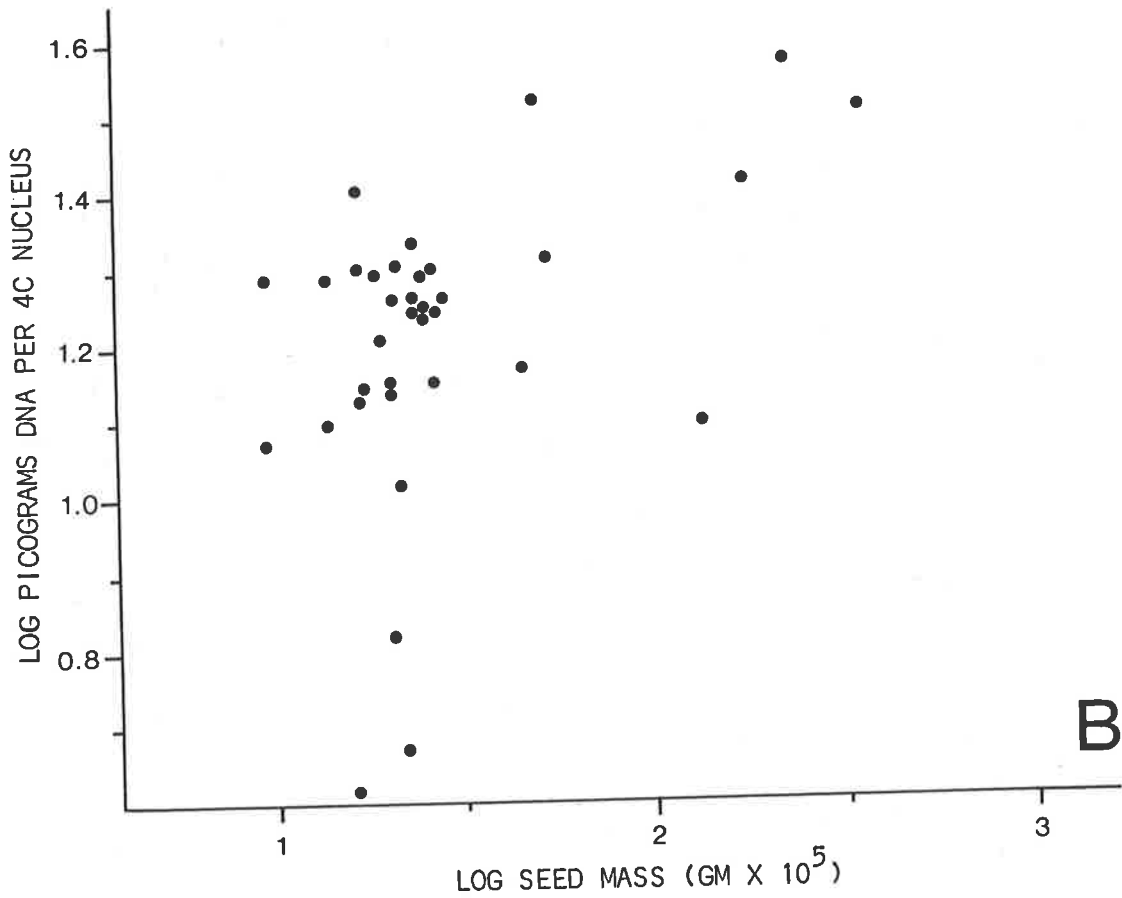
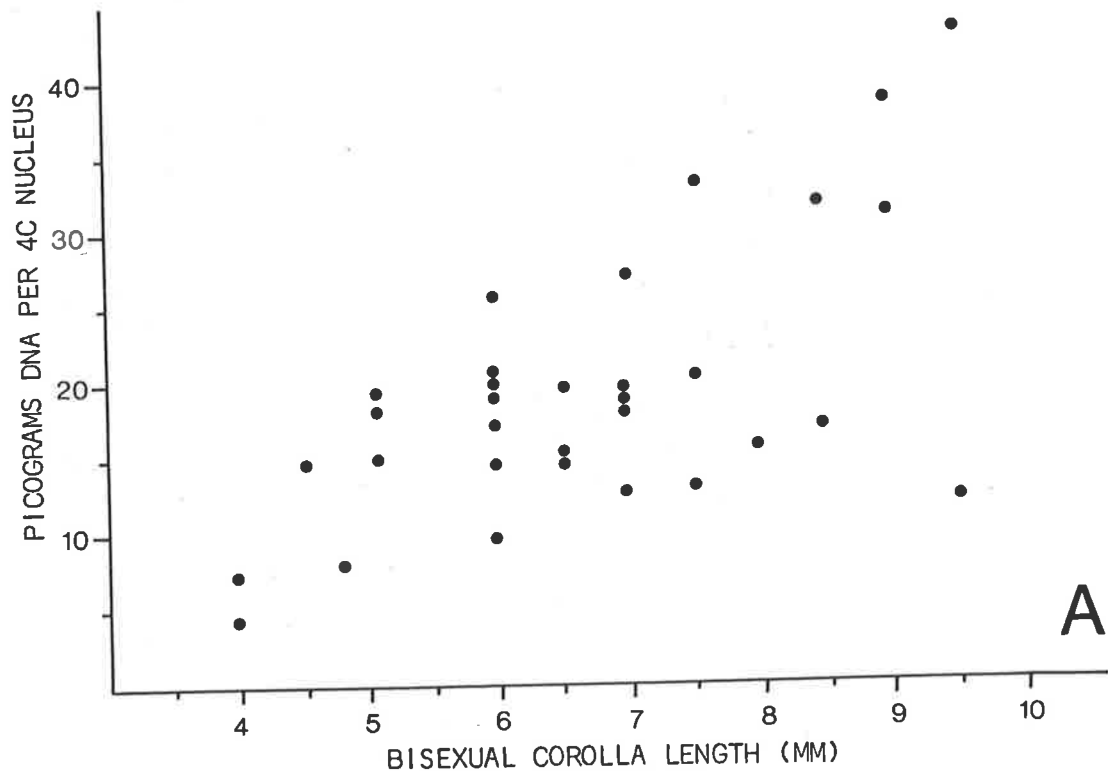


Fig. 6.4 Relationships between size of multicellular structures and DNA amounts of 34 *Senecio* species.



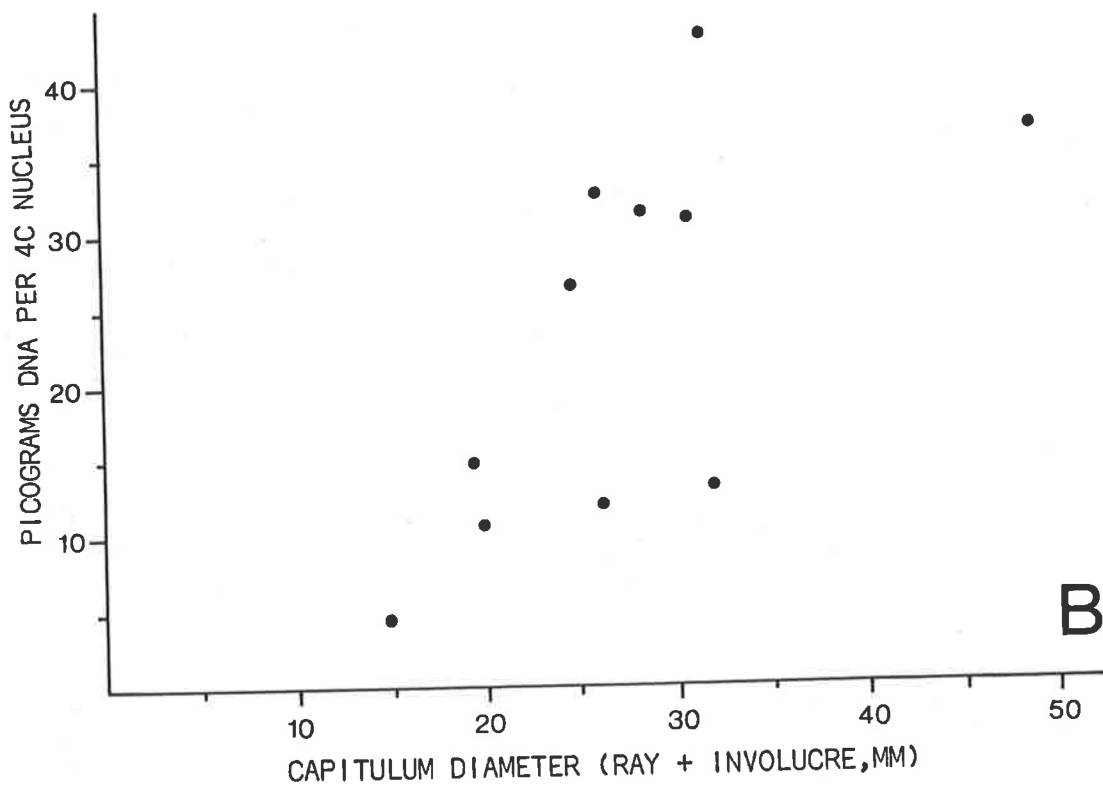


Fig. 6.5 Relationships between size of multicellular structures of *Senecio* species (A: 34 species. B:11 species).

polyploids may be longer than (Evans et al. 1970), equal to (Yang and Dodson 1970, Friedburg and Davidson 1970) or shorter than (Gupta 1969, Bennett and Smith 1972) the cell cycle times of their diploid progenitors. Cell cycle times were not examined in *Senecio* but speculations can be made from other data.

Senecio glossanthus consists of tetraploid and octaploid races which appear to have similar distributions and growth requirements. Both behave as ephemerals in the drier parts of Australia. In the glasshouse, tetraploid and octoploid plants raised from seed planted at the same time, also flowered and set fruit at the same time even though octoploid plants were taller. In this case, minimum generation time and perhaps also cell cycle time does not appear to be affected by polyploidy.

Among alpine species of *Senecio* there is considerable variation in DNA per 4C nucleus but less variation in DNA per genome. Four species, *S. lautus* subsp. *alpinus* (2N=40), *S. gunnii* (2N=40), *S. linearifolius* var. A (2N=60) and *S. pectinatus* (2N=80) occur in alpine environments and DNA amounts per 4C nucleus are 10.74pg, 14.04 pg, 16.62 pg and 31.09 pg. However, DNA amounts per genome are 1.34 pg, 1.76 pg, 1.38 pg and 1.95 pg. Bennett (1972) suggested that the effect of DNA amount on the rate of development is proportionately magnified at low temperatures, yet the above-mentioned perennial species can all flower in the first season - even though there is a 3-fold difference in 4C DNA amounts. One could speculate that among alpine species of *Senecio* rate of development may be more closely correlated with genome size than with total DNA amount, and therefore that polyploidy does not greatly affect cell cycle times. If this is the case, then polyploidy could be considered as a means of increasing cell size without significantly altering cell cycle time and the rate of development in *Senecio*.

A very different strategy proposed by Nagl (1974) is that some types of heterochromatin may act to shorten cell cycle times without any change in DNA amounts. Nagl and Ehrendorfer (1974) and Nagl (1974) found that in the Anthemideae, there are annual species with both lower and higher DNA amounts than perennials. However, mitotic cycle times and developmental rates of all annuals were shorter than those of perennials. Nagl (1974) also found that the proportion of heterochromatin increased dramatically in annuals with high DNA amounts, and on this basis, Nagl and Ehrendorfer (1974) proposed three evolutionary trends among annuals:

1. Heterochromatization of genome portions which have become useless in the annual habit.
2. Elimination of otherwise superfluous or reiterative DNA sequences.
3. Parallel increases in nuclear DNA content and heterochromatin.

In this study evidence of extensive heterochromatin in interphase nuclei was found only in the ephemeral *S. gregorii* (Fig. 6.6). Significantly *S. gregorii* has 60-90% more nuclear DNA than two other ephemerals at the same ploidy level. *S. gregorii* might therefore correspond to the first evolutionary trend proposed by Nagl and Ehrendorfer (1974) while other ephemerals have followed the second and more commonly observed trend.

6.3.6.3 Minimum generation times.

Minimum generation time was defined by Bennett (1972) as "the duration of the period from germination until first production of seeds." Perennials were divided to distinguish those that behave as annuals and set seed in their first year (facultative perennials) and those that require more than one year to set

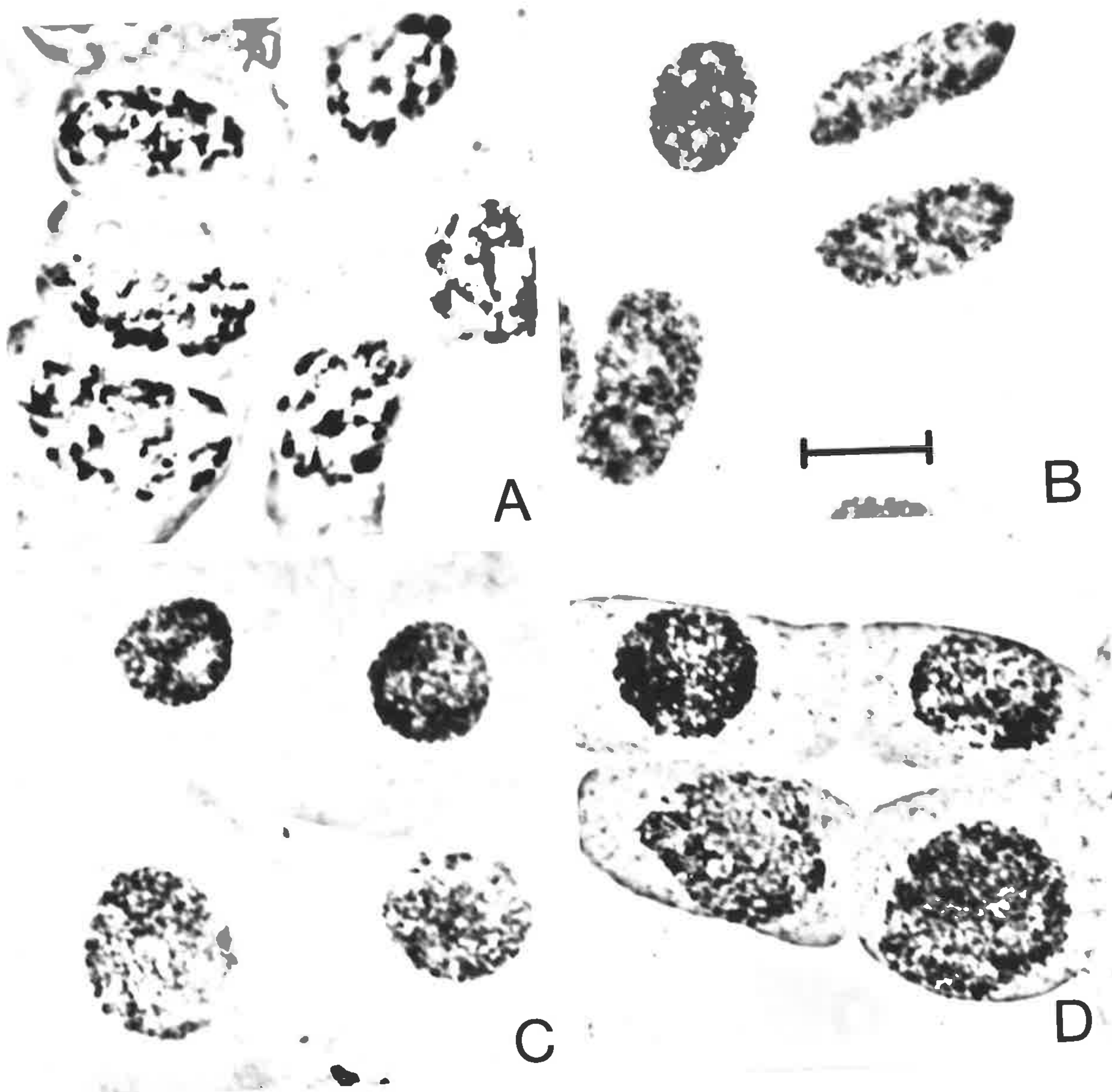


Fig. 6.7 Interphase nuclei of four _Senecio_ species.
A. _S. gregorii_ with dense regions suggesting heterochromatin.
Nuclei of remaining species of uniform density. B. _S. vulgaris_.
C. _S. glossanthus_. D. _S. spathulatus_. All figures at same
magnification. Scale 20 μm.

seed (obligate perennials). Among annuals, ephemerals with minimum generation times of a few months were considered as a distinct subgroup. Bennett (1972) compared DNA amounts and minimum generation times of 271 higher plants to determine if the nuclear DNA amount of many species is adapted to their minimum generation time or vice versa. Results indicated that:

1. ephemerals had the lowest DNA contents (1.1 - 13.5 pg per 4C nucleus);
2. values for facultative perennials and for annuals were not significantly different (6.3 to 38.1 pg and 5.7 to 37.9 pg respectively per 4C nucleus);
3. obligate perennials have the greatest range of values (5.3 to 393.3 pg per 4C nucleus).

The implication is that species with more than 14 pg of DNA per 4C nucleus are unlikely to be ephemerals and species more than 38 pg are unlikely to be annuals or facultative perennials.

Although none of the species included in this study are obligate perennials the remaining three categories are represented. The range of DNA amounts in each category is in close agreement with the results obtained by Bennett (Table 6.7). The maximum DNA amount of an ephemeral is 14.95 pg (compared with 14 pg found by Bennett) of an annual is 42.90 pg (compared with 38 pg) and of a facultative perennial is 37.48 pg (compared with 38 pg). Furthermore, ephemeral species of Senecio have the lowest DNA contents while those of annuals and facultative perennials are not significantly different. Bennett (1972) concluded that there is apparently a maximum limit to the mass of nuclear DNA for species which can complete development within a given time. As results for Senecio are very similar, the same conclusion most probably applies.

TABLE 6.7

Mean and Range of DNA Amounts in Ephemeral, Annual and Perennial species of *Senecio*

Growth Form	4C DNA Amount Mean ± s.e.	Range
ephemerals	11.26 ± 1.83	6.71 - 14.95
annuals	23.00 ± 2.46	9.79 - 42.90
facultative perennials	18.81 ± 2.06	4.22 - 37.48

6.3.7 The Nature of Changes in DNA Amount

DNA amounts of *Senecio* have in the past been altered both by polyploidy and by changes within genomes. The general opinion is that changes in genome size are caused by lengthwise repetition or deletion of chromosome segments (see reviews by Rees 1972, Rees and Jones 1972, Sparrow et al. 1972, Hinegardner 1976, Price 1976). Changes may be localized and sufficiently large to be observed microscopically - for example, as pachytene loops in *Lolium* hybrids (Rees and Jones 1967) or as localized increases in band sizes in polytene chromosomes of *Chironomous* (Keyl 1965). Alternatively, lengthwise changes may be small and numerous, leading to gradual changes in genome size. The logarithmic normal distribution of DNA amounts of various conifers (Price et al. 1974) and amphibians (Bachmannet al. 1972) is thought to support this hypothesis. Assumptions are that all species have

been derived from one well adapted genotype (the modal DNA amount) and that "tolerable" changes in DNA amounts are proportional to the preexisting DNA amount. Distributions are therefore skewed to the right of a modal value, but produce a normal distribution if DNA amounts are converted to logarithmic form.

Frequency distributions of DNA amounts of Senecio are shown in Figure 6.7. Distributions of DNA amounts per 4C nucleus (Fig. 6.7A) confound changes due to polyploidy (apparent as peaks at 12 pg and 19 pg) and changes within genomes. It is therefore necessary to examine the distribution of genome sizes (Fig. 6.7B). The distribution is not precisely logarithmic normal (shown as a dotted curve in Figure 6.7B), assuming that the modal value is 1.5 picograms and the range is 0.8 - 4.6 pg. It would therefore appear that changes in genome sizes of Senecio have occurred by some form of lengthwise repetition, but that the changes do not follow the assumptions of a logarithmic normal distribution. There are two major departures from the latter. Firstly, there are too many species with very high DNA amounts and secondly there are too many species at the modal DNA amount. One assumption of a logarithmic normal distribution is that there is a single modal value or population involved. However, phylogenetic interpretations based on morphology (see Chapter 3) suggest that at least two ancestral species migrated to Australia. One of the evolutionary lines consists of species with continuous stigmatic surfaces and the majority of these have very high DNA amounts per genome. However, if these species are removed as a separate population, the distribution still has too many species with a modal value. I believe that polyploidy may account for this phenomenon as it is possible that changes in genome size following polyploidy are limited (see discussion in following section).

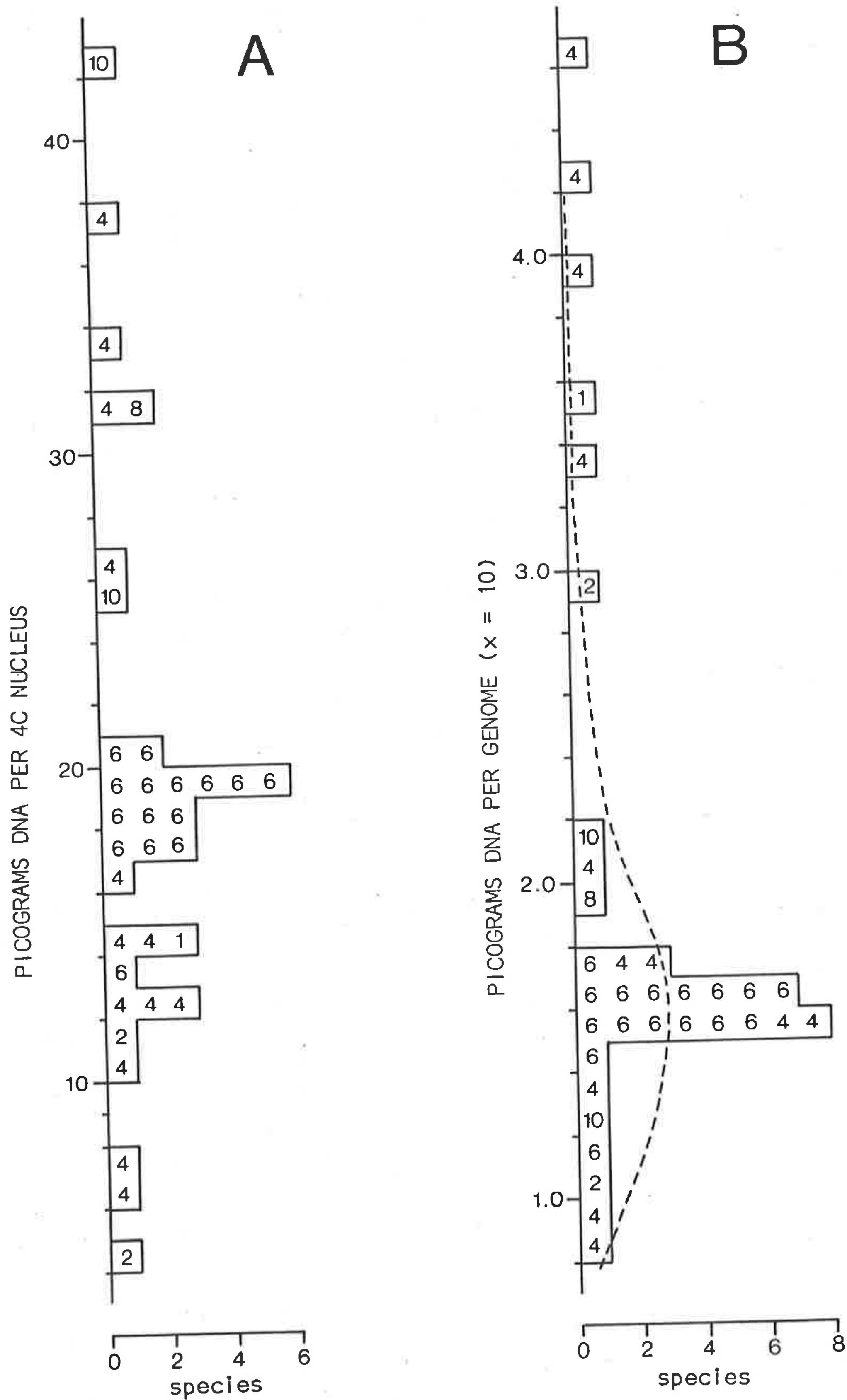


Fig. 6.7 Distributions of DNA amounts per nucleus (A) and per genome (B) among 34 *Senecio* species. Dotted line in B is logarithmic normal distribution for observed range with mode of 1.5 pg. Numbers within blocks equal ploidy levels of each species.

The distribution of genome sizes in Senecio therefore suggests that changes in genome size have occurred by lengthwise repetition of chromosome segments, that two different populations may be involved, and that the rate of change may not be proportional to the preexisting DNA amount because of polyploidy.

6.3.8 Speculations on the Direction of Changes in DNA Amount

Although instances of reduction in ploidy level have been recorded (deWet 1968, 1971), Stebbins (1980) commented that polyploid phylogeny can still be regarded as usually unidirectional from lower to higher levels. One trend in Senecio is therefore an increase in 4C DNA amounts by polyploidy. However, the direction of changes at the genomic level are not as clear. A change in values presupposes that one value is "basic" (as in polyploid series) and that others are derived at a later stage in evolution. Observations based largely on diploid species suggest that changes in genome size can proceed in either direction but that in general, a reduction in genome size most often accompanies evolutionary advancement among related species (Rees and Jones 1972, Nagl and Ehrendorfer 1974, Price 1976, Hinegardner 1976). In the majority of cases, advanced species are annuals and primitive species are perennials. The reduction in DNA amount is therefore largely associated with a reduction in cell cycle time and minimum generation time. However, it is possible that in Senecio polyploidy increases nuclear DNA amounts without greatly affecting cell cycle times (see Section 6.3.6.2). It follows that if selection favours a particular cell cycle time then increases in DNA amounts by polyploidy may be independent of changes within genomes. On the other hand, if selection is for a certain cell size then selection will act on any change in DNA amount irrespective of whether it is caused by polyploidy or

changes in genome size. All Australian species of Senecio are polyploids; most are either tetraploid or hexaploid but octoploids and decaploids also occur.

To interpret evolutionary changes at the genomic level it is necessary to consider what happens to genome size after polyploidy has occurred. It has frequently been suggested that polyploidy leads to a decrease in genome size (Pai et al. 1961, Grant 1969, Kadir 1974) as the polyploids examined in each case were found to have less DNA per genome than their diploid progenitors. Another suggestion is that only those species with the smallest chromosomes are likely to form polyploids (Chooi 1971). However, such an hypothesis must apply to species within genera as polyploids do occur in genera with very high DNA amounts - for example, in Frittilaria with about 35 pg per genome of 12 chromosomes (Bennett and Smith 1976).

I suggest that a third (but not entirely independent) hypothesis is that changes in genome size may proceed at a much slower rate following polyploidy. If this is the case then genome sizes of species at lower ploidy levels may change while the higher polyploid genome size remains relatively constant. There are two reasons why this might be the case. The first is based on the genotypic (rather than nucleotypic) evidence that polyploidy favours intermediate genotypes and restricts selection of extreme genotypes (Chapter 5.3.1.3). If an environmental change favours an extreme genotype as well as a change in genome size, then a high polyploid may be unable to respond because of genotypic (rather than nucleotypic) resistance to change. For example increasing aridity might favour plants with faster cell cycles and therefore with smaller genome sizes, but if the change also requires a modification of the genotype (which is more than likely) then the higher polyploids may be limited in their ability

to respond.

The second reason is that an effective change in the genome size of a polyploid requires loss or gain of more nuclear DNA than in a diploid. For example, a diploid with 10 pg per genome must increase its 2C DNA amount by 6 pg to achieve a 3 pg increase in genome size. However, a tetraploid must increase its 2C DNA amount by 12 pg, and an octoploid would require 24 pg to increase the genome size by 3 pg. As there is a direct relationship between nuclear DNA amount and cell size, the same increase in genome size will lead to progressively larger cells at higher ploidy levels. In many environments such a change might not be advantageous.

If polyploid species of *Senecio* (above the tetraploid level) are examined within related groups then a number of trends are apparent.

1. Hexaploid species of the erechthitoid group (Table 6.6) have remarkably consistent genomic DNA amounts. Seven species are within the range of 1.60 - 1.68 pg and one has 1.17 pg per genome. Furthermore, the range of hexaploid genome sizes falls within the range of genome sizes in the four related tetraploid species (i.e. 1.59-2.02 pg). The evidence suggests that in the erechthitoid group, gradual changes in genome sizes were generally restricted after hexaploids formed.

2. In the radiate group 2B, the octoploid form of *S. glossanthus* has slightly but significantly ($P<0.001$) more DNA than the tetraploid form - 0.94 pg and 0.84 pg respectively. As *S. glossanthus* is an ephemeral, there is presumably strong selection for a reduced genome size. It is therefore unlikely that the octoploid genome size has increased. A more likely explanation is that subsequent changes in the genome size of the octoploid were limited, but the tetraploid genome size continued

to decrease.

3. There are two high polyploids, S. pectinatus and S. vagus, among the morphologically primitive species of the radiate group 1A. Both have very much less DNA per genome then have the four related tetraploids. The evidence could support either theory of polyploid genome evolution - reduction of genome size in polyploids or restrictions on subsequent changes in polyploids. However, a low or high basic genome size in the genus depends upon which theory is correct. As evidence in the previous two cases supports restrictions rather than reductions in genome size following polyploidy, I have chosen the former as a general rule in Senecio.

Figure 6.8 shows genomic DNA amounts of native species of Senecio plotted against evolutionary advancement on a morphological basis (see Chapter 3). There is a general trend from large genome sizes among primitive species to smaller genome sizes among more advanced species. One might therefore infer that the primitive or basic genome size in Senecio was very high. However, if conclusions drawn from polyploid genome sizes are correct then the basic genome size of Senecio was comparatively small (1.5-1.8 pg). The genome size of most primitive species has therefore increased with little morphological advancement, whereas genome sizes of other species has remained more or less constant or has decreased with increasing morphological advancement. On this basis, evolutionary changes both in nuclear size and in the genome size of species of Senecio are shown diagrammatically in Figure 6.9.

According to the theory of "selfish" or "parasitic" DNA (Doolittle and Sapienza 1980, Orgel and Crick 1980) the C-value paradox can be explained in terms of a universal constant tendency for C-values to be increased by the multiplication of

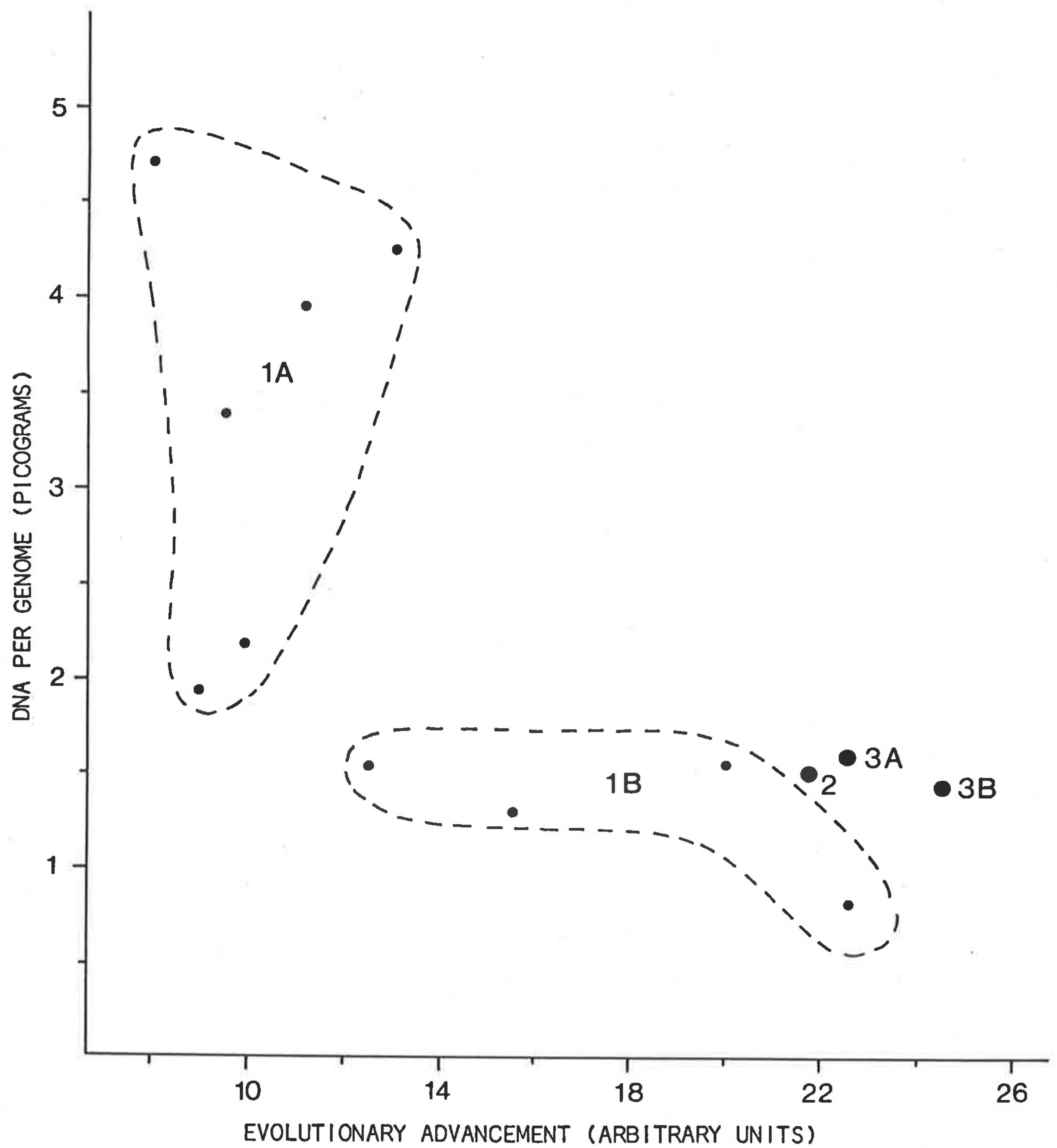


Fig. 6.8 Genome size plotted against evolutionary advancement for morphological groups of Australian *Senecio* species (see text for explanation).

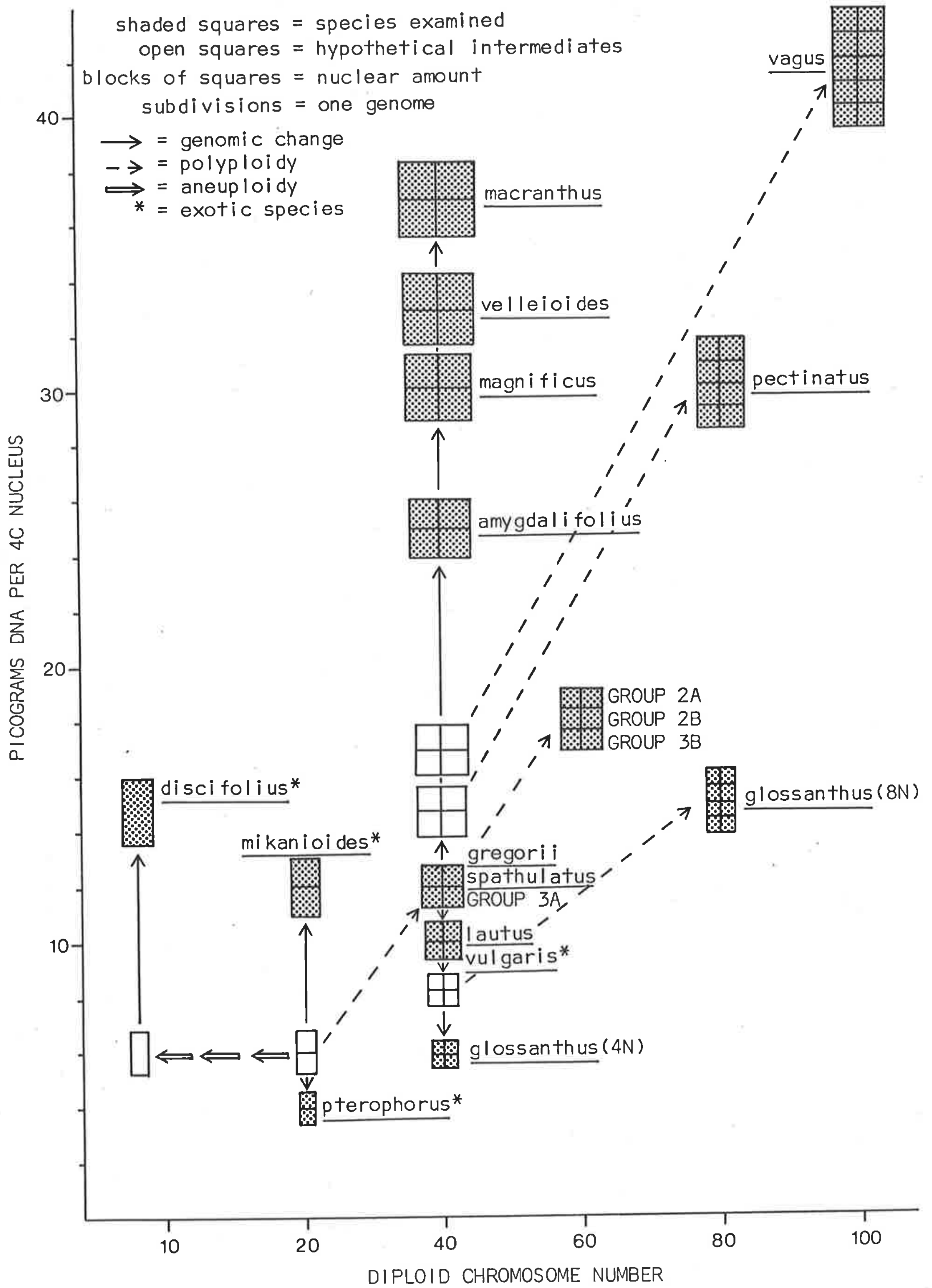


Fig. 6.9 Postulated scheme of evolution of nuclear size and genome size in Senecio (see text for explanation).

selfish DNA sequences, and selection against such increases, which would be more intense in smaller and more rapidly reproducing cells. If this is the case then it is difficult to explain why very large increases in genome size are restricted to only one morphological group of Senecio species - that is, outcrossing radiate species with continuous stigmatic surfaces. Alternatively, if Cavalier-Smith's (1978) theory that genome sizes are selected for because of their nucleotypic effects is correct, then the large genome and nuclear sizes of primitive species are advantageous. Excluding the high polyploids within this group, three of the four remaining species occur only in wet sclerophyll forests and therefore in a highly competitive and congested environment. In such conditions greater height and larger floral structures (as all are outcrossing) would be advantageous. The fourth species, S. magnificus, occurs only in arid inland areas but most frequently near watercourses. S. magnificus might therefore be considered a secondary adaptation to drier conditions. Price and Bachmann (1976) presented an alternative model based on nucleotypic effects which suggested that among annual species, high diploid DNA amounts may be another way of differentiating a larger mass in a given period of time. Their model is based on evidence that nuclear DNA content has a greater nucleotypic effect on cell size than it does on the mitotic cycle time. It is possible that in the case of Senecio their model is illustrated among perennials, and that perennials with large genome sizes can differentiate more mass in a given time than those with smaller genomes. Such an ability would be most advantageous in crowded forest conditions. A question remains as to why polyploidy was not favoured instead of increased genome sizes, as polyploidy increases cell size with little or no effect on cell cycle times. However, it is possible that species with very large chromosomes

cannot form viable polyploids (Chooi 1971) or that the proportional but massive increase in such an event is unfavourable. Polyploidy can therefore occur if the genome size is comparatively small, as in *S. pectinatus* (1.95 pg) and *S. vagus* (2.19 pg), but among larger genomes the only alternative is to further increase genome size.

By comparison reductions in genome sizes are also relatively large if the basic genome size is between 1.5 and 1.8 picograms. Reductions to 0.8-0.9 pg are proportionate to increases to 3.0-3.6 picograms. The smallest genome occurs in *S. glossanthus*, and is explicable in terms of selection for a smaller genome size and faster cell cycle in an ephemeral species. An alternative strategy in the ephemeral *S. gregorii* may be a reduction in cell cycle time by extensive formation of heterochromatin - as suggested by Nagl and Ehrendorfer (1974) for annual species of Anthemideae.

Exotic species of *Senecio* included in this study can also be interpreted as having evolved from a genome size of about 1.5-1.8 pg. Although *S. vulgaris* and *S. pterophorus* differ in longevity, both are capable of very rapid development and are either garden or agricultural weeds. Both have reduced genome sizes (0.98 and 1.06 pg respectively). *S. mikanioides* is a weedy liana, succeeding by shading out other species. Genomic DNA (2.95 pg) and presumably also cell size is increased. The ephemeral *S. discifolius* with N=5 most probably evolved by aneuploid reduction from a species with N=10. It would therefore be expected that the genome of 5 chromosomes in *S. discifolius* would be equal to or smaller than one of 10 chromosomes. However, 5 chromosomes of S. discifolius represent 3.57 pg of DNA - twice the expected value. If the genome has increased in size, then *S. discifolius* can be compared with annual species of

Microseridinae in which an increased genome size is thought to be potentially capable of differentiating more mass in a given time.

Although a great deal of this section is speculative and is based on many assumptions, I feel the data can generally be explained in terms of selection both for larger and smaller genome sizes. The alternative thesis of a universal tendency for genome sizes to increase independently of phenotypic selection cannot be disproven without more detailed research, but in the case of *Senecio* I believe that such a model overlooks Cavalier-Smith's (1978) simpler explanation - that genome sizes may be selected for.

6.4 Conclusions

When compared with other genera the variation in DNA amounts per 4C nucleus and per genome in *Senecio* are comparatively large. Polyploidy accounts for some of the variation, but there is also extensive variation at the genomic level.

A comparison of DNA amounts and minimum generation times in *Senecio* corresponds with a previous survey of 271 plants by Bennett (1972). In both, ephemerals have a lower mean DNA amount per nucleus and a maximum of about 14 pg whereas annuals and facultative perennials have a higher mean DNA amount and a maximum of about 38 pg (although annuals and facultative perennials differ in longevity, they have the same minimum generation time). Bennett (1972) suggested that as DNA amount and cell cycle time are positively correlated, larger DNA amounts are most often associated with perennial life forms. Although this

may generally be the case, Price and Bachmann (1976) suggested that among annuals an increase in DNA amount may be an alternative way of differentiating a given mass very quickly. Their suggestion is based on evidence that an increase in DNA amount (at the diploid level) has a greater nucleotypic effect on cell size than on mitotic cycle time. I believe this model may also explain the very large genome sizes of outcrossing species of Senecio in congested environments, the liana S. mikanioides and the ephemeral S. discifolius. An alternative way of achieving rapid development may be the extensive formation of heterochromatin, thought by Nagl and Ehrendorfer (1974) to reduce mitotic cycle times without changing DNA amounts. The ephemeral S. gregorii supports this suggestion.

Evidence in Senecio suggests that changes in genome size may be restricted at higher ploidy levels. On this basis, higher ploidy levels may reflect earlier genome sizes. The primitive or basic genome size of Senecio is therefore thought to lie between 1.5 and 1.8 pg of DNA.

Although the model of genome increase by multiplication of "selfish" DNA segments cannot be conclusively disproven by evidence of DNA amounts in Senecio, the general correlation between DNA amount, life form and environmental conditions suggests that plants in different conditions need different genome sizes, and therefore that nuclear DNA amounts are the product of natural selection.

CHAPTER 7

Karyotypes

7.1 Introduction

7.2 Materials and Methods

7.2.1 Karyotype construction

7.2.2 Interspecific comparisons

7.3 Results and Discussion

7.3.1 Illustration of karyotypes

7.3.2 Comparison of karyotypes

7.3.2.1 Size of significant differences

7.3.2.2 Interpretation of data

7.3.2.3 Relationships deduced from percentage similarities

7.3.2.4 Satellite chromosomes

7.3.3 Karyotype symmetry and evolutionary advancement

7.3.4 Changes in absolute chromosome size

7.3.5 The basic chromosome number of *Senecio*

7.3.5.1 Karyotype symmetry and absolute chromosome size

7.3.5.2 The number of satellite chromosomes

7.4 Conclusions

7.1 Introduction

The underlying assumption of a study of karyotypes is that karyotypes of closely related species and groups are likely to be similar. However, because of exceptions to the rule, authors such as Stebbins (1971), Jackson (1971) and Jones (1978) have stressed the importance of combining other evidence with a study of karyotypes. For example, there are many examples where investigators cling to the concept of symmetrical karyotypes being most primitive, even when the phenotype argues in the opposite direction (Jones 1978). Similarly, the number of satellite chromosomes is often used as an indicator of ploidy level, but there are cases in which satellite numbers are more than or less than expected on the basis of ploidy level alone (Stebbins 1971). In spite of the problems, the value of karyotypes in determining evolutionary relationships has long been recognised.

The major objective of including karyotypes in this study was to see if additional evidence might clarify the phylogenetic position of inbreeding erechthitoid species. A second objective was to determine the basic chromosome number of *Senecio* from chromosome morphology. As chromosome numbers of the majority of native species of *Senecio* are comparatively high (2N = 40 to 2N = 100), no attempt was made to determine precise structural changes between species. Instead karyotypes were compared by determining percentage similarities, absolute chromosome size and karyotype symmetry. In view of the statistical errors that arise when chromosomes are similar in length or lack distinguishing features such as satellites (Mattern and Simark 1969) a more precise comparison of species with high chromosome numbers is probably not possible.

7.2 Materials and Methods

Karyotypes were determined for only one population of each taxon using the same material as for DNA estimates (see collection numbers listed in Table 5.2). The method of slide preparation is described in Lawrence (1980, copy bound with thesis), and karyotypes were constructed by the method described by Martin and Hayman (1965). The basic assumption of the method is that, in an interspecific comparison, the ratio of total chromosome lengths will be the same as the ratio of amounts of DNA per chromosome complement. Each chromosome arm is first expressed as a mean percent length (the mean value obtained from a number of nuclei) of the total length of all chromosomes in the complement. Mean percent lengths are then expressed as a proportion of the total DNA amount per nucleus (see previous chapter). As the size of chromosome arms expressed as an amount of DNA is an absolute measurement, the size of chromosomes belonging to different species can be compared. The assumptions involved are recognised, but were considered justified in view of the general way in which the data were to be used.

7.2.1 Karyotype Construction

The construction and comparison of karyotypes of Australian species of Senecio was complicated by the comparatively high diploid chromosome numbers; most species have either 40 or 60 chromosomes but species with 80 and 100 also occur. For this reason the number of complements measured for each species was eventually limited to six. Preparations were photographed at maximum magnification using Agfa Copex film and were printed on Ilfobrom paper at maximum enlargement (total magnification was approximately 4270x). Chromosome arms were measured to the

nearest 0.1 mm using a Peak Scale Lupe x7 magnifying lens fitted with a number 2 graticule. Each chromosome arm was measured twice, the values corresponding to chromatids if these were apparent, or otherwise to each side of the chromosome arm. No attempt was made to pair chromosomes before they were measured. Instead, chromosomes were arbitrarily numbered from 1 to 2N on a sheet of tracing paper placed over the photograph and measurements were recorded in that order. In most instances, satellites were too small or too diffuse to be assigned an accurate length measurement, but their position was noted. Large satellites were measured separately. When 6 to 8 complements had been measured, the photographs were inverted on a light table and the original measuring sequence transferred to the back of each chromosome. The chromosomes of each complement were then cut out and arranged in pairs in descending order of length. After the complements had been aligned (i.e. rows corresponding to complements and columns corresponding to apparently identical chromosomes), each complement was carefully turned over and the "correct order" recorded from the reverse side.

A Fortran computer program was written to analyse results. Input data consisted of two matrices with rows corresponding to all duplicate complements. Matrix 1 contained all arm lengths entered in the order in which chromosomes were measured. Matrix 2 contained the "correct order" of chromosomes determined by visual matching - every two columns (i.e. columns 1 and 2, 3 and 4, etc.) corresponded to all locations in Matrix 1 of original measurements of one chromosome in the karyotype. The total length of each complement was first determined and the original arm lengths converted to a percentage of the total. As absolute chromosome sizes were required, percentage arm lengths were multiplied by the 4C DNA amount of that species. Percentage arm lengths

(expressed in picograms) were taken from Matrix 1 in the order specified by the columns of Matrix 2, and the mean percentage length and standard error of each arm in the karyotype calculated.

7.2.2 Interspecific Comparisons

Interspecific differences between karyotypes of *Senecio* might be caused by one or more of the following events:

1. changes in nuclear DNA amounts - leading to differences in absolute chromosome size;
2. structural rearrangements - leading to differences in relative chromosome size and in chromosome arm ratios;
3. polyploidy - leading to genome duplication and differences in chromosome number;
4. hybridization - leading to different combinations of genomes.

Changes in nuclear DNA amounts were accounted for by proportionately adjusting chromosome arm lengths of a karyotype so that their total length equalled half the 4C DNA amount of that taxon. However, by this method it is impossible for two taxa with the same chromosome number but very different DNA amounts to have highly similar karyotypes. An alternative approach would have been to equate total arm length with a standard value per genome. Such a recalibration and comparison of karyotypes would indicate if differences are largely due to changes in absolute chromosome size, but results could be misleading as a high similarity between karyotypes need not reflect a high degree of homology between chromosomes. For this reason analyses were completed using absolute chromosome size. It was still possible to compare karyotypes independently of their DNA amounts as arm ratios and the ratio of the longest divided by the shortest chromosome (see discussion in part 7.3.3) are two karyotypes features that

do not depend on DNA amounts.

As 35 karyotypes with an average of 25 chromosomes in each were to be compared, a Fortran computer program was written to complete the analysis (a listing is provided in Appendix 3). The data input consisted of the absolute size and standard error of each chromosome arm. With each execution of the program, the last karyotype in the data list was compared with each preceeding karyotype. In any one comparison, chromosome numbers were first examined. If these differed, the species with fewer chromosomes was designated as A and the species with more chromosomes as B. Each chromosome in A was then compared with all chromosomes in B and matching chromosomes recorded. Two chromosomes were said to match if the short chromosome arms and long chromosome arms were both equal ($P > .05$) using a Students t test.

An example of the program output is shown in Table 7.1. Thirteen chromosomes of each set match uniquely with a previously unmatched chromosome in the other set. Percentages at the bottom of the output are the proportion of uniquely matching chromosomes. Although the number of unique matches is constant in any one comparison, percentages will differ if the chromosome numbers of species A and B differ. In the example given, a unique match of 13 chromosomes represents 65% of A (N=20) and 43.33% of B (N=30). Chromosome 11 in set A and chromosome 13, 26 and 27 in set B do not have unique matches and therefore represent duplicates of the uniquely matching set. If these chromosomes are included, the percentages become 70 and 53.33%, respectively, and represent all chromosomes in one set matching with any chromosome in the other. The need to calculate total (as opposed to unique) matches was not foreseen until all species had been analysed. Total matches were therefore calculated by hand from the program outputs.

TABLE 7.1

Example of the Output of a Program

to Compare Karyotypes

Set A *S. vulgaris* N=20 vs. Set B *S. bipinnatisectus* N=30

Identical chromosomes (P >0.05)

Set A	Set B	All matches in B			
1					
2					
3	9	9	11		
4	11	9	11	13	
5	16	16			
6	19	19	20		
7	20	19	20	21	
8	21	19	20	21	
9	17	17			
10	15	15	17		
11		15	17		
12	23	20	21	23	
13	24	23	24	26	27
14	25	25	26	27	
15					
16					
17	28	28	29	30	
18	30	30			
19					
20					

65.00% of set A matches with set B

43.33% of set B matches with set A

Although a comparison of any two karyotypes can produce four percentage similarities (total and unique matches of both species A and species B) no one of the four adequately represents karyotype similarity when polyploidy is involved. A system combining the four percentages was therefore devised, and is illustrated by examples in Table 7.2. As the majority of Australian species of _Senecio_ are either tetraploid or hexaploid, only these ploidy levels are represented in the table. In the examples shown it is assumed that species are newly-formed polyploids with no chromosomes common between genomes (e.g. to both X and Y). In examples 1,5,6,8, 12 and 13 species A and B share at least four genomes. Although represented as autopolyploids, species in these examples might also be allopolyploids provided the four genomes are identical in both A and B. Duplications of the X genome in examples 2,7,9, 14, 15 and 16 must be due to autopolyploidy, or the case will resemble another listed example.

As no one of the four possible percentage similarities (Table 7.2, columns 1 to 4) can distinguish between all cases, a fifth parameter - the total percentage similarity (TPS) - was generated by adding together columns 1 and 2. The TPS values therefore represents the sum of unique matches in A and B plus any duplicate matches in either A or B. TPS values are shown in column 5, and have different amounts in all but examples 7 and 8. However, these cases can be separated by their UPS values (column 6) - the sum of unique percent matches (columns 3 and 4). A difference in TPS and UPS values therefore indicates that either species A or species B contains duplicates of the uniquely matching chromosomes. The percentage of each complement containing duplicates was determined by subtracting column 3 from 1 (for A) and column 4 from 2 (for B). It was useful to determine duplicates in terms of genomes so that species with different chromosome numbers

TABLE 7.2

Percentage Similarities of Tetraploid and Hexaploid Karyotypes (See text for explanation)

Example	Genomic Composition (matches underlined) Species A	Species B	1 Total Match A in B	2 Total Match B in A	3 Unique Match A in B	4 Unique Match B in A	5 TPS (1+2)	6 UPS (3+4)	7 Duplicate Genomes (in karyotypes) of A	8 Duplicate Genomes (in karyotypes) of B
				A = B = Tetraploid						
1	XXXX	XXXX	100	100	100	100	200	200	0	0
2	XXXX	XXYY	100	50	50	50	150	100	1	0
3	XXYY	XXZZ	50	50	50	50	100	100	0	0
4	XXXX	YYYY	0	0	0	0	0	0	0	0
				A = B = Hexaploid						
5	XXXXXX	XXXXXX	100	100	100	100	200	200	0	0
6	XXXXXX	XXXXYY	100	67	67	67	167	134	1	0
7	XXXXXX	XXYYYY	100	33	33	33	133	66	2	0
8	XXXXYY	XXXXZZ	67	67	67	67	134	134	0	0
9	XXXXYY	XXZZZZ	67	33	33	33	100	66	1	0
10	XXYYYY	XXZZZZ	33	33	33	33	66	66	0	0
11	XXXXXX	YYYYYY	0	0	0	0	0	0	0	0

Table 7.2 - continued

Example	Genomic Composition (matches underlined) Species A	Species B	1 Total Match A in B	2 Total Match B in A	3 Unique Match A in B	4 Unique Match B in A	5 TPS (1+2)	6 UPS (3+4)	7 Duplicate Genomes in Karyotype of A	8 Duplicate Genomes in Karyotype of B
			A = Tetraploid		B = Hexaploid					
12	XXXX	XXXXXX	100	100	100	67	200	167	0	1
13	XXXX	XXXXYY	100	67	100	67	167	167	0	0
14	XXYY	XXXXXX	50	100	50	33	150	83	0	2
15	XXXX	XXYYYY	100	33	50	33	133	83	1	0
16	XXYY	XXXXZZ	50	67	50	33	117	83	0	1
17	XXYY	XXZZZZ	50	33	50	33	83	83	0	0
18	XXXX	YYYYYY	0	0	0	0	0	0	0	0

to duplicate genomes by dividing the former by the percentage of the karyotype (haploid) represented by one genome - 50% in the case of tetraploid karyotypes and 33% for hexaploid karyotypes.

Using TPS, UPS and duplicated genome values it is possible to predict the genomic composition of any species pair listed in Table 7.2. However, it is also necessary to consider cases in which some chromosomes are common to different genomes. Such a situation might arise if hybridization occurs between closely related species or if structural rearrangements occur in an autopolyploid. TPS, UPS and duplicate genome values may then be intermediate to those listed in Table 7.2, but the overall pattern will remain the same. For example, if genome X contains 10 chromosomes and shares four with genome X', then the comparison XXXX - XXX'X' will have a TPS value of 170, a UPS value of 140, and 0.6 and 0 duplicated genomes, respectively.

7.3 Results and Discussion

7.3.1 Illustration of Karyotypes

Karyotypes of 33 species and 5 subspecies of *Senecio* and the karyotype of *Erechtites valerianaefolia* are shown in Figure 7.1 (1 to 39). Preparations suitable for analysis were not obtained for *Senecio pectinatus* (2N = 80), *Arrhenechtites mixta* (2N = 100) and *Bedfordia salicina* (2N = 60). As described in the Methods, the size of chromosome arms was determined by first calculating the mean percent length of each arm and then converting this value to a proportionate amount of the 4C DNA content. A complete listing of arm lengths is given in Appendix 1.

Chromosome numbers of most species are comparatively high (2N = 40 to 2N = 100) and are undoubtedly polyploid derivatives of species with smaller chromosome numbers. One would therefore expect that in higher polyploids each chromosome of the genome would be duplicated several times forming a group of homologous chromosomes. However, when aligning cut out photographs of chromosomes it was very difficult to accurately group chromosome pairs. In many instances pairs could be aligned in a series of decreasing size - with little apparent difference between immediate neighbours but with large differences between the first and last pair of the series. Although such series may have represented several subgroups of homologous chromosomes, each differing slightly in size, it was not possible to determine boundaries between groups. For this reason no attempt was made to group chromosomes and then average the lengths within groups. This omission undoubtedly introduced errors, but I believe errors caused by grouping of pairs would have been equally great.

Fig. 7.1 Karyotypes of 33 species and 5 subspecies of *Senecio*, and of *Erechtites valerianaefolia*

7.1-1 to 7.1-39 Line drawings of all karyotypes appearing in the order listed on the next page.

7.1-40 to 7.1-43 Representative photographs of karyotypes.

Chromosomes in each complement are arranged in descending order of the combined 'length' (DNA amount) of both arms. The total arm length of each karyotype (haploid complement) therefore equals half the 4C DNA amount in picograms. Satellites are shown attached to one chromosome arm and are drawn as a circle if they were too small to measure accurately. All karyotypes are drawn at the same scale (1 cm = 0.1 picograms) so that visual comparison is possible.

List of Fig. 7.1 karyotypes in their order of appearance.

GROUP 1A

1 *Senecio magnificus*

2 *S. velleioides*

3 *S. amygdalifolius*

4 *S. macranthus*

5 *S. vagus* subsp. *eglandulosus*

BROUP 1B

6-10 *S. lautus* subspp.

11 *S. spathulatus*

12 *S. glossanthus*

13 *S. gregorii*

GROUP 2A

14 *S. hypoleucus*

15 *S. odoratus*

16 *S. cunninghamii*

17 *S. anethifolius*

18 *S. gawlerensis*

GROUP 2B

19 *S. linearifolius*

20 *S.* sp. A

GROUP 3A

21 *S. quadridentatus*

22 *S. gunnii*

23 *S.* aff. *apargiaefolius*

24 *S. runcinifolius*

GROUP 3B

25 *S.* sp. B

26 *S. squarrosus*

27 *S. bipinnatisectus*

28 *S. minimus*

29 *S. picridioides*

30 *S. glomeratus*

31-32 *S. hispidulus* vars.

33 *S.* sp. C

34 *S. biserratus*

EXOTIC SPECIES

35 *S. pterophorus*

36 *S. vulgaris*

37 *S. mikanioides*

38 *S. discifolius*

OTHER GENERA

39 *Erechtites valerianaefolia*

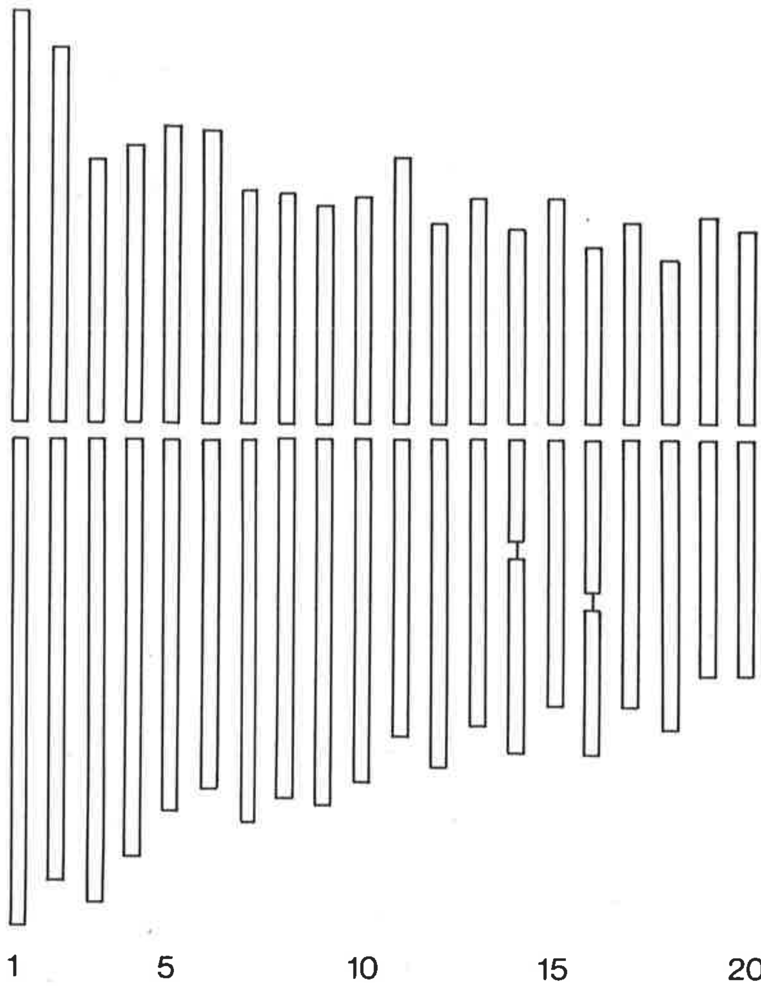
REPRESENTATIVE PHOTOGRAPHIC TREATMENTS

40 *Senecio vagus* ssp. *eglandulosus*

41 *S.* sp. A

42 *S. velleioides*

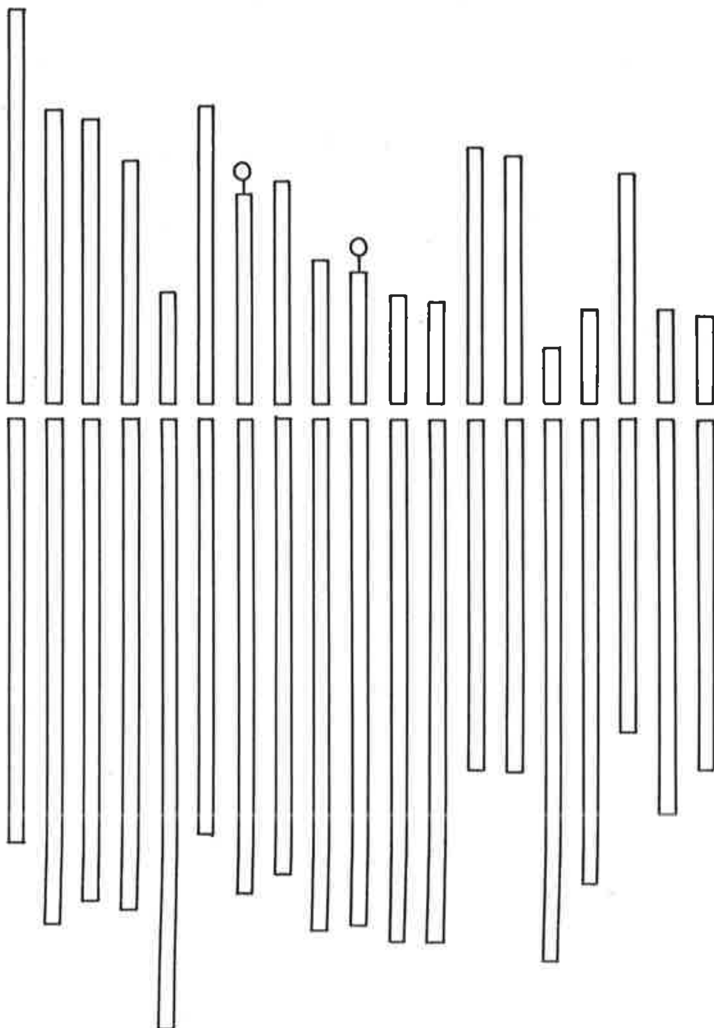
43 *S. lautus* ssp. *dissectifolius*



1. S. *magnificus*

N = 20

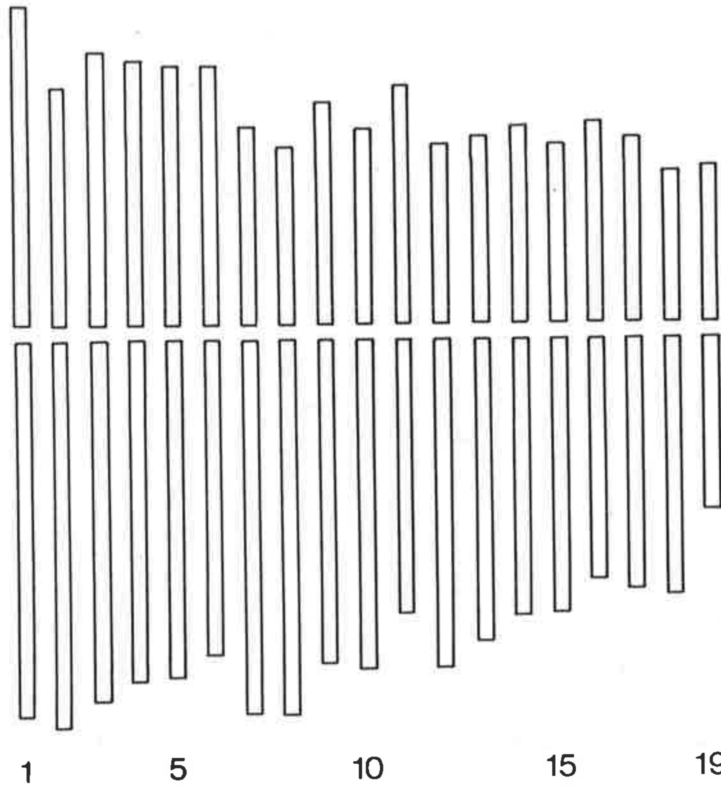
4C DNA = 31.7 pg.



2. S. *velleioides*

N = 19

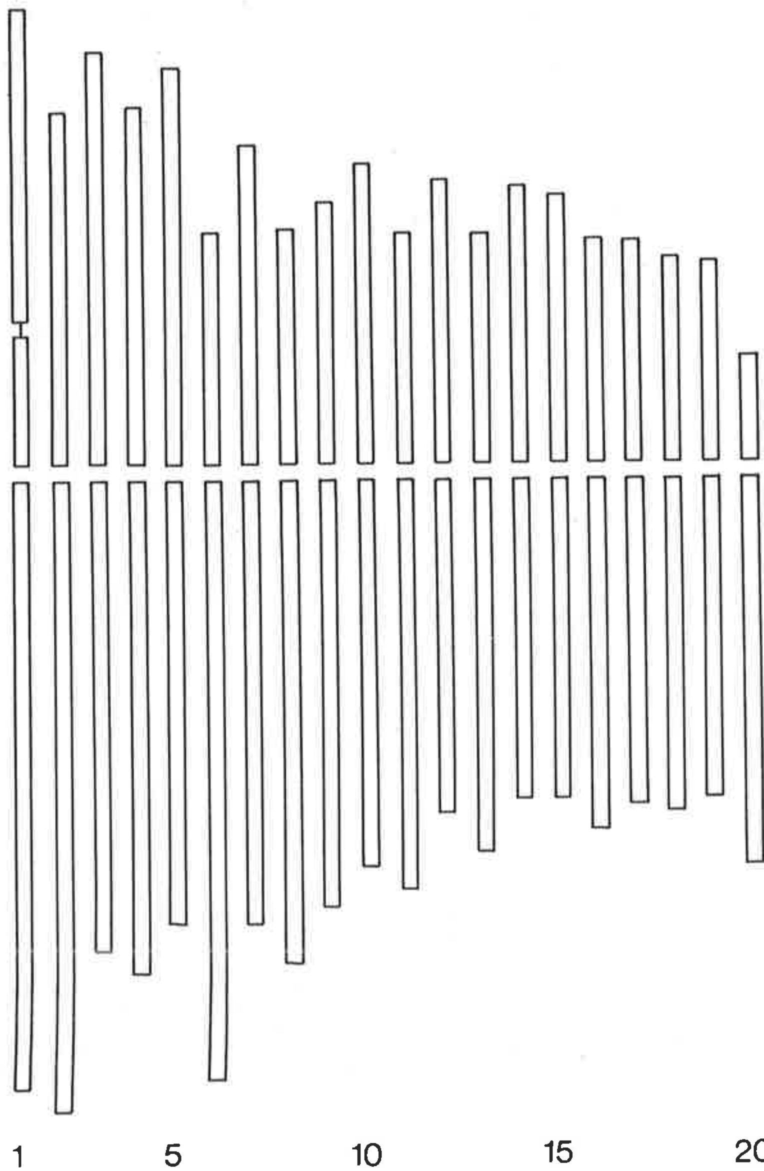
4C DNA = 33.1 pg.



3. <u>S</u>. <u>amygdalifolius</u>

N = 19

4C DNA = 26.9 pg.

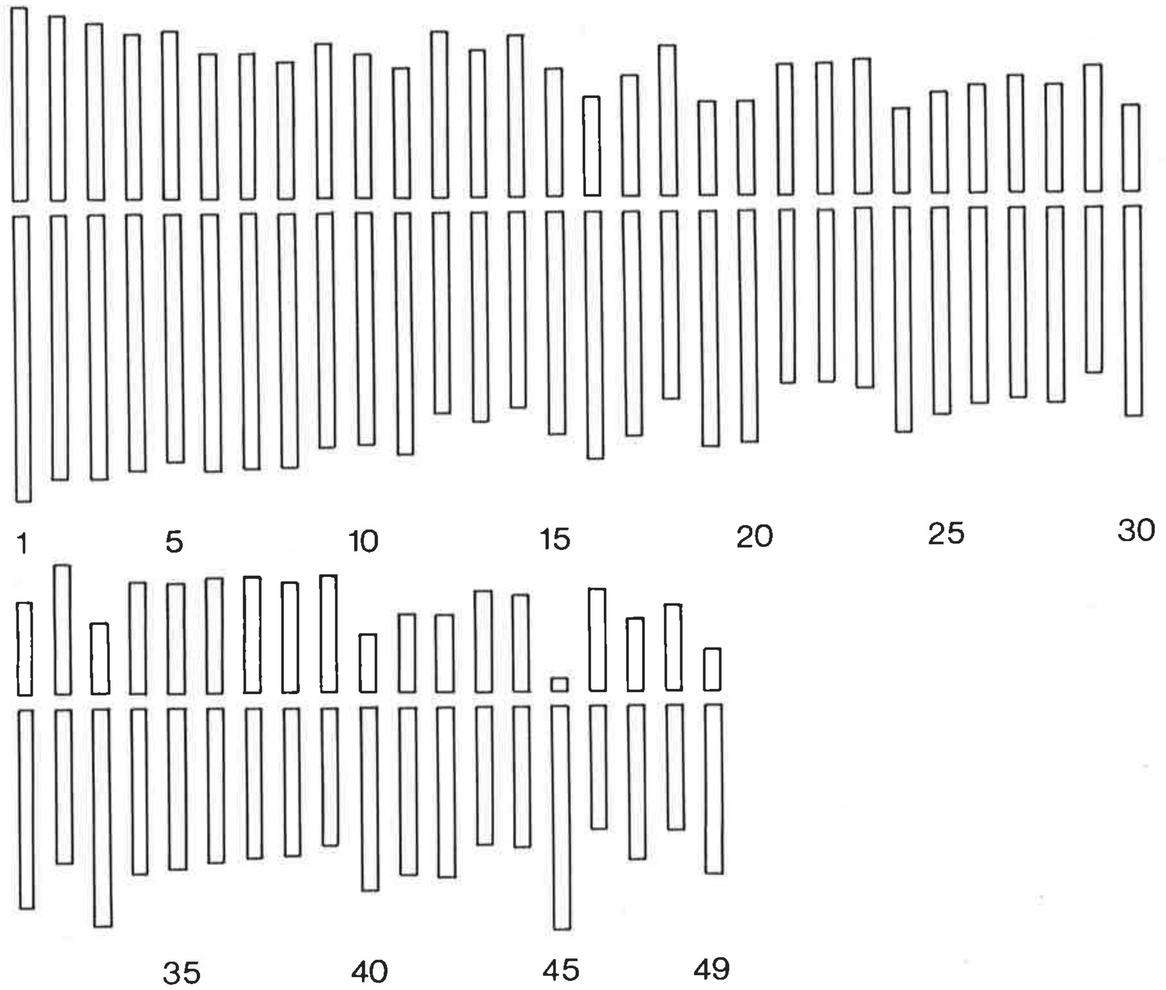


4. <u>S</u>. <u>macranthus</u>

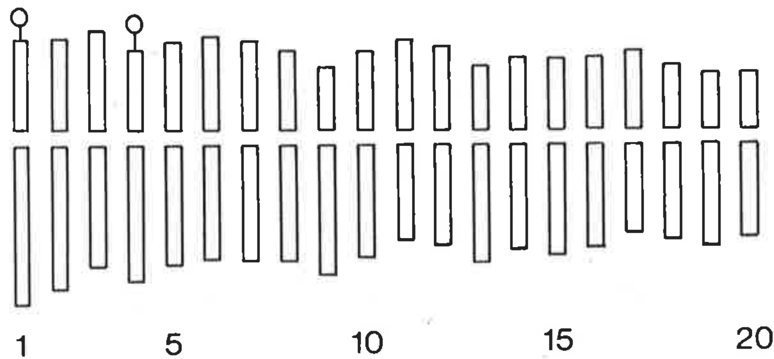
N = 20

4C DNA = 37.48 pg.

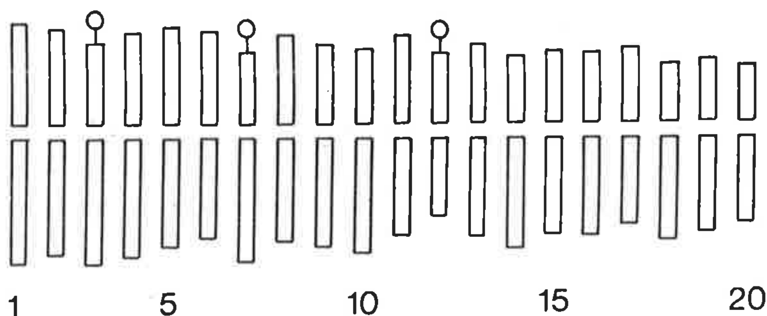
5. *S. vagus* subsp. *eglandulosus* N = 49 4C DNA = 42.90 pg.



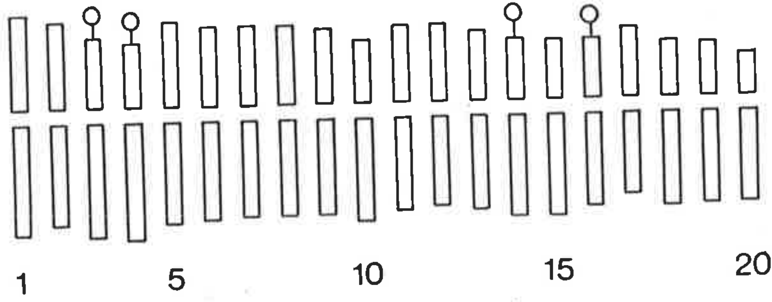
7. *S. lautus* subsp. *dissectifolius* N = 20 4C DNA = 10.63 pg.



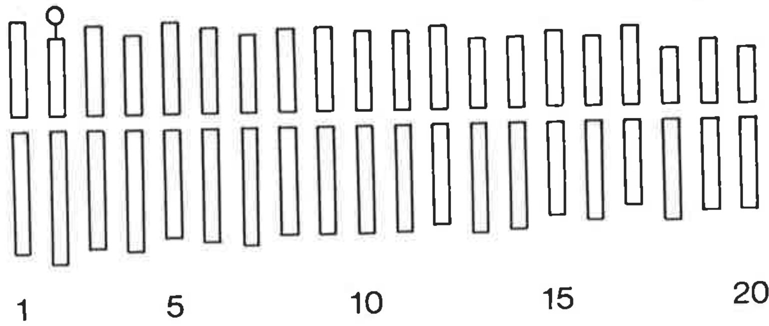
6. *S. lautus* subsp. *lautus* N = 20 4C DNA = 10.81 pg.



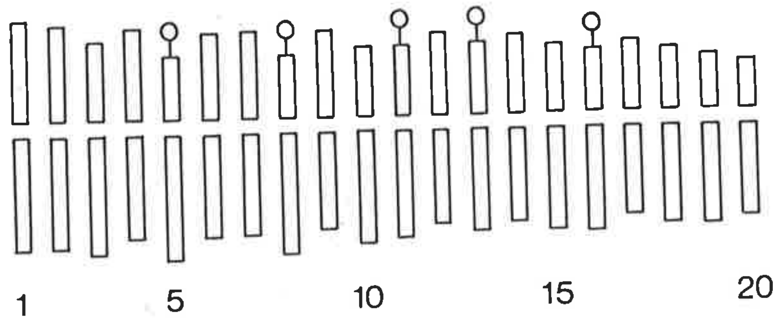
8. *S. lautus* subsp. *maritimus* N = 20 4C DNA = 10.19 pg.



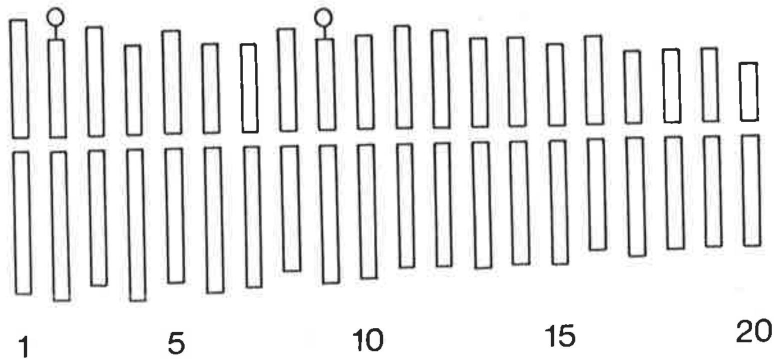
9. *S. lautus* subsp. *alpinus* N = 20 4C DNA = 10.74 pg.



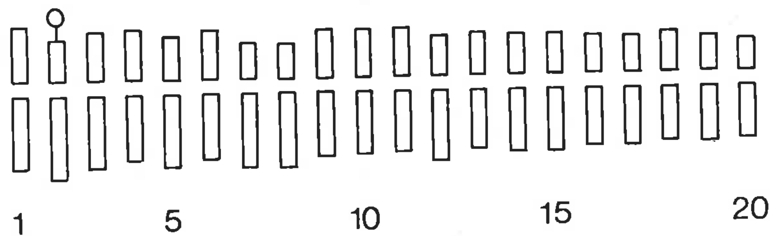
10. *S. lautus* subsp. *lanceolatus* N = 20 4C DNA = 9.79 pg.



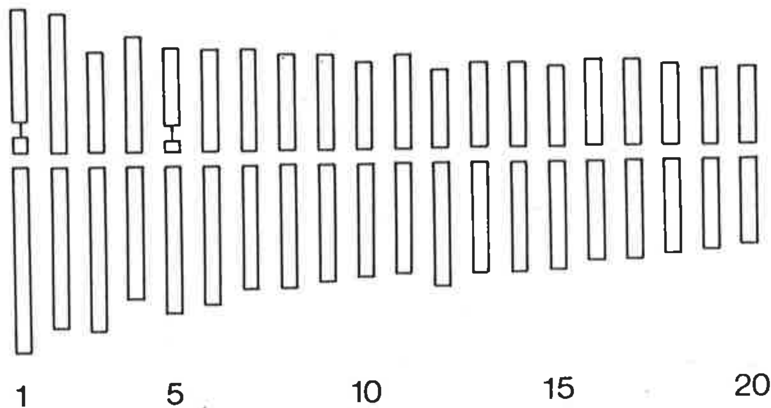
11. *S. spathulatus* N = 20 4C DNA = 12.28 pg.



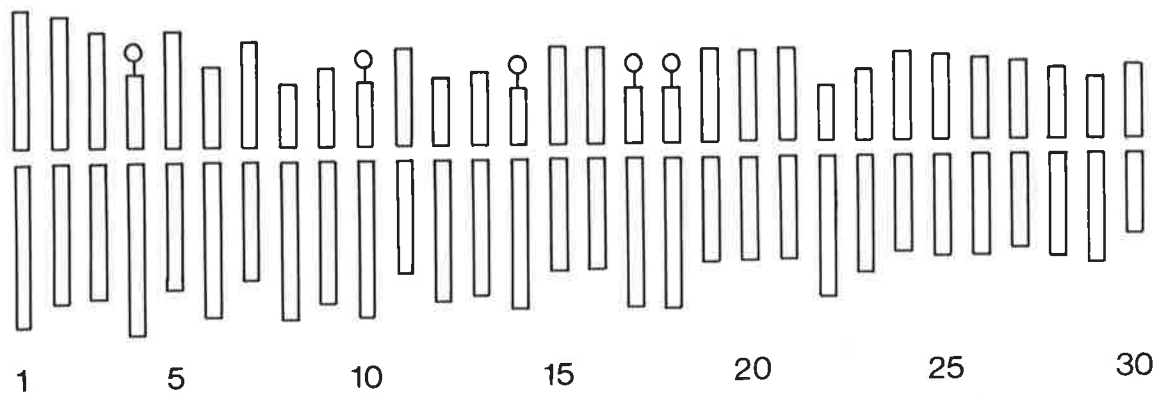
12. *S. glossanthus* N = 20 4C DNA = 6.71 pg.



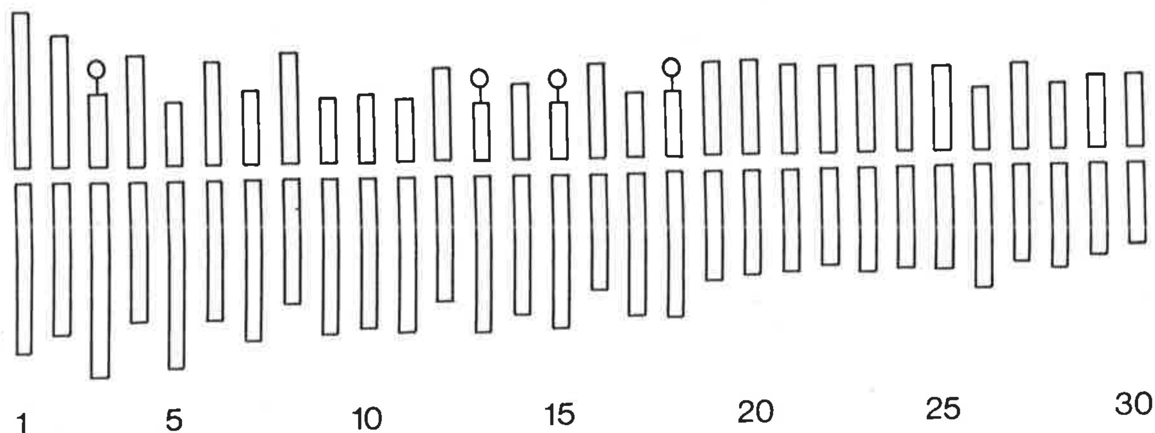
13. *S. gregorii* N = 20 4C DNA = 12.55 pg.



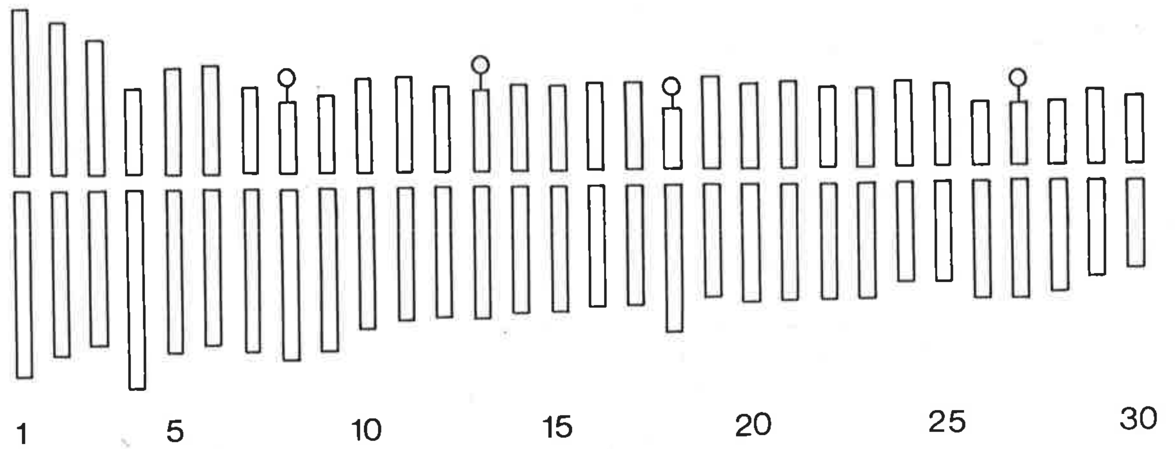
14. *S. hypoleucus* N = 30 4C DNA = 17.92 pg.



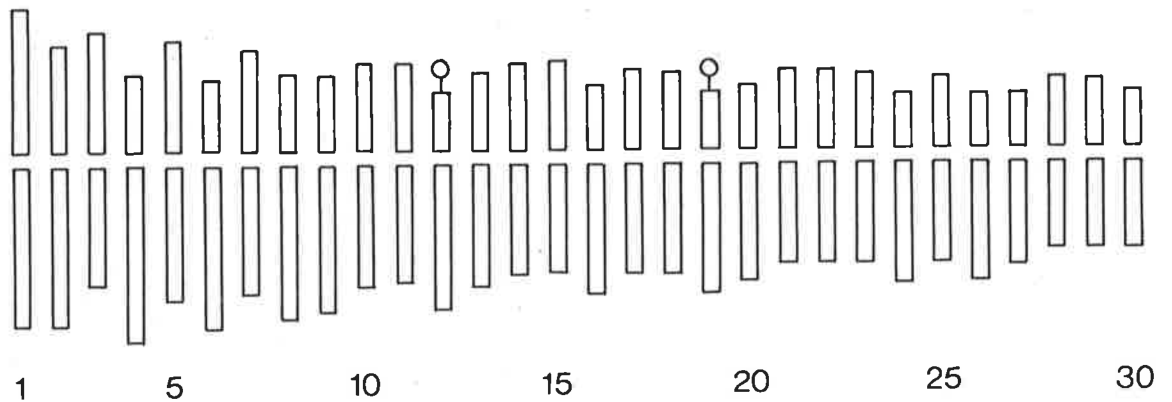
15. *S. odoratus* N = 30 4C DNA = 18.14 pg.



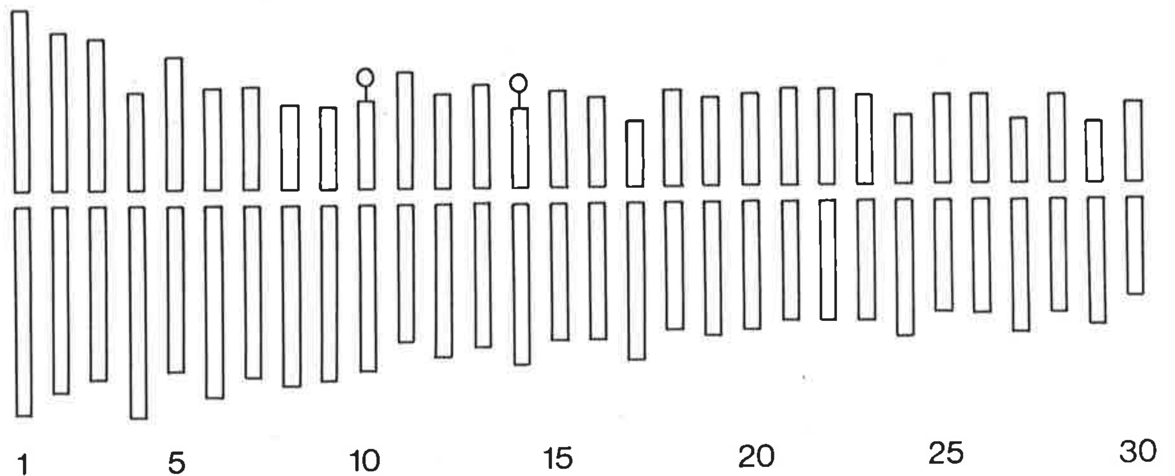
16. S. cunninghamii N = 30 4C DNA = 18.90 pg.



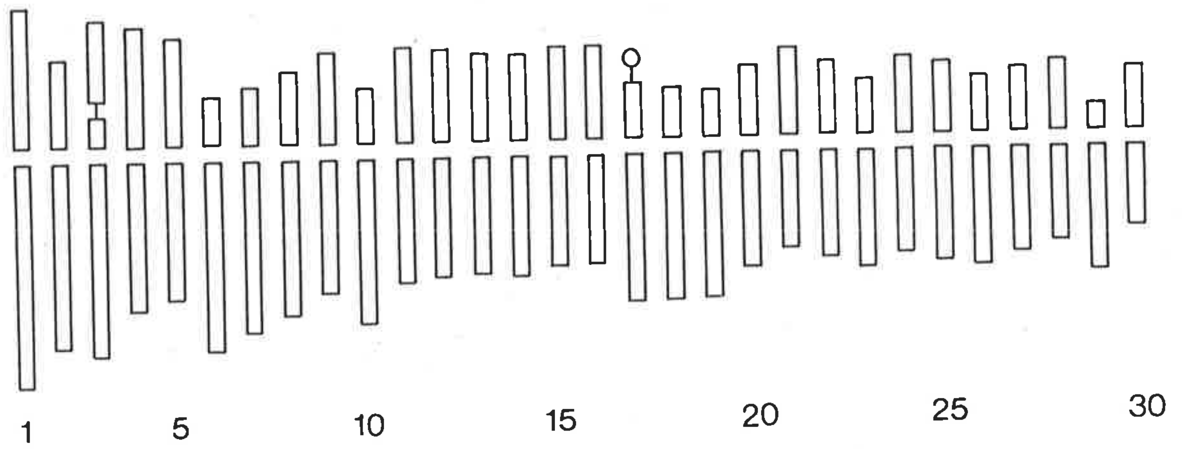
17. S. anethifolius N = 30 4C DNA = 17.35 pg.



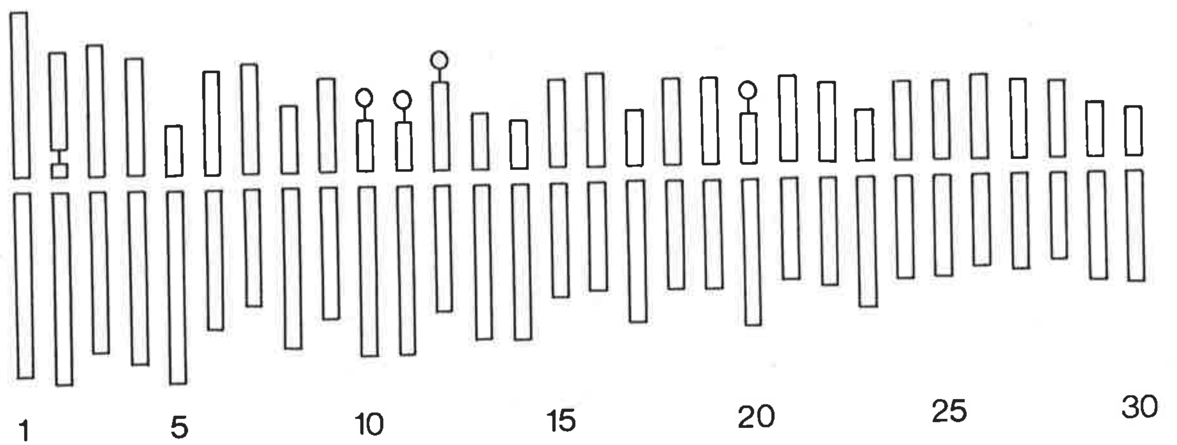
18. S. gawlerensis N = 30 4C DNA = 20.39 pg.



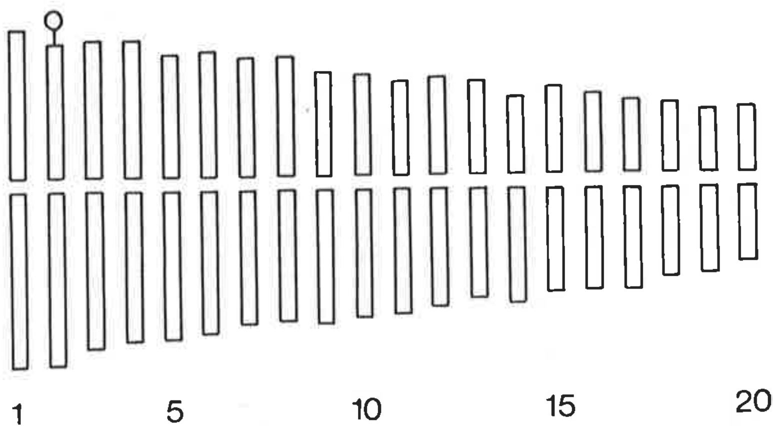
19. *S. linearifolius* N = 30 4C DNA = 18.02 pg.



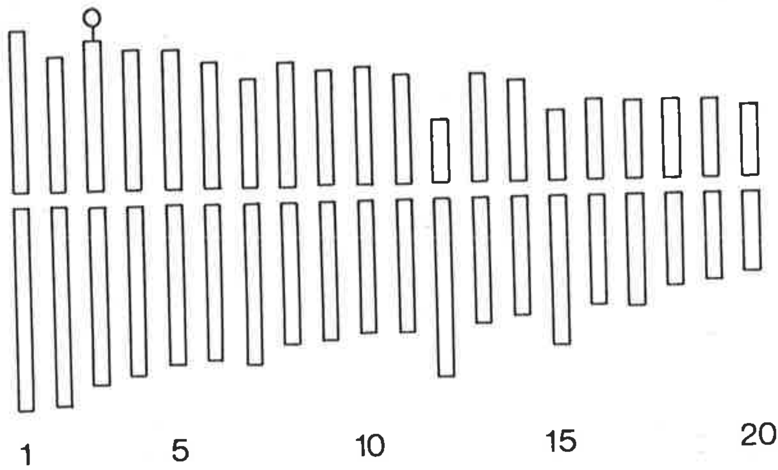
20. *S.* sp. A N = 30 4C DNA = 18.03 pg.



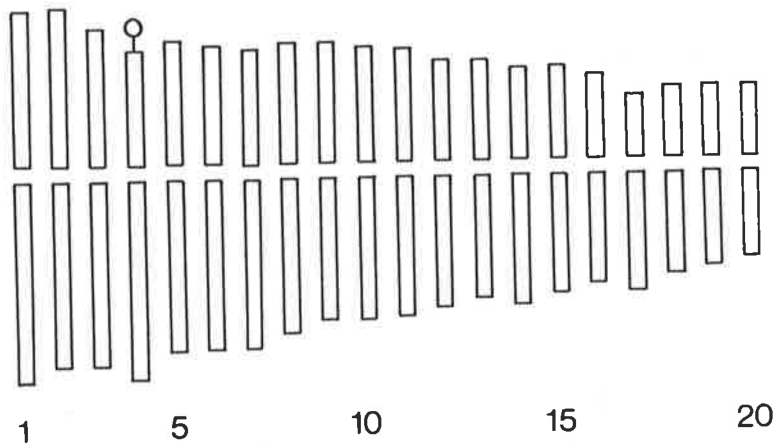
21. *S. quadridentatus* N = 20 4C DNA = 12.75 pg.



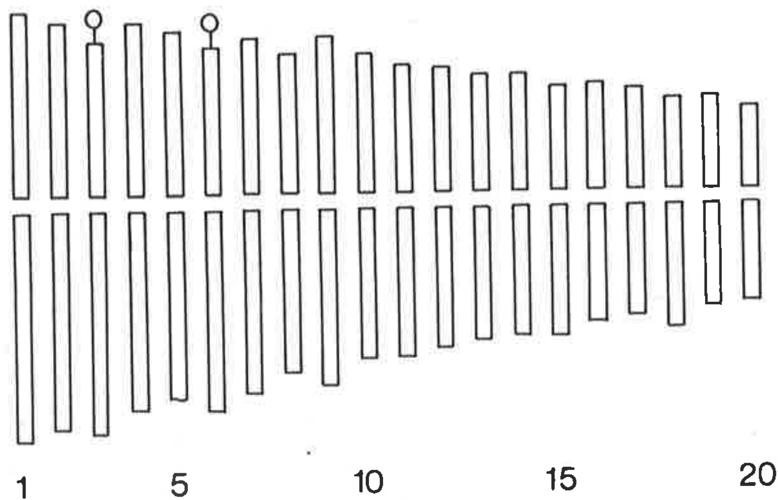
22. *S. gunnii* N = 20 4C DNA = 14.04 pg.



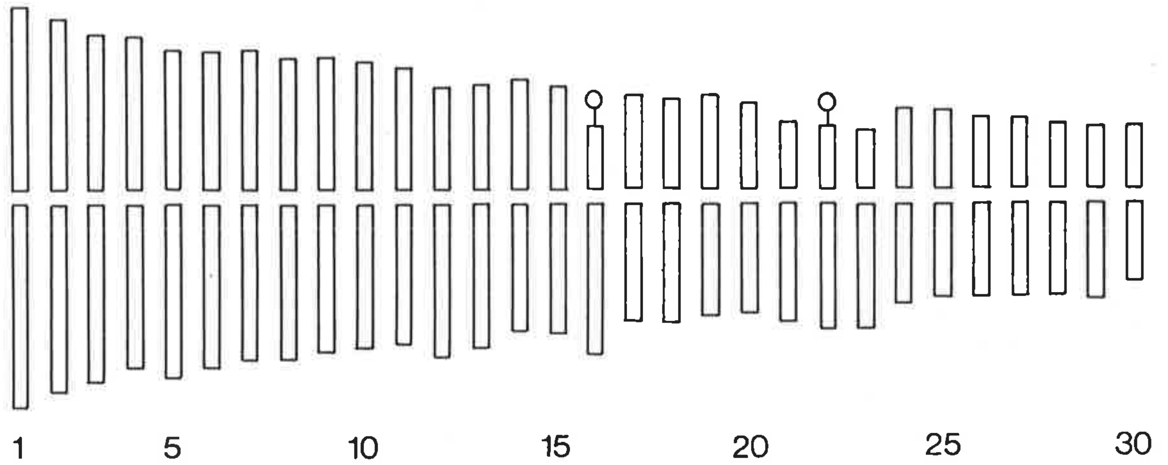
23. *S.* aff. *apargiaefolius* N = 20 4C DNA = 14.10 pg.



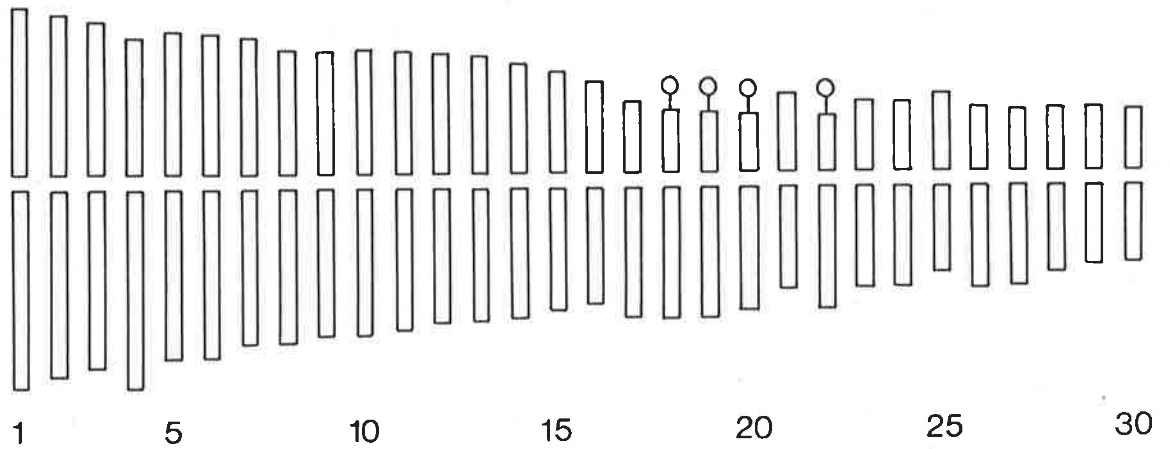
24. *S. runcinifolius* N = 20 4C DNA = 16.15 pg.



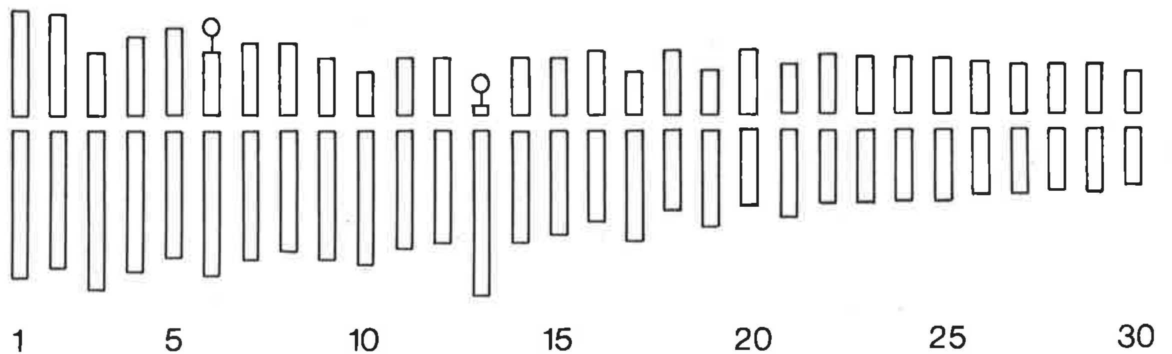
25. S. sp. B N = 30 4C DNA = 19.94 pg.



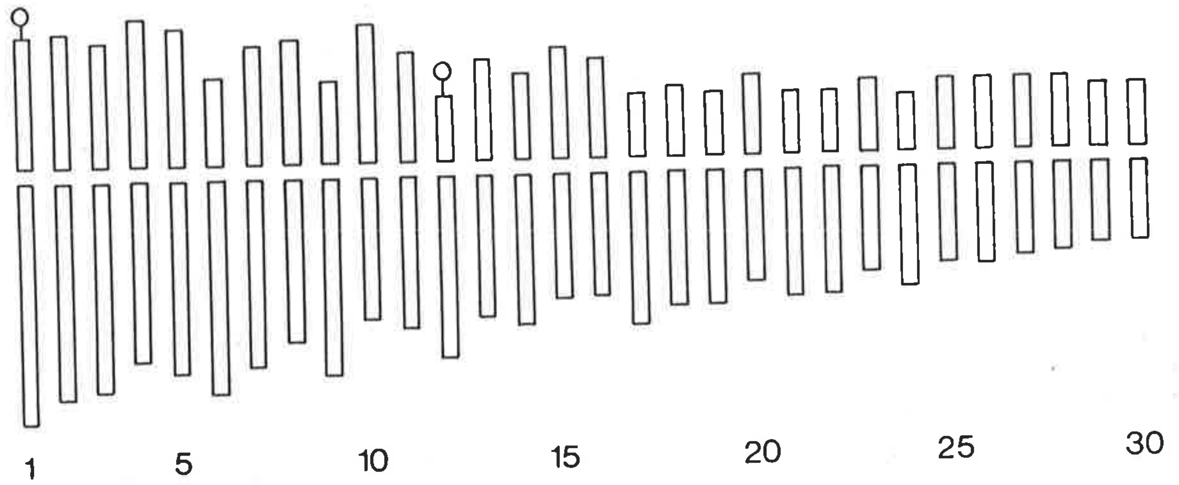
26. S. *squarrosus* N = 30 4C DNA = 19.81 pg.



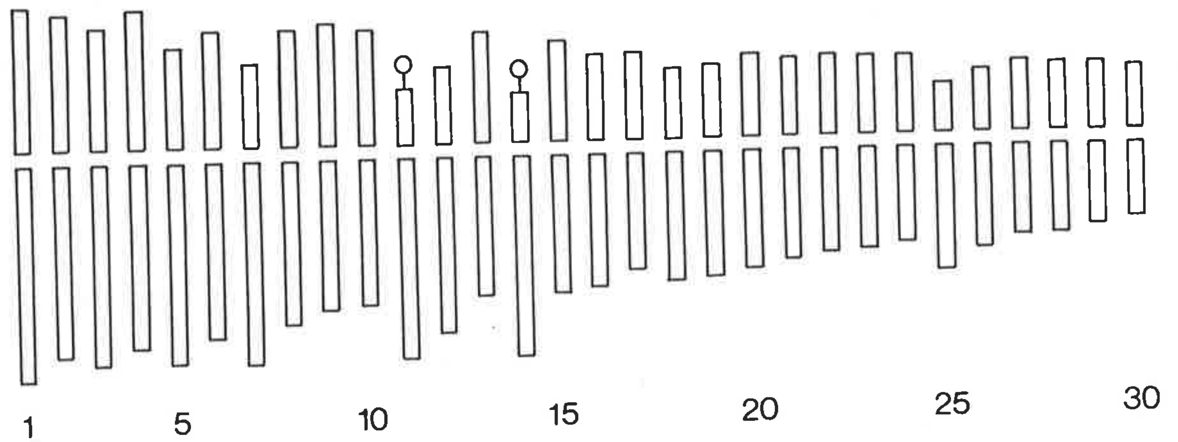
27. S. *bipinnatisectus* N = 30 4C DNA = 13.98 pg.



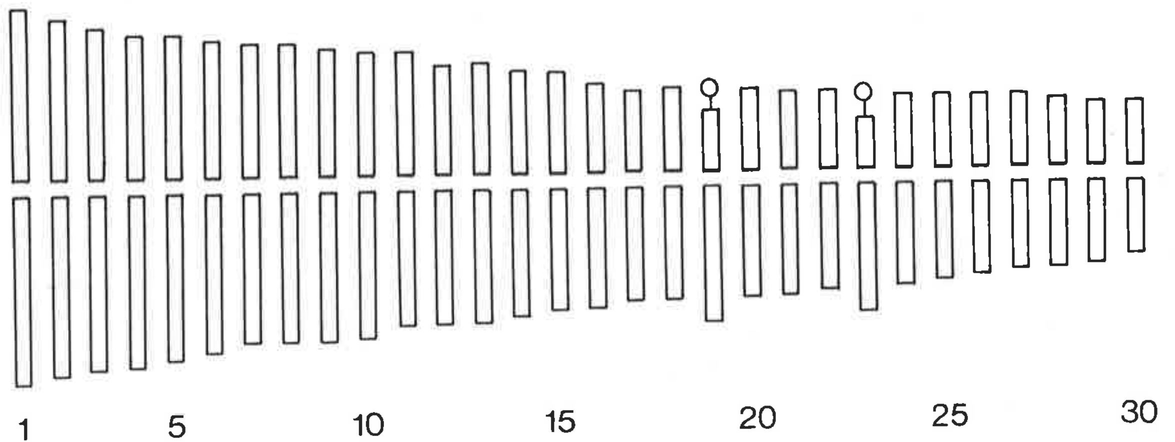
28. S. minimus N = 30 4C DNA = 19.82 pg.



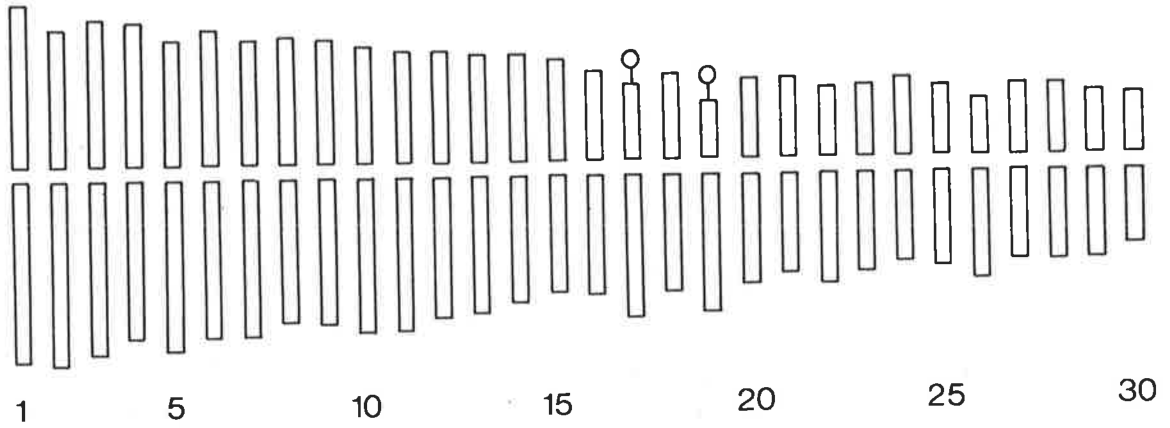
29. S. picridioides N = 30 4C DNA = 19.68 pg.



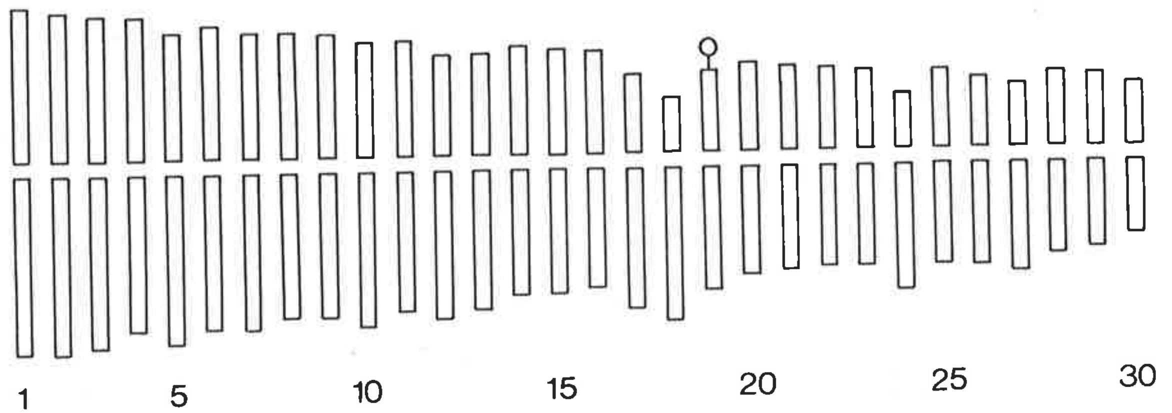
30. S. glomeratus N = 30 4C DNA = 19.18 pg.



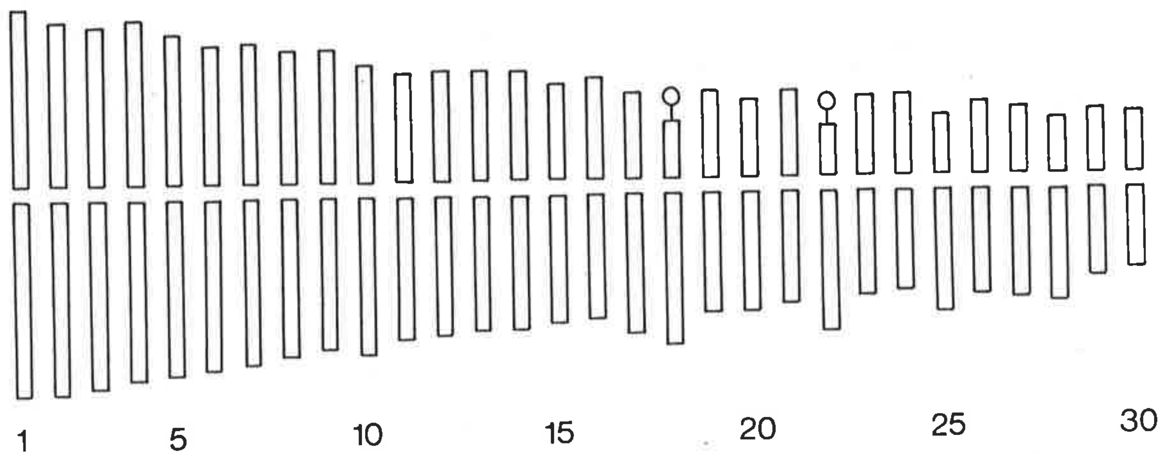
31. *S. hispidulus* var. *hispidulus* N = 30 4C DNA = 19.11 pg.



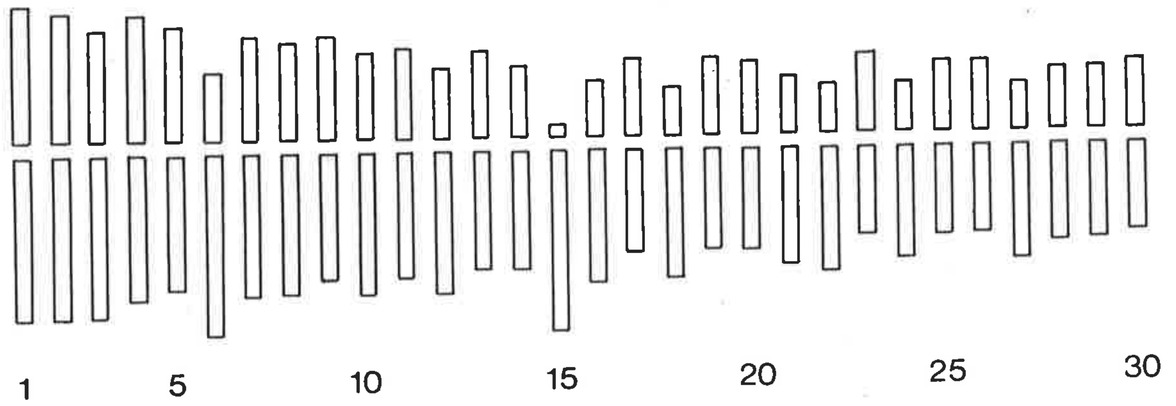
32. *S. hispidulus* var. *dissectus* N = 30 4C DNA - 19.41 pg.

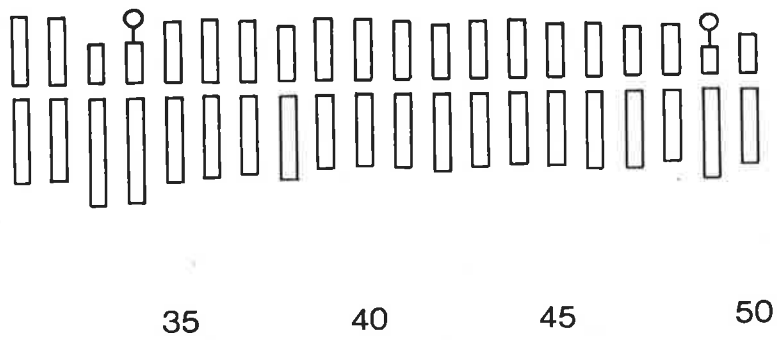


33. *S.* sp. C N = 30 4C DNA = 20.12 pg.



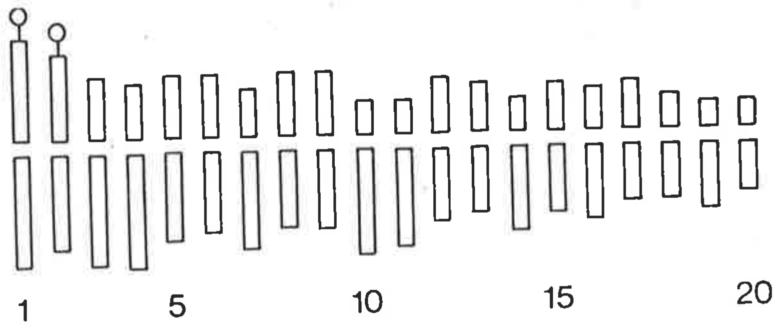
34. S. biserratus N = 50 4C DNA = 25.27 pg.





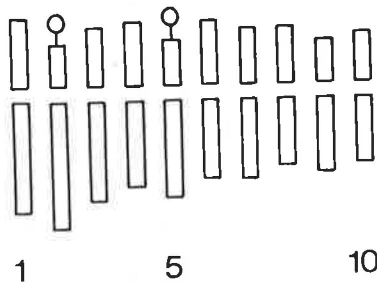
35. *S. vulgaris* N = 20

4C DNA = 7.82 pg.



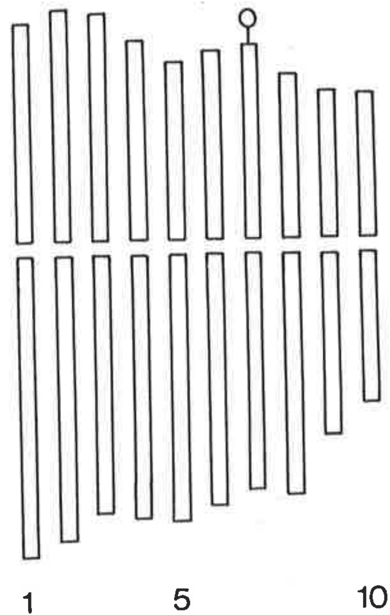
36. *S. pterophorus* N = 10

4C DNA = 4.22 pg.



37. *S. mikanioides* N = 10

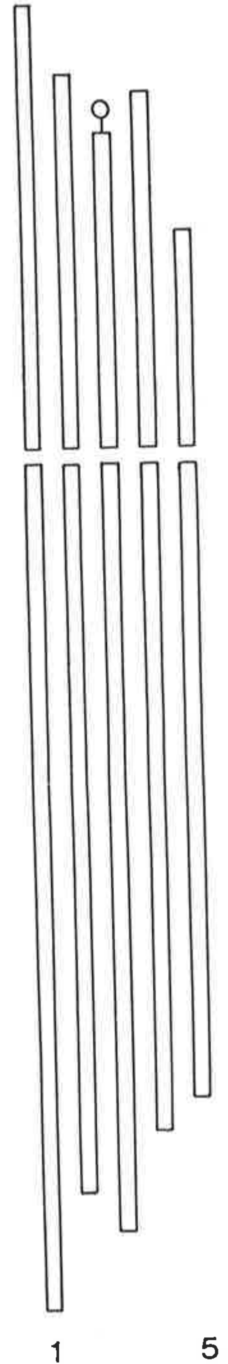
4C DNA = 11.78 pg.



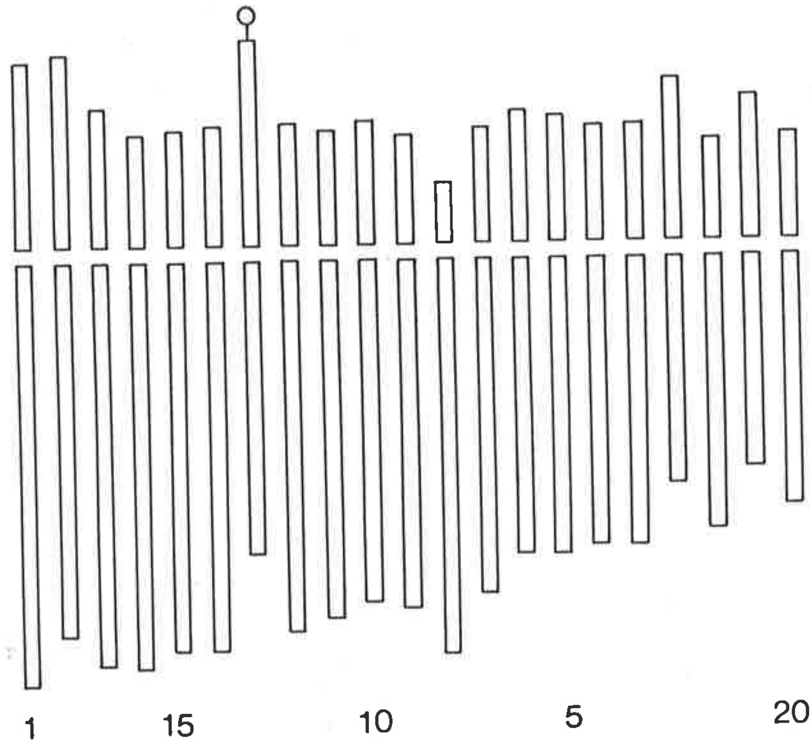
38. *S. discifolius*

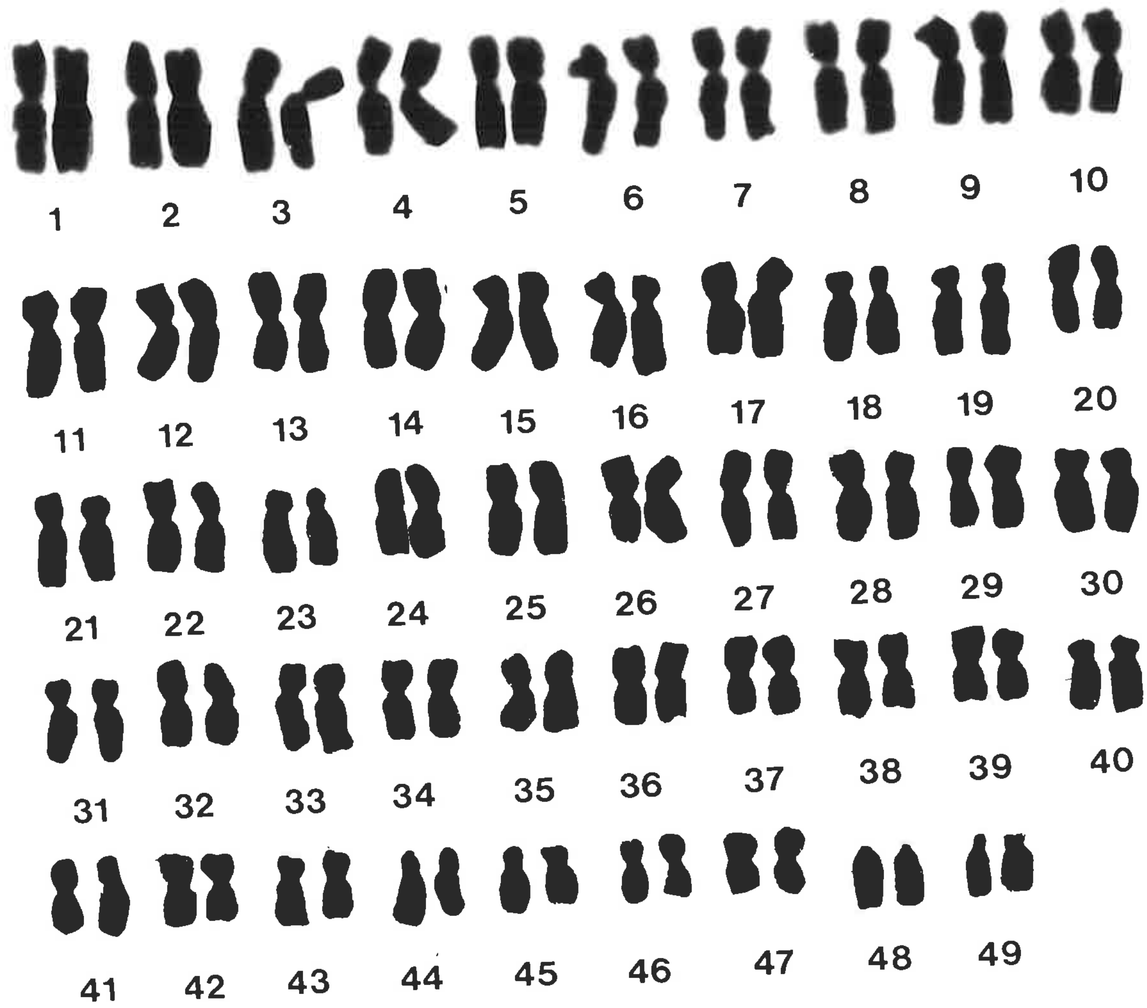
N = 5

4C DNA = 14.27 pg.



39. <u>Erechtites valerianaefolia</u> N = 20 4C DNA = 25.02 pg.





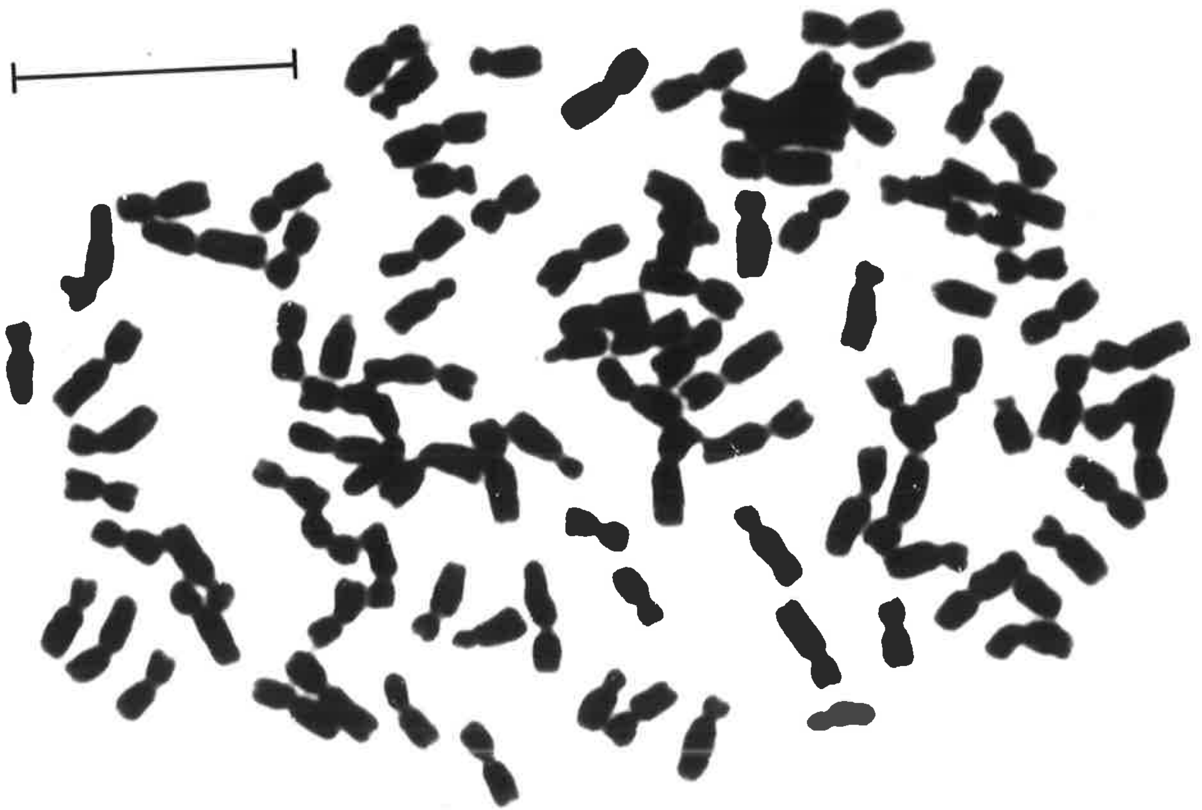
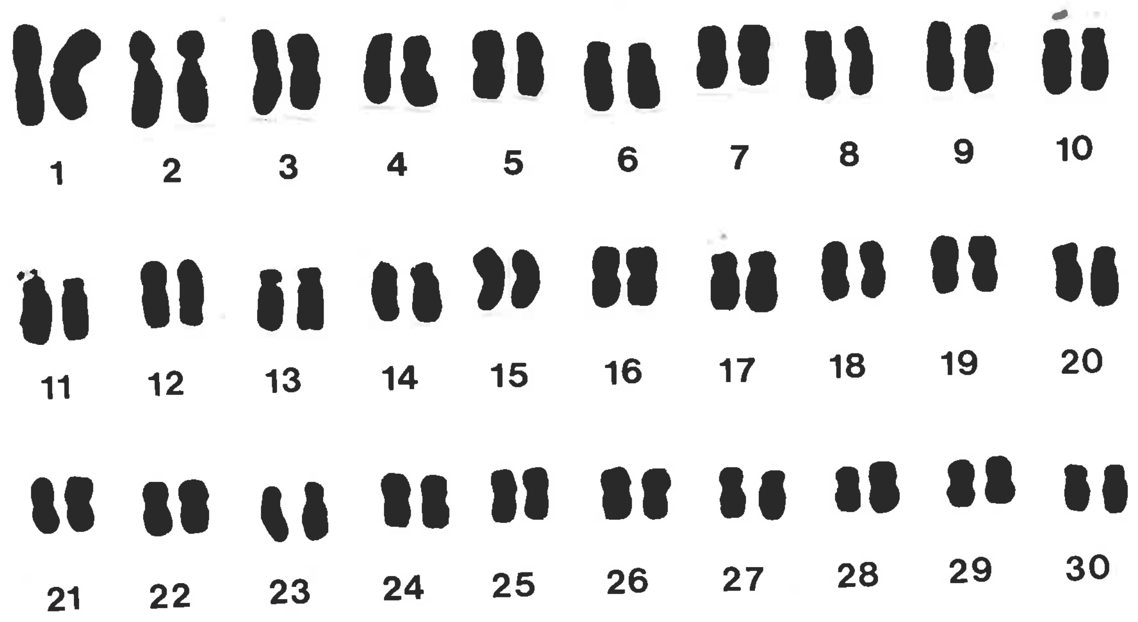


Fig. 7.1-40 <u>Senecio</u> <u>vagus</u> subsp. <u>eglandulosus</u>, N=49, 4C DNA = 42.90 pg.

Scale 10 μm.



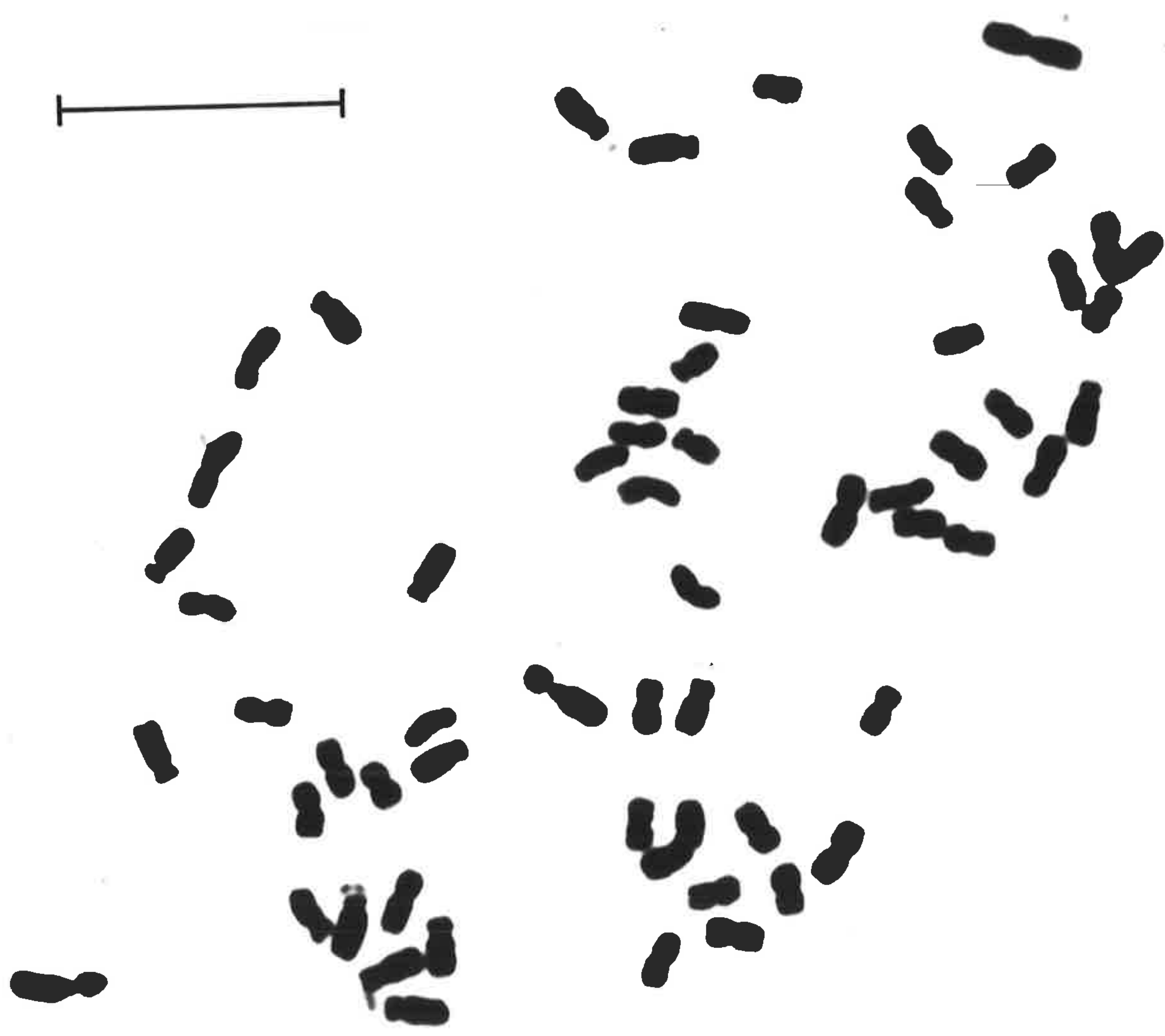


Fig. 7.1-41 Senecio sp. A, N=30, 4C DNA = 18.03 pg. Scale 10 μm.

1 2 3 4 5 6 7 8 9 10

11 12 13 14 15 16 17 18 19

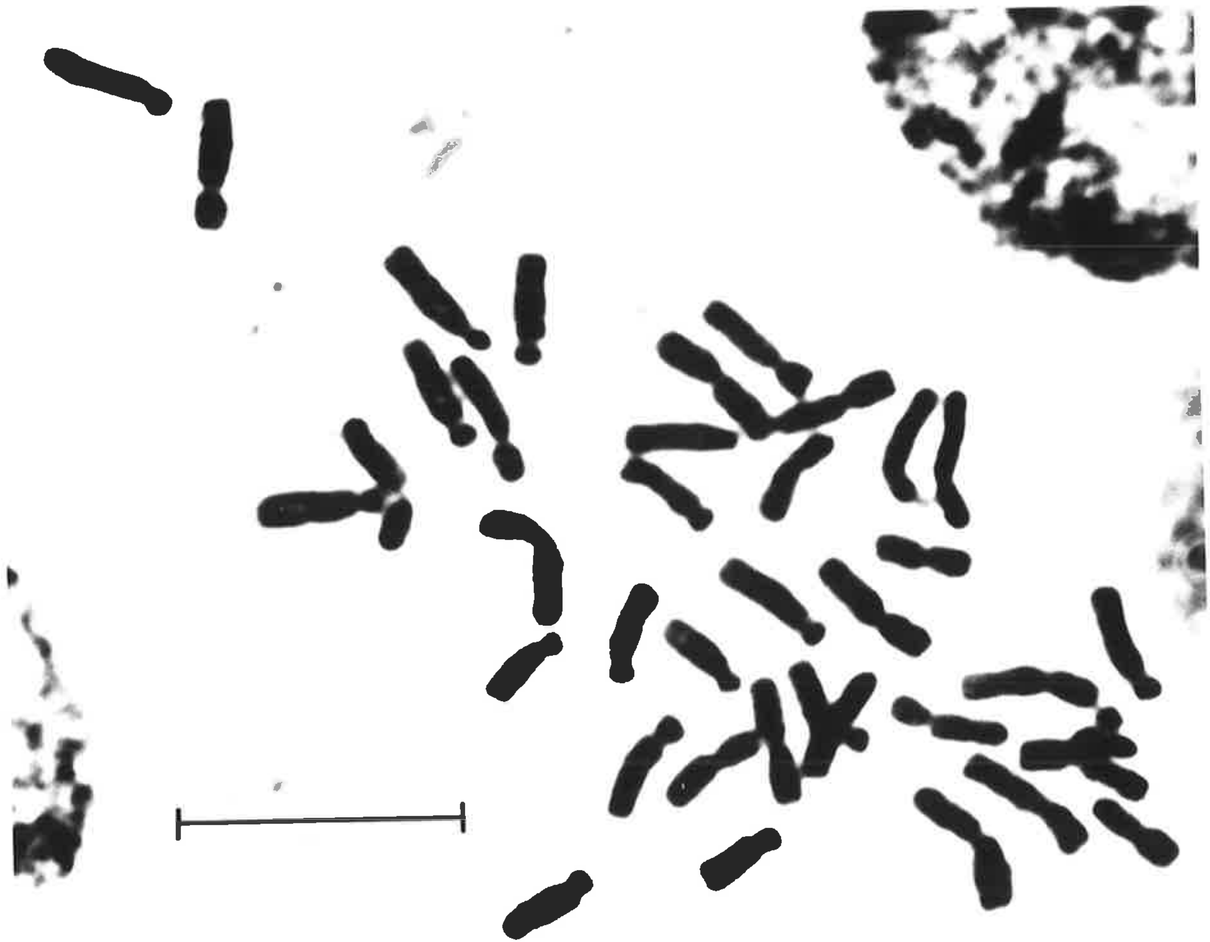
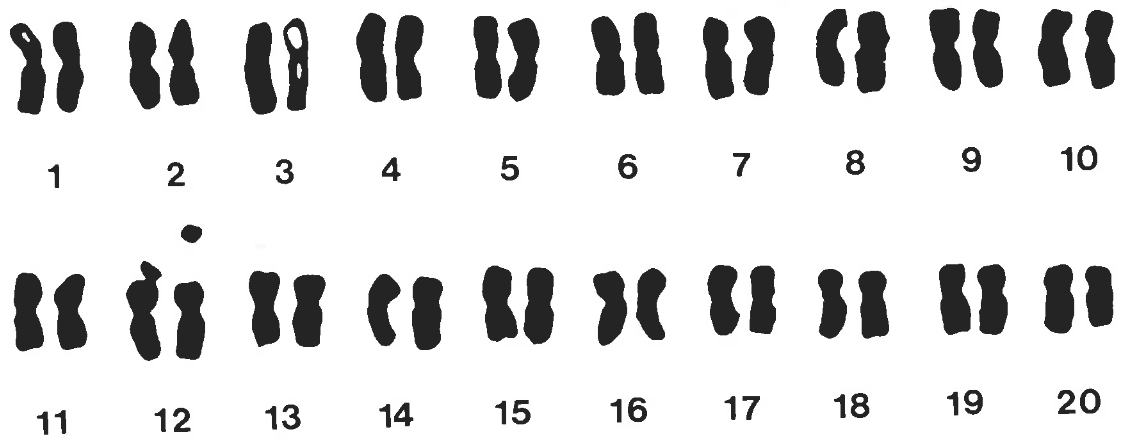


Fig. 7.1-42 *Senecio velleioides*, N=19, 4C DNA = 26.9 pg. Scale 10 μm.



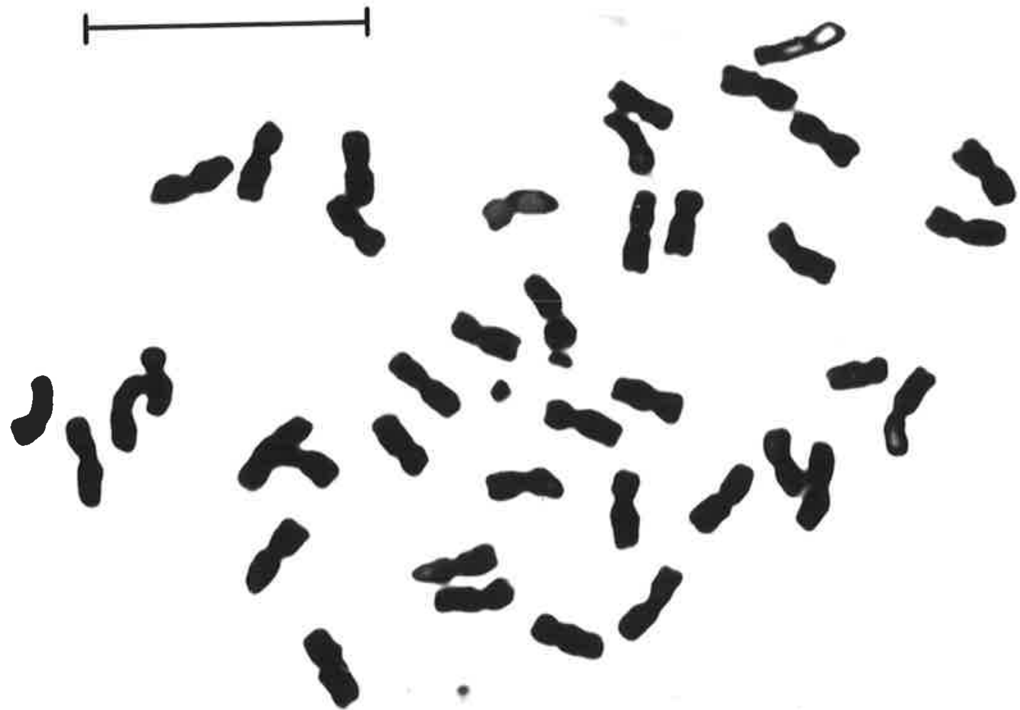


Fig. 7.1-43 Senecio lautus subsp. dissectifolius, N=20, 4C DNA = 10.63 pg.

Scale 10 μm.

As the main purpose of constructing karyotypes for Senecio was to determine similarities between species, the lack of grouping of chromosomes should not be a serious problem.

7.3.2 Comparison of Karyotypes

7.3.2.1 Size of significant differences.

Standard errors were calculated for the mean length of each shromosome arm (see part 7.2.1). As all standard errors were between 1.5 and 3% of respective arm lengths, standard errors are not shown in Appendix 1. In karyotype comparisons, two chromosomes were said to match if the short and long chromosome arms were both equal ($P > .05$) using a Students t test. The size of standard errors meant that any two chromosome arms differing in length by more than 4% to 9% were significantly different ($P > .05$).

7.3.2.2 Interpretation of data.

A complete listing of total percentage similarities (TPS), unique percentage similarities (UPS) and duplicated genome values for each species pair are given in Appendix 2. Derivations of TPS, UPS and duplicated genomes are described in part 7.2.2. The above-mentioned data are also summarized in Figures 7.2 to 7.4 as shaded representatives - darker squares indicating higher values. Only native species with at least one TPS value greater then 100% (half of the range) are shown in Figures 7.2 and 7.3. Excluded are the exotic species *S. pterophorus*, *S. vulgaris*, *S. mikanioides* and *S. discifolius*; the native species *S. macranthus* *S. magnificus*, *S. velleioides* and *S. amygdalifolius* with large chromosomes, and *S. glossanthus* with very small chromosomes. For reference, TPS and UPS values are also shown in the form of phenograms (Figures 7.5 and 7.6). However, shaded representative diagrams will be referred to in the following discussion as their

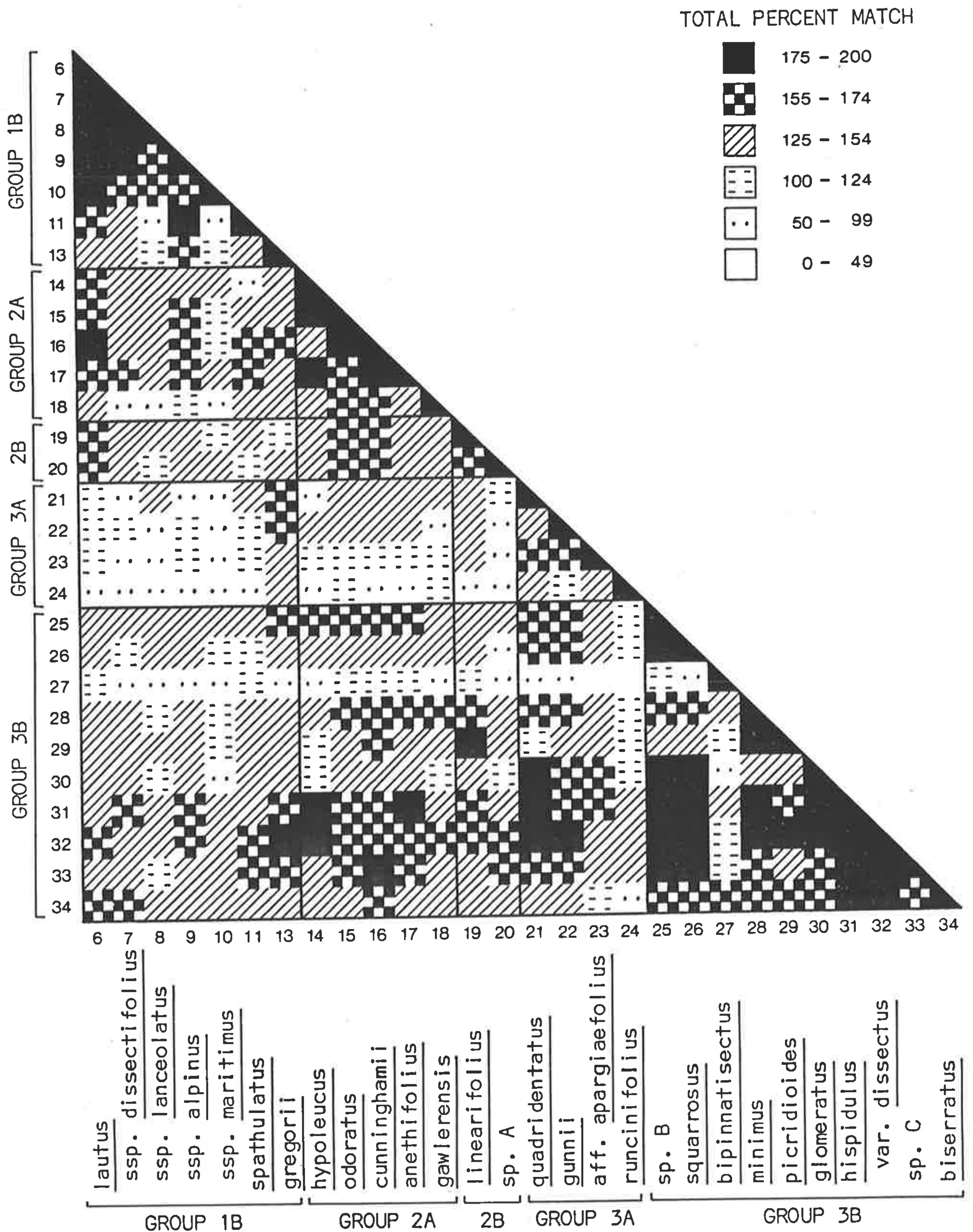


Fig. 7.2 Shaded representative of Total Percent Match (TPS) values of species of Senecio with some Unique Percent Match values greater than 100.

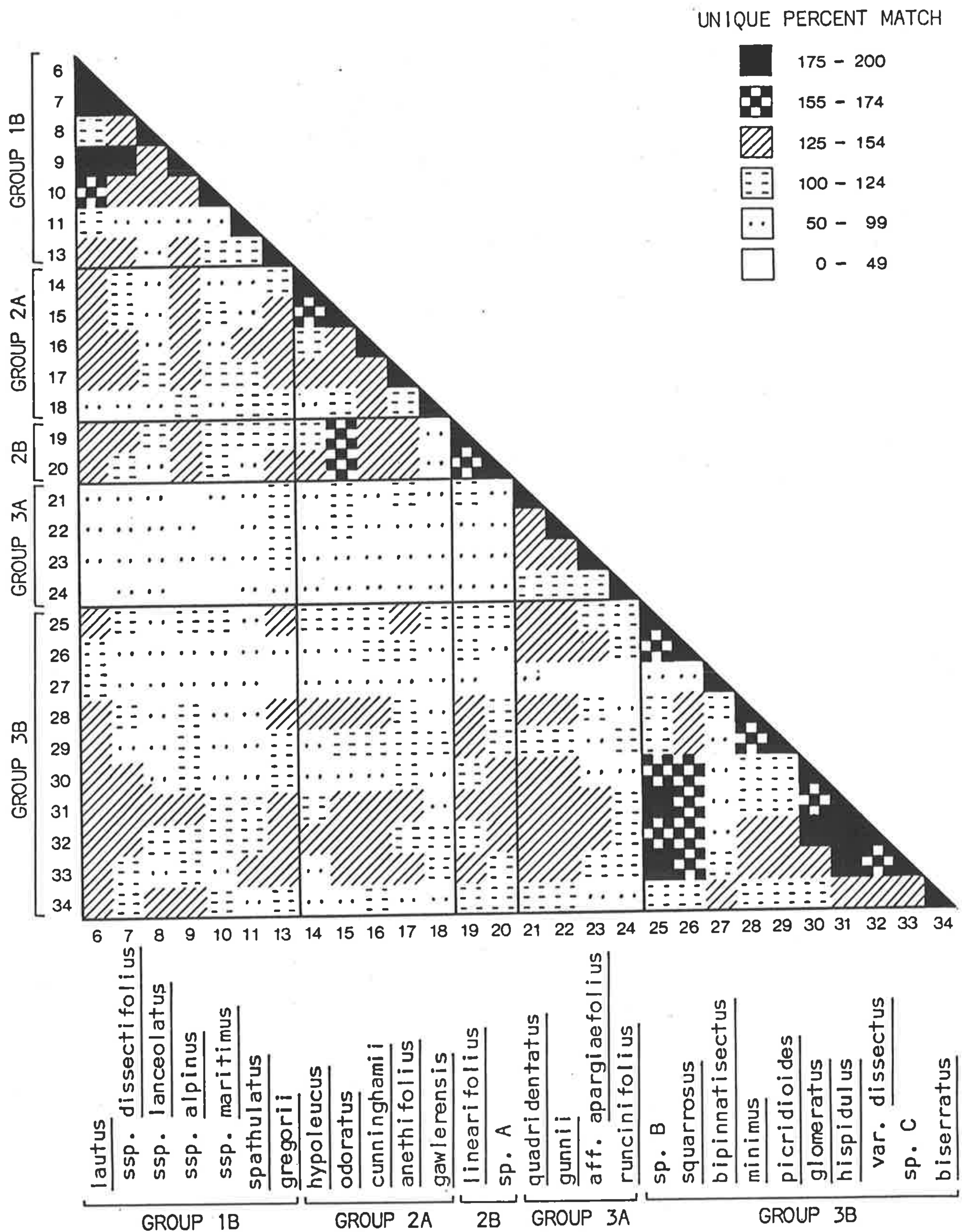


Fig. 7.3 Shaded representative of Unique Percent Match (UPS) values of species of Senecio with some UPS values greater than 100.

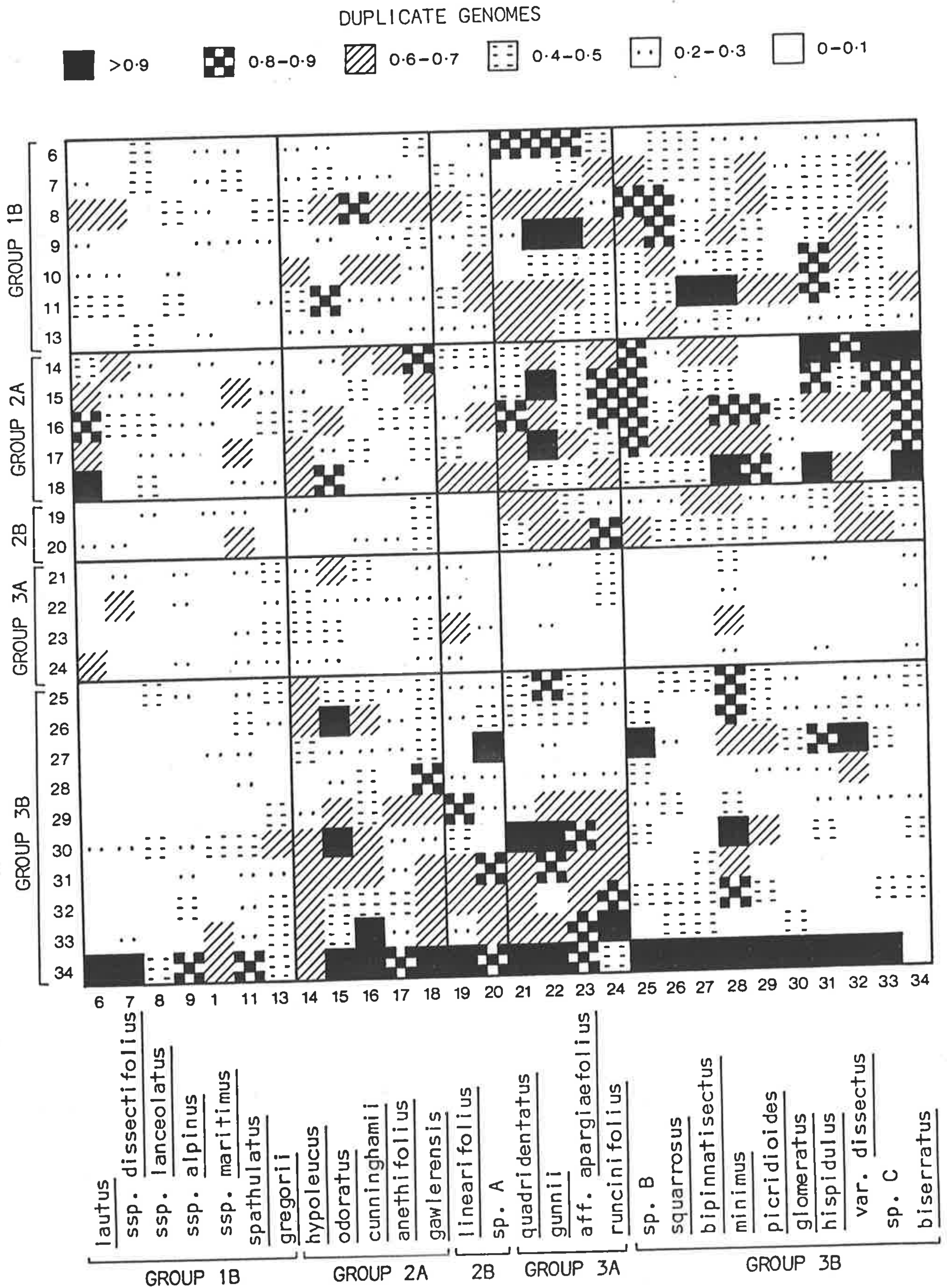


Fig. 7.4 Shaded representative of Duplicate Genome values of species of Senecio with some Unique Percent Match values greater than 100.

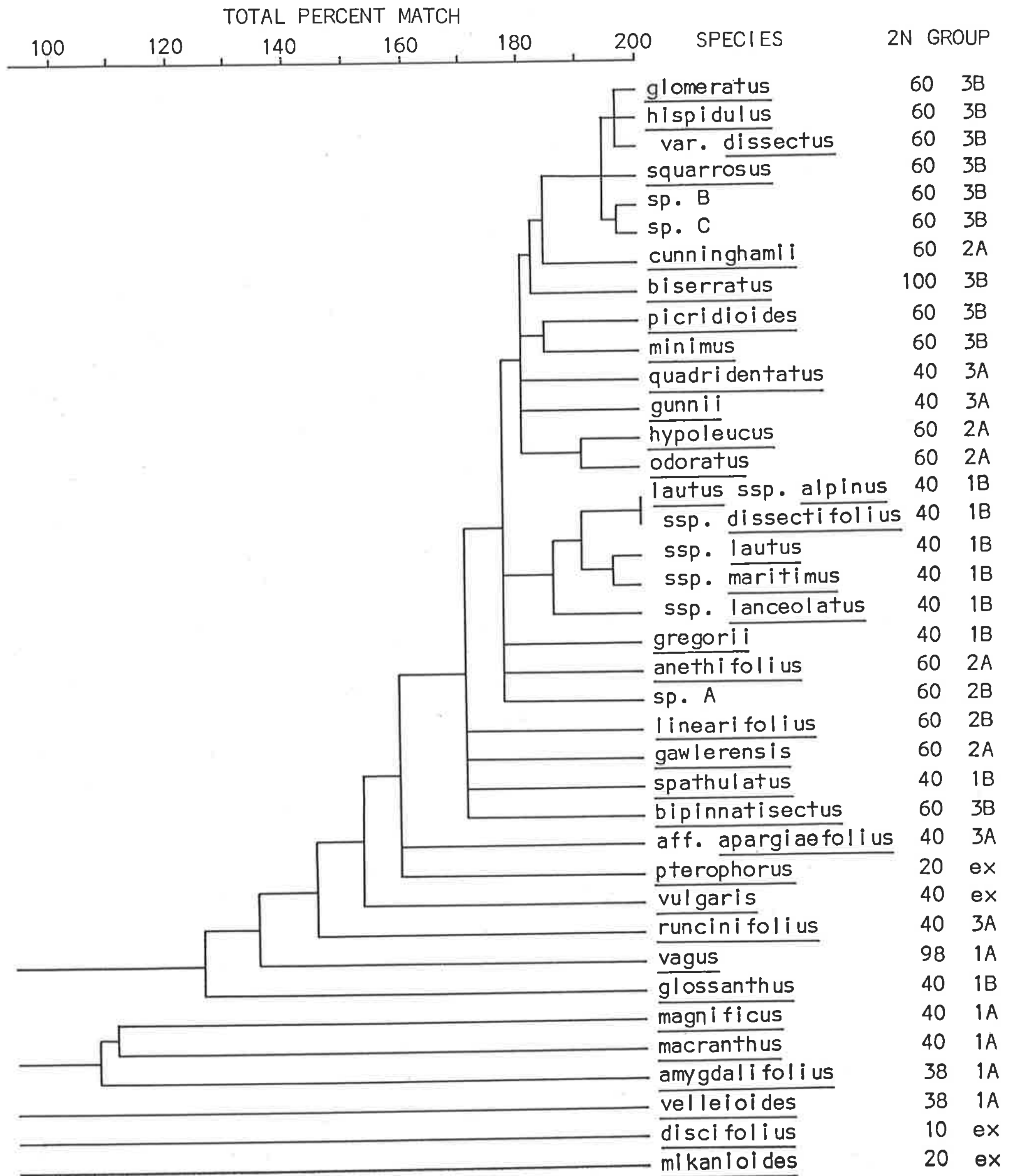


Fig. 7.5 Phenogram of 33 Senecio species based on Total Percent match (TPS) values of karyotypes. Diploid chromosome numbers and morphological groups are given in right-hand columns (ex = exotic).

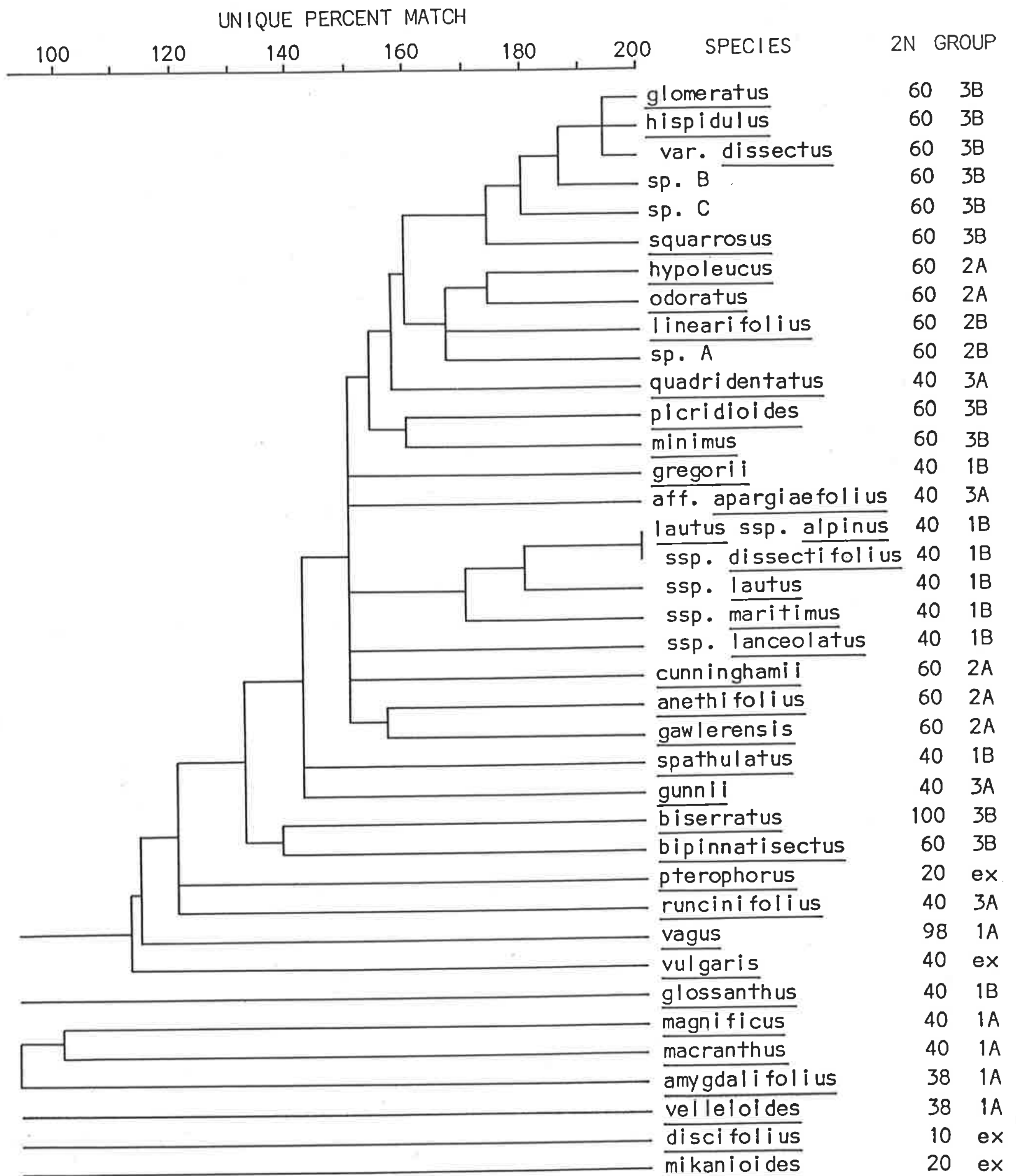


Fig. 7.6 Phenogram of 33 *Senecio* species based on Unique Percent Match (UPS) values of karyotypes. Diploid chromosome numbers and morphological groups are given in right-hand columns (ex = exotic).

information content is much higher.

Numbers along the axes of Figures 7.2 and 7.3 correspond with karyotypes illustrated in Figure 7.1. Horizontal and vertical lines within Figures 7.2 and 7.3 delimit boundaries between morphological groups and subgroups discussed in Chapter 3.6. The five large triangular shapes along the diagonal margins therefore contain within-subgroup comparisons whereas the rectangular shapes in the remainder of the figures contain between-subgroup comparisons. Duplicate genomes shown in Figure 7.4 should only be interpreted by rows. For example, row 34 contains the duplicates in species 34 when compared to all other species. Column 34 contains the duplicates in all other species when compared with species 34. The duplicates referred to are duplicate chromosomes of the uniquely matching set of chromosomes in any comparison.

A general comparison of Figures 7.2 and 7.3 indicates that the overall distribution of values is similar, but that TPS values are generally larger than their corresponding UPS value. As TPS values represent the UPS values plus any duplicates of either species A or B, Figures 7.2 and 7.3 indicate that the majority of *Senecio* species contain duplicates of their unique matches. Such a result is not unexpected as all Australian species are polyploids.

7.3.2.3 Relationships deduced from percentage similarities.

The greatest proportion of very high TPS values (solid black squares in Figure 7.2) occurs within groups 1B and 3B. Furthermore, the high TPS values within these groups are largely unique matches as the same pattern is evident in the shaded representative of UPS values (Figure 7.3). If chromosome similarity is indicative of the relationship between taxa then taxa 6 to 10 in

Group 1B and taxa 25, 26, and 30 to 34 in Group 3B form two associations of closely related taxa. Karyotypes 6 to 10 in Group 1B are the five subspecies of *Senecio lautus*. The high karyotype similarity is therefore in agreement with very similar morphology. The same is true of the closely related karyotypes 30 to 32 of Group 3B. These correspond to *S. glomeratus*, *S. hispidulus* var. *hispidulus* and *S. hispidulus* var. *dissectus*, which overlap in some of their morphological characteristics. Karyotypes of *S.* sp. A, *S. squarrosus*, *S.* sp. C and *S. biserratus* are also closely related to each other and to the above-mentioned species of Group 3B, but differ considerably in external morphology *S. squarrosus*, for example, differs from all other erechthitoid species (Group 3) in having 16 or more involucral bracts and few capitula per inflorescence. Karyotype similarity need not therefore correspond exactly with morphological similarity. The three remaining species of Group 3B, *S. bipinnatisectus*, *S. minimus* and *S. picridioides* are not as closely related, but the highest TPS value of each is with another Group 3B species. Karyotype evidence therefore supports the maintenance of the morphologically based Group 3B. Figure 7.4 indicates that *S. biserratus* (34) and *S. bipinnatisectus* (27) both contain duplicates of their unique matches with other Group 3B species. As *S. biserratus* is a decaploid and other Group 3B species are hexaploid, duplicates are not unexpected. It is also apparent that most species of Group 3B contain duplicates of *S. minimus* (and to a lesser extent of *S. picridioides*). Using this evidence and the size of TPS values it is possible to predict genomic compositions as follows:

Group 3B (most species)	AABBBB
S. minimus, *S. picridioides*	AABBCC
S. bipinnatisectus	$A^1A^1B^1B^1B^1B^1$

Symbols have been chosen so as not to conflict with evidence from

other groups. Genomes represented by different letters are thought to share no more than two chromosomes whereas numbered genomes (e.g. A^1) share three or more chromosomes with genomes designated by the same letter. S. bipinnatisectus is shown as a modification of AABBBB. If A^1 has three chromosomes in common with A and 3 duplicates of the common chromosomes, and if a similar relationship exists between B^1 and B, then TPS, UPS and duplicate genome values similar to those actually observed are generated. Alternative compositions result in either too high a UPS value or too many duplicates. Similarly the genomic composition of S. minimus indicates that two genomes (A and B) match with most other karyotypes of Group 3B and that the latter contain one duplicate genome (B) of the unique match.

Possible genomic compositions of other subgroups can be determined with reference to Group 3B. A discussion of interrelationships between all species would be time consuming, and unnecessary in view of the aims of this section. However, treatment of groups rather than species means that some results do not correspond exactly with genomic compositions suggested for groups.

Considering Group 2A, Figure 7.4 indicates that most contain 0.8 to 1 or more duplicate genomes of unique matches with 3B and vice versa. Furthermore, comparisons of Group 2A with most karyotypes of Group 3B have UPS values between 110 and 140, suggesting that at least two genomes are common to both (see Table 7.2). If Group 2A is represented as AAAABB then karyotype comparisons with a duplicate of 1 genome in both Group 2A and Group 3B. As Group 3B species have their highest UPS value with Group 2A, a similar genomic composition was assigned. A notable difference between Group 2A and 2B is that the latter contains less duplicates of matches with Group 3B. However, alternative genomic compositions created greater problems than the one suggested.

Group 3A differs from all other subgroups in having very few or no duplicates of matches with other subgroups, whereas all other subgroups contain duplicates of 3A. The evidence suggests a general composition of AABB for Group 3A, so that Groups 2A and 3B both contain one duplicate genome of the unique match with 3A. However, the size of unique matches (Fig. 7.3) suggests that Group 3A has less in common with Group 2A than with Group 3B. Group 3A is therefore represented as A^2A^2BB.

There are some unique matches between Group 1B and all other groups, although those with Group 3A are fewer. Similarly Group 1B contains some duplicates of matches with all other groups, with a higher proportion of duplicates generally occurring in comparisons with Group 3A and 3B species. Genomes of Group 1B must therefore have a few chromosomes common to both the A and B genomes and some chromosomes that are duplicates of the common chromosomes. The suggested composition of Group 1B species is therefore $A^3A^3B^3B^3$ when the A^3 and B^3 genomes comprise 2 sets of similar chromosomes. Species of Group 1A were not shown in Figures 7.2 to 7.4 as their chromosomes are very large and match with few chromosomes of other subgroups. It is likely that the genomic composition of Group 1A species is quite different to that of groups already discussed.

A summary of the genomic compositions of groups other than Group 1A is shown below.

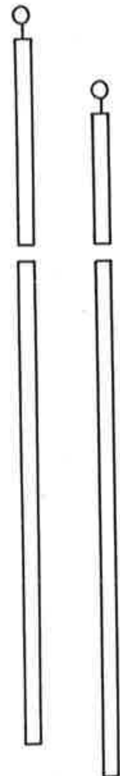
Group 3B (most species)	AABBBB
S. minimus, *S. picridioides*	AABBCC
S. bipinnatisectus	$A^1A^1B^1B^1B^1B^1$
Group 3A	A^2A^2BB
Group 2A and 2B	AAAABB
Group 1B	$A^3A^3B^3B^3$

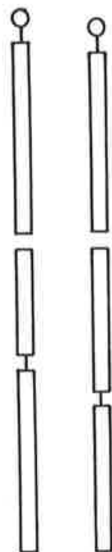
Genomes are shown as either A, B or C to indicate genome-sized groups of different chromosomes. Differences might have arisen by hybridization with an unknown species, or by extensive structural modification of part or all of an existing genome. The latter event is supported by the almost continuous range of TPS, UPS and duplicate genome values - distributions best explained by frequent structural rearrangements.

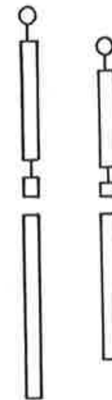
Although results of all species differ in some respects, the general similarity of karyotypes within groups originally defined by external morphology lends support to these groupings. The $A^3A^3B^3B^3$ genomes suggested for Group 1B are likely to represent two sets of largely similar chromosomes, as Group 1B karyotypes have low matches but comparatively high duplications when compared with species of other subgroups. If other genomes are modifications of the Group 1B "type" karyotype, then karyotype evidence supports a previously proposed phylogeny (Chapter 3.6) in which discoid (Group 2A and 2B) and erechthitoid species (Group 3A and 3B) were derived from a radiate ancestor similar to species of Group 1B. Karyotype similarity also suggests a comparatively close relationship between Groups 2A, 2B, 3A and 3B, but does not indicate their phylogenetic positions.

7.3.2.4 Satellite chromosomes.

Precise comparison of homologous chromosomes from different karyotypes of *Senecio* is complicated by comparatively high chromosome numbers and by differences in ploidy levels. However, it is possible to compare the shape and size of chromosomes distinguished by a secondary constriction and associated satellite of chromatin. Satellite chromosomes from each complement are illustrated separately in Figure 7.7. In the majority of cases, satellites were attached to the shorter chromosome arm and







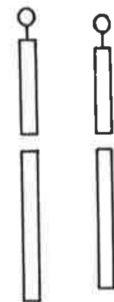
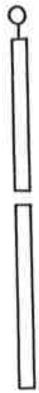


Fig. 7.7 Satellite chromosomes of Australian *Senecio* species. (All chromosomes drawn to same scale as in karyotypes - Fig.7.1).

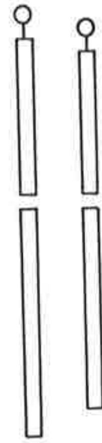
quadridentatus
N = 20

gunnii
N = 20

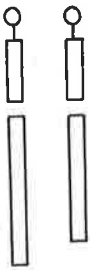


aff. apargiaefolius
N = 20

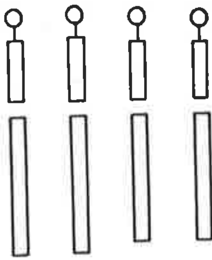
runcinifolius
N = 20



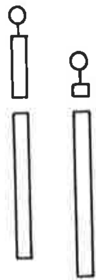
sp. B
N = 30



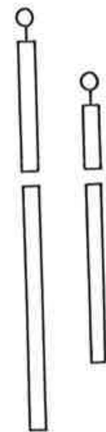
squarrosus
N = 30



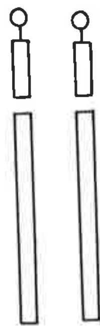
bipinnatisectus
N = 30



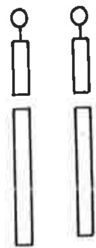
minimus
N = 30



picridioides
N = 30

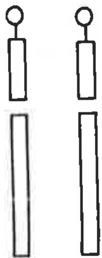


glomeratus
N = 30



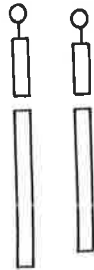
hispidulus var.

hispidulus
N = 30

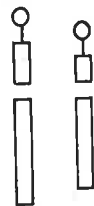


dissectus
N = 30

sp. C
N = 30

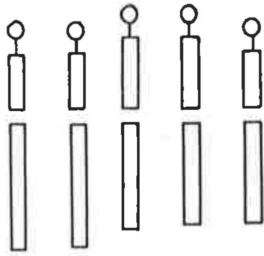


biserratus
N = 50

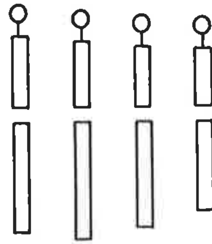


lautus subspecies N = 20

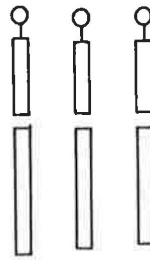
maritimus



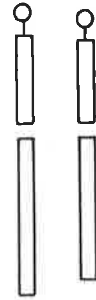
lanceolatus



dissectifolius



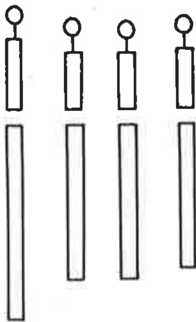
lautus



alpinus

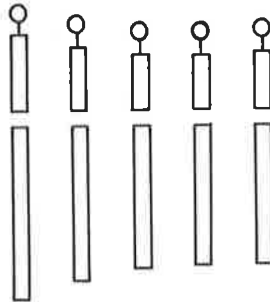
odoratus

N = 30



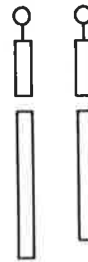
hypoleucus

N = 30



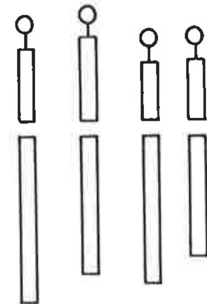
anethifolius

N = 30



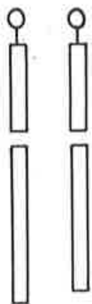
cunninghamii

N = 30



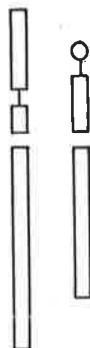
gawlerensis

N = 30



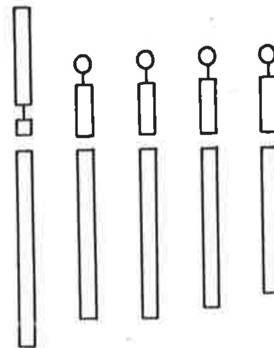
linearifolius

N = 30



sp. A

N = 30



appeared as minute globose portions of chromatin with a diameter smaller than the chromosome width. These satellites were not included in measurements of chromosome length and are shown in Figure 7.7 as small circles. In a few cases, secondary constrictions occurred closer to the centromere producing long and measurable satellites. These are drawn as rectangles in Figure 7.7. It should be noted that intraspecific variation in satellite numbers occurred in most species of Senecio examined. For example, satellite numbers in the eight measured complements of S. hypoleucus were 0, 6, 6, 8, 8, 9, 10 and 11. Because of possible position errors when aligning chromosomes it was therefore decided only to represent a satellite in the karyotype if two or more "homologous" chromosomes from different complements both had satellites. In the case of S. hypoleucus, five chromosomes are shown with satellites, but this need not imply that ten satellites were observed in each of the replicate complements.

Chromosomes with large satellites occur only in S. macranthus, S. magnificus (both Group 1A), S. gregorii (Group 1B), S. linearifolius and S. sp. A (both Group 2B). As these species are morphologically very diverse, one might postulate that the large satellites represent five independent instances of an inversion involving most of the short arm. However, the five species do have an outer row of ray florets in common. In Chapter 3.6.4 it was suggested (on the basis of external morphology and geographic distribution) that S. linearifolius and S. sp. A might be products of introgression between a radiate and a discoid species. The chromosome with a large satellite in karyotypes of S. linearifolius and S. sp. A might therefore have been derived from a radiate species. Significantly, other satellite chromosomes of these two species correspond most closely with satellite chromosomes of truly discoid species (Group 2B). Distributions

of arm ratios of satellite chromosomes from each morphological group are shown in Figure 7.8. S. linearifolius and S. sp. A form Group 2B, and distributions of arm ratios suggest affinities with both the radiate Group 1A and the discoid Group 2A. The satellite chromosomes of Group 2B therefore support the hypothesis that these species were derived by introgression between a radiate and a discoid species.

Figure 7.8 also indicates that discoid species of Group 2A have satellite chromosomes most similar to those of annual erechthitoid species of Group 3B - both having arm ratios between about 2 and 2.7. However, perennial erechthitoid species of Group 3A are more closely related to radiate species of Group 1B than to annual erechthitoid species. Unless similarities between satellite chromosomes are assumed to be coincidental, it is difficult to justify part of the phylogenetic scheme proposed in Chapter 3.6. A modified phylogeny shown in Figure 7.9 is in greater agreement with karyotype evidence (the modified scheme is also supported by chromosome numbers and by karyotype symmetry discussed in the next section).

If the original phylogeny (Fig. 7.9A) based only on external morphology is correct, then the similar achene morphology, chromosome number, satellite morphology and karyotype symmetry of erechthitoid annuals (Group 3B) and discoid perennials (Group 2A) must have evolved independently. In the modified phylogeny (Figure 7.8B), these affinities are explained by a common ancestry. Evidence from karyotypes therefore suggests that erechthitoid species evolved from two different self-incompatible ancestors - one a tetraploid and the other an hexaploid.

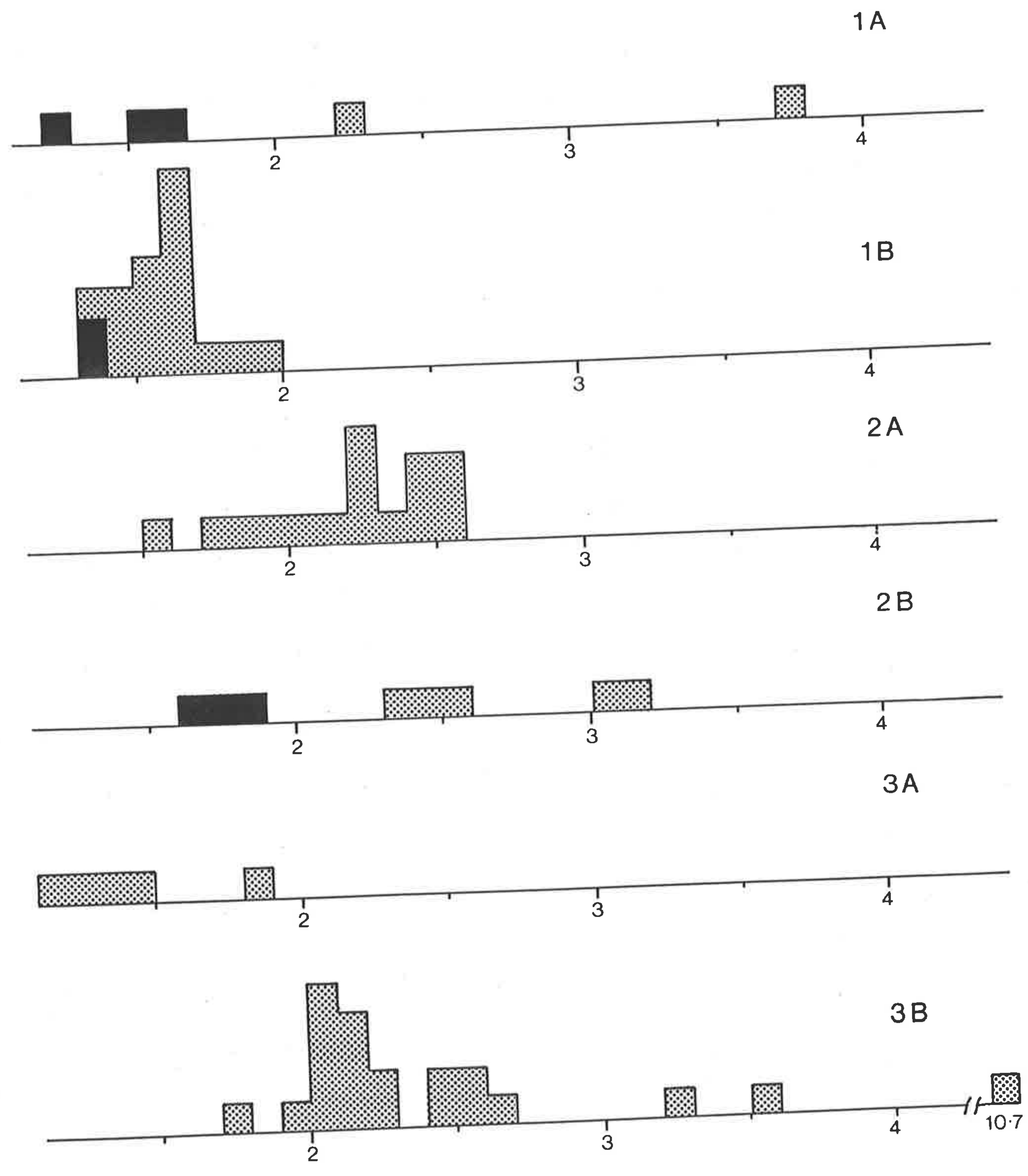


Fig. 7.8 Arm ratios of satellite chromosomes of Australian <u>Senecio</u> species from 6 morphological subgroups (groups indicated by 1A, 1B, etc; see text for explanation).

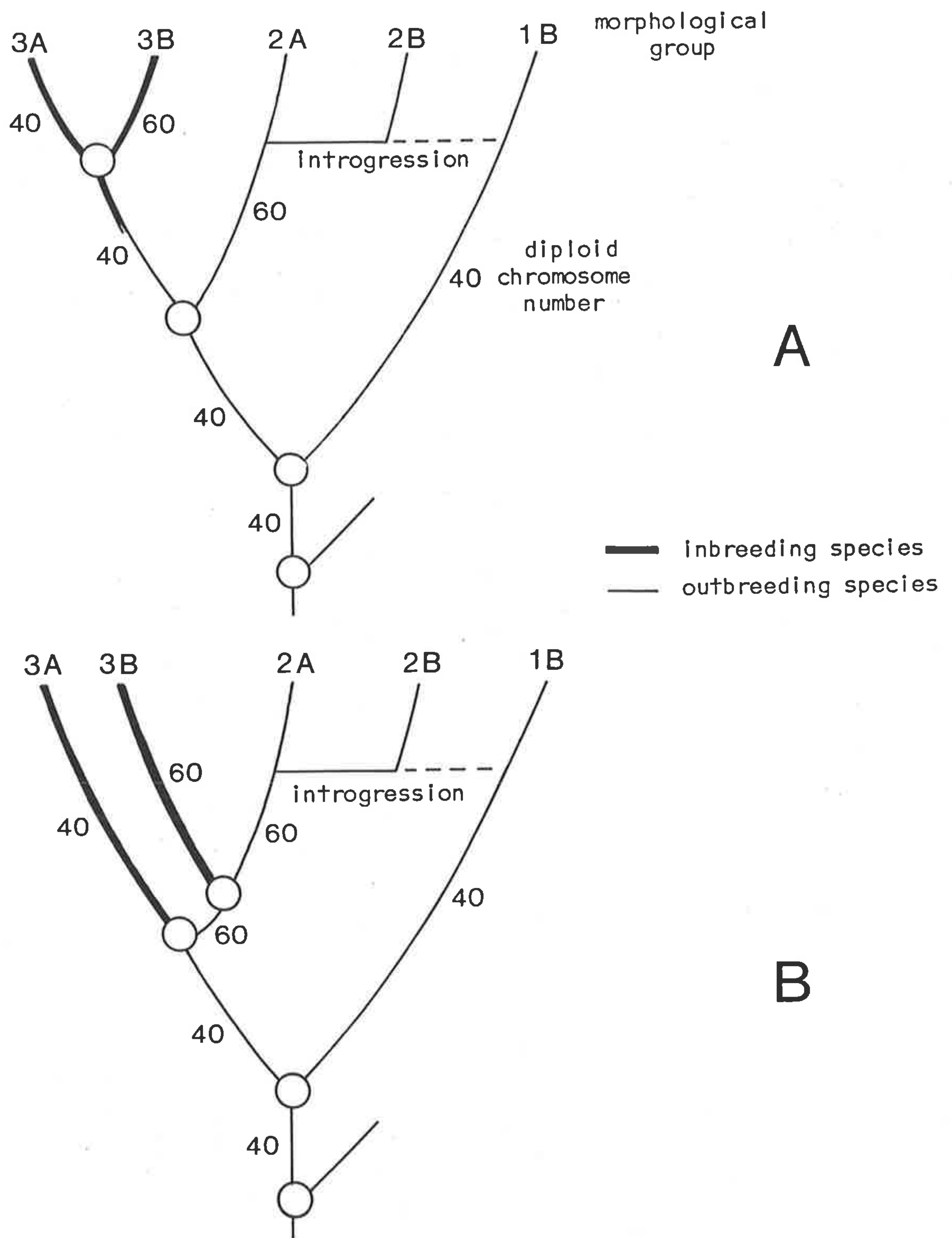


Fig. 7.9 A. Original phylogeny of Australian Senecio species (Chapter 3.6) based on external morphology. B. Modified phylogeny based on external morphology and karyotype evidence.

7.3.3 Karyotype Symmetry and Evolutionary Advancement

In a review of chromosome evolution in higher plants, Jones (1978) indicated the importance of the concept of primitive symmetry and advanced asymmetry of karyotypes, but also suggested that some investigators cling to the concept even when the phenotype argues in the opposite direction. Levitsky (1931a, b) was first to suggest the concept based on evidence from studies of the tribe Helleboreae. The trend, as evolution progressed in the Helleboreae, was from symmetrical karyotypes of largely metacentric chromosomes to asymmetrical ones with submetacentric and acrocentric chromosomes. Stebbins (1958, 1971) reported similar trends in other plant genera, and devised a classification scheme for karyotypes based on the proportion of chromosomes with an arm ratio greater than 2:1 and the ratio of the longest chromosome divided by the shortest (see Table 7.3). Parameters of relevance to karyotype symmetry in _Senecio_ are given in Table 7.2 along with the karyotype class according to the scheme presented by Stebbins. Stebbins (1958) suggested that in tribe Cichorieae, karyotype asymmetry may have evolved in response to selection for reduced recombination in species of unstable habitats. Stebbins also suggested that pericentric inversions occurring in metacentric chromosomes, followed by chromosome elimination, would restrict recombination and also increase karyotype asymmetry, so that in Cichorieae, evolutionary advancement is associated with a reduction in chromosome number and an increase in karyotype asymmetry.

Among species of _Senecio_, polyploidy rather than aneuploid reduction is the predominant evolutionary trend. The relationship between chromosome number and karyotype symmetry in _Senecio_ is shown in Table 7.4, and in contrast to Stebbins' (1958)

TABLE 7.2

Karyotype Features of 33 Species of Senecio

and of Erechtites valerianaefolia

(Typical varieties analysed unless otherwise specified.)

Species and Karyotype No.	4C DNA Amount (pg)	2N	Lengths mean	Lengths L/S	Arm Ratios mean	Arm Ratios %>2	Class*
GROUP 1A - Radiate species with continuous stigmatic surfaces							
1 S. magnificus	31.7	40	.792	2.07	1.41	0	1b
2 S. velleioides	33.1	38	.870	1.82	3.09	58	3a
3 S. amygdalifolius	26.9	38	.714	2.06	1.46	5	2b
4 S. macranthus	37.5	40	.937	1.96	1.59	10	2a
5 S. vagus subsp. eglandulosus	42.9	98	.439	2.17	1.89	24	2b
GROUP 1B - Radiate species with discrete stigmatic surfaces							
6 S. lautus ssp. lautus	10.8	40	.270	1.52	1.41	0	1a
7 ssp. dissectifolius	10.6	40	.266	1.56	1.34	0	1a
8 ssp. maritimus	10.2	40	.255	1.47	1.38	0	1a
9 ssp. alpinus	10.7	40	.269	1.41	1.36	0	1a
10 ssp. lanceolatus	9.8	40	.243	1.47	1.37	0	1a
11 S. spathulatus	12.3	40	.307	1.49	1.41	0	1a
12 S. glossanthus	6.7	40	.163	1.44	1.25	0	1a
13 S. gregorii	12.6	40	.309	1.78	1.25	0	1a
GROUP 2A - Discoid species without marginal ray florets							
14 S. hypoleucus	17.9	60	.295	1.73	1.55	23	2a
15 S. odoratus	18.1	60	.303	2.01	1.61	33	2b
16 S. cunninghamii	18.9	60	.315	2.17	1.48	10	2b
17 S. anethifolius	17.4	60	.289	2.02	1.53	17	2b
18 S. gawlerensis	20.4	60	.340	2.11	1.52	10	2b
GROUP 2B - 'Discoid' species with marginal ray florets							
19 S. linearifolius	18.0	60	.300	2.28	1.77	27	2b
20 S. sp. A	18.0	60	.304	2.05	1.72	33	2b

Table 7.2 - continued

Species and Karyotype No.	4C DNA Amount (pg)	2N	Lengths mean	L/S	Arm Ratios mean	%>2	Class*
GROUP 3A - Erechthitoid species, perennials with slender achenes							
21 *S. quadridentatus*	12.8	40	.319	2.15	1.21	0	1b
22 *S. gunnii*	14.0	40	.351	2.25	1.33	5	2b
23 *S.* aff. *apargiaefolius*	14.1	40	.353	2.14	1.31	0	1b
24 *S. runcinifolius*	16.2	40	.404	2.21	1.19	0	1b
BROUP 3B - Erechthitoid species, annuals with plump achenes							
25 *S.* sp. B	19.9	60	.333	2.56	1.30	3	2b
26 *S. squarrosus*	19.8	60	.330	2.48	1.35	10	2b
27 *S. bipinnatisectus*	14.0	60	.233	2.31	1.90	20	2b
28 *S. minimus*	19.8	60	.331	2.52	1.55	13	2b
29 *S. picridioides*	19.7	60	.328	2.45	1.58	17	2b
30 *S. glomeratus*	19.2	60	.320	2.49	1.27	7	2b
31 *S. hispidulus* var. *hispidulus*	19.1	60	.319	2.36	1.32	7	2b
32 var. *dissectus*	19.4	60	.324	2.25	1.31	7	2b
33 *S.* sp. C	20.1	60	.335	2.47	1.38	7	2b
34 *S. biserratus*	25.3	60	.253	2.42	1.72	20	2b
EXOTIC SPECIES							
35 *S. pterophorus*	4.2	20	.209	1.49	1.56	10	2a
36 *S. vulgaris*	7.8	40	.196	2.52	1.63	20	2b
37 *S. mikanioides*	11.8	20	.586	1.72	1.26	0	1a
38 *S. discifolius*	14.3	10	1.427	1.51	2.17	40	2a
OTHER GENERA							
39 *Erechtites valerianaefolia*	25.0	40	.628	1.67	2.62	80	3a

* Class according to Stebbins (1958), see Table 7.3.

TABLE 7.3

Distribution of Karyotypes of *Senecio* According to Their Degree of Asymmetry (Classifications in parentheses after Stebbins (1958)).

Ratio Longest ÷ Shortest	Proportion of Chromosomes with Arm Ratio >2:1			
	0.0	0.01-0.5	0.51-0.99	1.0
<2:1	(1a) 9	(2a) 4	(3a) 1	(4a)
2:1 - 4:1	(1b) 4	(2b) 20	(3b)	(4b)
>4:1	(1c)	(2c)	(3c)	(4c)

Table 7.4

Relationship Between Karyotype Symmetry and Chromosome Number in *Senecio*

2N	Type of Symmetry* 1a	2a	1b	2b	3a	Total
10		1				1
20	1	1				2
38				1	1	2
40	8	1	4	2		15
60		1		15		16
98				1		1
100				1		1
Total	9	4	4	20	1	38

*as defined in Table 7.3

findings, karyotypes of species with higher chromosome numbers are generally more asymmetrical than those with lower numbers. Two factors may have contributed to such a trend. First of all, it is perhaps more likely that structural rearrangements would be tolerated at higher ploidy levels as each chromosome of the genome is represented several times. A second factor is that most of the species at higher ploidy levels are either hexaploid (2N=60) or decaploid (2N=100). If these ploidy levels were formed by events including hybridization, then asymmetry in the high polyploid would be increased if the parental karyotypes differed in absolute size.

To compare evolutionary advancement with karyotype asymmetry, the mean arm ratio of each karyotype was plotted against the ratio of the longest chromosome divided by the shortest chromosome (Fig. 7.10). On the basis of morphological evidence (Chapter 3) it was concluded that outcrossing radiate species with continuous stigmatic surfaces are most primitive, yet these species (Group 1A) have comparatively asymmetrical karyotypes (Fig. 7.10). However, species of Group 1A also have more DNA per genome than any other group the the asymmetry is apparently due to unequal increases in chromosome arm lengths (see following discussion). If the remaining groups are compared, then there is a general increase in karyotype asymmetry with evolutionary advancement. Radiate species of Group 1B are morphologically most primitive and also have the most symmetrical karyotypes. In terms of the modified phylogeny shown in Figure 7.9B, it would appear that the primitive symmetrical karyotype has been maintained in Group 1B and to a lesser extent in Group 3A, but that asymmetry has increased among discoid species of Group 2 and annual erechthitoid species of Group 3B. Although the increased asymmetry might be due to high ploidy levels rather than to

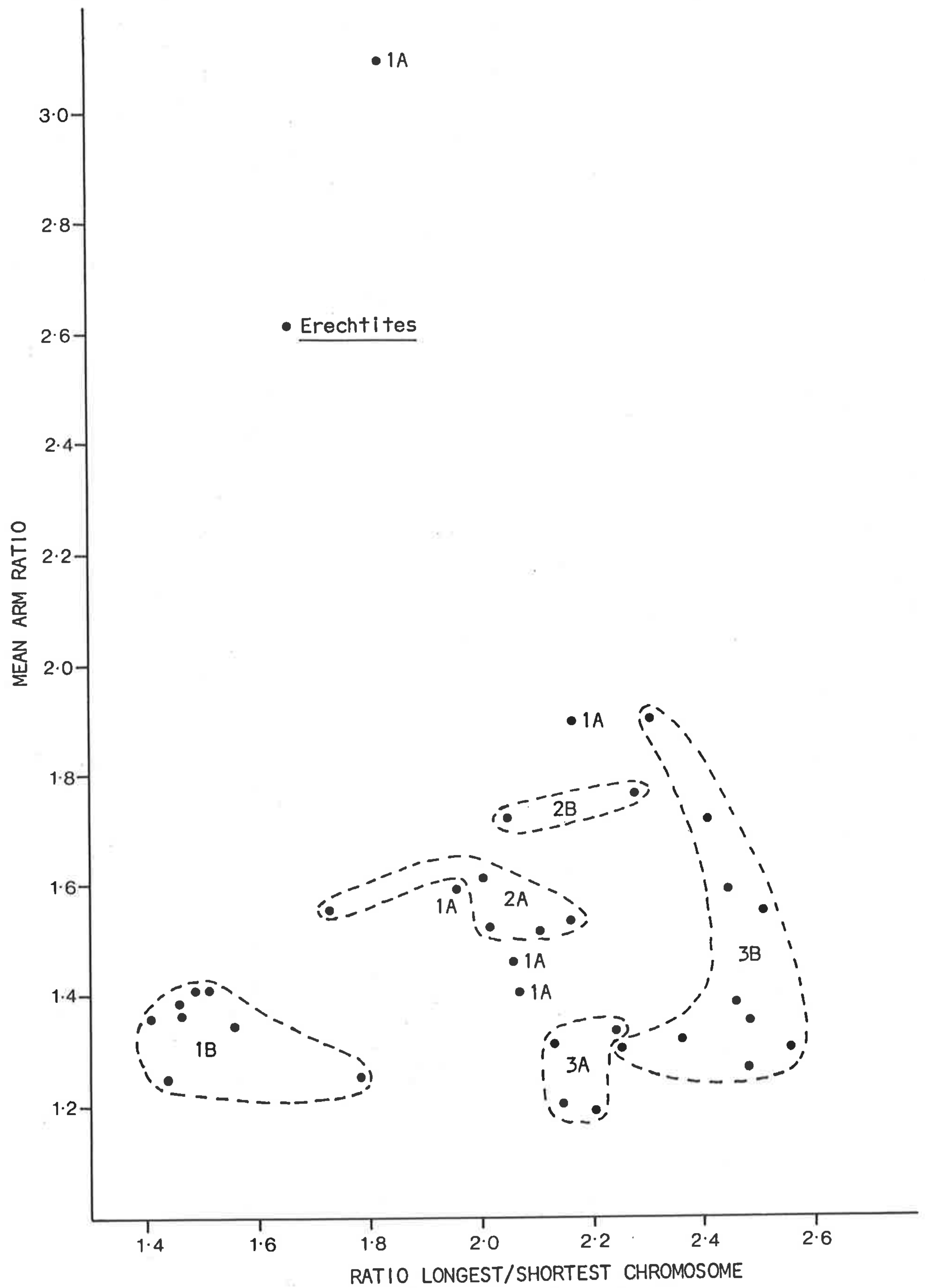


Fig. 7.10 Relationship between karyotype symmetry and groups based on external morphology (1A, 1B, 2A, etc. indicate morphological groups).

evolutionary advancement, the fact remains that the most symmetrical karyotype occurs in a comparatively primitive group.

7.3.4 Changes in Absolute Chromosome Size

In the previous chapter it was concluded that the ancestral species of *Senecio* probably had about 1.5 pg of DNA per genome of 10 chromosomes or about 0.15 pg per chromosome. In Table 7.2 the mean chromosome length of each taxon corresponds to the mean DNA amount per chromosome, but values are doubled as total chromosome length was equated with the 4C DNA amount per nucleus. The ancestral chromosome size is therefore represented by 0.30 pg. Among native species of *Senecio* the greatest departure from this amount occurs in species of Group 1A with chromosomes up to three times as large, and in *S. glossanthus* with chromosomes about half the ancestral or primitive size.

If the mean arm ratios of Group 1A species are compared with those of other groups (Table 7.3) then it is apparent that in the case of *S. magnificus*, *S. amygdalifolius* and *S. macranthus* there was little change in the mean arm ratio as chromosome size increased. However, the mean arm ratios of *S. vagus* and *S. velleioides* are higher than those other species of *Senecio*. In the latter case it would appear that proportionately more DNA has been added to the long chromosome arm than to the short chromosome arm. Alternatively, it could be suggested that pericentric inversions have occurred more frequently. However, in the case of *S. velleioides*, inversions must have been comparatively large and have occurred in at least 50% of the complement to obtain the observed difference in arm ratios. One might therefore expect to see some evidence of inversion heterozygosity during meiosis, but meiosis was quite normal in all populations of *S. velleioides*

and *S. vagus* examined. Although by no means conclusive, I believe the evidence favours unequal increases in the size of chromosome arms as the total DNA amount of *S. vagus* and *S. velleioides* increased.

S. glossanthus has chromosomes reduced to half the ancestral chromosome size, but the mean arm ratio of this species is comparable with that of related species (Group 1B) with much larger chromosomes. In the case of *S. glossanthus* DNA has apparently been lost from all chromosome arms in equal proportions. Another species with comparatively small chromosomes is *S. bipinnatisectus* (Group 3B). Although the mean arm ratio of *S. bipinnatisectus* is considerably higher than that of other species in the same group, the increase is due largely to the presence of one acrocentric chromosome with an arm ratio of 10.7:1. If this chromosome is omitted then the mean arm ratio of the remaining chromosomes becomes 1.59, a value comparable with *S. minimus* and *S. picridioides* of the same group. It is therefore likely that the karyotype of *S. bipinnatisectus* evolved by loss of DNA from all chromosome arms and by an event involving at least one major structural change.

Although there is a 5.7-fold difference in the mean chromosome size of native species of *Senecio*, evidence suggests that in most cases changes in chromosome size have affected all chromosome arms more or less equally so that karyotype symmetry is unchanged. Exceptions are *S. velleioides* and *S. vagus* in which the long chromosome arms have apparently increased to a greater extent than the short chromosome arms.

7.3.5 The Basic Chromosome Number of *Senecio*

Although it is generally agreed that the basic chromosome number of Senecioneae is x=10 (Solbrig 1977, Nordenstam 1977,

Lewis 1980), there is some disagreement as to the basic chromosome number of Senecio. Chromosome counts of over 400 species and varieties of Senecio have been reported in the literature, and of these, 0.7% have a haploid number of N=5, 9% have N=10 and 41% have N=20. Turner and Lewis (1965) suggested that the absence of species with N= 6, 7 and 8 supports a basic number of x=5 for Senecio. However, Ornduff et al. (1963, 1967) concluded that x=10 is basic as the three species with N=5 are annual - a feature common in taxa at the lower end of a reduction series - and "hexaploid" (N=15) and "decaploid" (N=25) species do not occur in the genus. Of the 63 genera of Senecioneae for which chromosome numbers have been determined (Nordenstam 1977), N=5 occurs only in Emilia and Senecio. Nordenstam (1977) suggested that N=5 evolved by aneuploid reduction in both cases, as Emilia also contains phylogenetically advanced annuals and reduction is evident in other derived annuals, namely Gymnodiscus (N=9) and Steriodiscus (N=10, 8). However, Olyrode (1973) has shown conclusively that a species of Emilia with N=10 is an allopolyploid of two species with N=5. Although x=10 is apparently the basic number of Senecioneae, N=10 is obviously derived in the case of Emilia. It would seem unlikely that specialized annual species of Senecio with N=5 could give rise to less specialized perennials with N=10 or more, but in view of the evidence from Emilia, the possibility cannot be completely discounted.

Three African species of Senecio with low chromosome numbers were included in this study to see if karyotypes could provide further evidence of the basic chromosome number. The lowest chromosome number among species of Senecio native to Australia is N=19, but the introduced weeds S. pterophorus and S.mikanioides both have N=10. None of the species with N=5 appear to occur outside of Africa, but it was possible to obtain seeds of

S. discifolius - one of three species with N=5.

7.3.5.1 Karyotype symmetry and absolute chromosome size.

Karyotypes of *S. pterophorus*, *S. mikanioides* and *S. discifolius* are shown in Fig. 7.1-35, -37 and -38. The most obvious difference is in absolute chromosome size. The mean chromosome size of *S. pterophorus* is 0.21 pg, of *S. mikanioides* is 0.59 pg and of *S. discifolius* is 1.43 pg. If *S. discifolius* evolved from an ancestor with N=10 by aneuploid reduction, then it might be expected that the chromosomes of *S. discifolius* would be at most twice the size of an ancestral chromosome - that is, 0.60 pg. However, the average chromosome size of *S. discifolius* is twice this amount. If aneuploidy is truly the mode of origin, then either *S. discifolius* was derived from a species with larger chromosomes, or the DNA amount of *S. discifolius* increased after the chromosome number was reduced. Alternatively, if N=5 is the basic chromosome number then the chromosome size of *S. discifolius* has increased along with the evolution of an annual habit. A similar trend was reported by Nagl and Ehrendorfer (1974) for some annual species of Anthemideae, although in these, the increase in nuclear DNA was accompanied by an increase in the proportion of heterochromatin.

General karyotype symmetry does not offer conclusive evidence. Karyotypes of the perennial *S. pterophorus* and *S. mikanioides* are both more symmetrical than the karyotype of *S. discifolius* - evidence that might suggest that *S. discifolius* was derived by aneuploid reductions. However, it could also be argued that the absolute chromosome size of *S. discifolius* increased by the addition of proportionately more DNA to the long chromosome arms - as appears to be the case for *S. velleioides* (see part 3.4 of this chapter).

7.3.5.2 The number of satellite chromosomes.

As each haploid chromosome complement normally has one satellite chromosome (Stebbins 1950, Jackson 1971), satellite number can be an indicator of ploidy level. However, McClintock (1934) demonstrated that fragmentation and interchange in *Zea mays* can lead to an increase in satellite number independently of polyploidy. A further difficulty is that at higher ploidy levels the number of observed satellites may in fact be reduced (Stebbins 1950).

The relationship between chromosome number and satellite number in *Senecio* is shown in Table 7.5. The number of satellite chromosomes among species with N=5 or 10 could support either x=5 or x=10 as the basic chromosome number (i.e. one satellite in the haploid set in each case). The majority of species with N=19 or 20 have either one or two satellite chromosomes, supporting x=10 as the basic number, but three (subspecies of *S. lautus*) do have higher numbers of satellites. Significantly, one subspecies has five satellite chromosomes, a higher than expected number for N=20 even if the basic chromosome number was x=5. Fragmentation and translocation must therefore have occurred in at least this case. Species with N=30 can again support either x=5 or x=10 as the basic number. Although a greater number of species do support x=10 as basic (with fragmentation accounting for higher satellite numbers) it is not possible to decide conclusively whether x-5 or x=10 is basic from the number of satellite chromosomes.

It is possible that examination of karyotypes of additional species of *Senecio* with N=5 and N=10 would provide more conclusive evidence, but if variation among the three species chosen is typical of the lower ploidy levels then the picture might become even more confusing. The most suggestive evidence

TABLE 7.5

Relationship Between Chromosome Number and Observed and Expected Satellite Number in Haploid Complements of Species and Varieties of *Senecio*

N	Expected satellite Number		Observed Satellite Number
	x=5	x=10	(number of taxa in parentheses)
5	1	1	1(1)
10	2	1	1(1), 2(1)
19, 20	4	2	1(7), 2(7), 3(1), 4(1), 5(1)
30	6	3	0(1), 2(10), 4(3), 5(2)
49, 50	10	5	0(1), 2(1)

is still the fact that all species with N=5 are specialized annuals whereas all primitive perennial herbs and shrubs have N=10 or higher. I therefore favour x=10 as the basic chromosome number of Senecio, and believe species with N=5 most probably evolved by aneuploid reduction.

7.4 Conclusions

As chromosome numbers of Australian species of Senecio are comparatively high (N=20 to N=50) karyotypes were compared by computer matching of chromosomes. Four values - two unique percent matches and two total percent matches - are possible when chromosome numbers differ. A system combining the four values was therefore devised in order to assess relationships. Results indicated that species grouped by their external morphology (Chapter 3.6) were, in the majority of cases, also grouped by their karyotype similarity. Percentage similarity values also indicated a degree of chromosome duplication both within and between karyotypes - an expected result in view of the polyploid nature of most taxa. However, the more or less continuous range of similarity values suggested that structural changes within karyotypes have been extensive. Differences in absolute chromosome size may account for some of the structural change but rearrangements of chromosome segments are also likely as the nuclear DNA amounts of many species are similar.

A comparison of satellite chromosome morphology provided evidence in support of the hypothesis that S. linearifolius and S. sp.A(Group 2B) are the product of introgression between a discoid and a radiate taxon. However, on the basis of karyotype evidence the phylogenetic relationship between the two erechthitoid

subgroups proposed in Chapter 3 was altered. A modified phylogeny in which the erechthitoid subgroups are derived from different ancestral species best fits the observations of karyotype morphology and external morphology.

Comparisons of karyotype symmetry suggest that the most primitive karyotype in *Senecio* was probably symmetrical, but that asymmetry is not necessarily correlated with advanced morphology or breeding systems. Instead, it is suggested that asymmetry is largely correlated with higher ploidy levels as it is more likely that structural changes will be "tolerated", species with 2N=60 and 2N=100 most probably formed by events including hybridization. so that asymmetry may in part be due to the combination of different parental karyotypes. It is possible that the asymmetrical karyotypes of *S. velleioides* and *S. vagus* are the result of unequal increases in the absolute size of chromosome arms, but in the majority of cases, changes in absolute chromosome size have not affected arm ratios of karyotypes.

Three African species with low chromosome numbers were included in an attempt to confirm the basic chromosome number of *Senecio*. However, variation in karyotype symmetry, absolute size of chromosomes and the number of satellites could support either x=5 or x=10 as basic. The occurrence of 2N=10 among specialized annuals is therefore still the most suggestive evidence - favouring x=10 as the basic chromosome number of *Senecio*.

CHAPTER 8

Natural and Synthetic Hybrids

8.1 Introduction

8.2 Materials and methods

8.3 Results and discussion

8.3.1 Natural hybrids

8.3.1.1 Characteristics of hybrid and parent plants

8.3.1.2 Evidence used to determine parent species

8.3.1.3 Pollen and seed development

8.3.2 Crossing programs

8.3.2.1 Program 1

8.3.2.2 Program 2

8.3.3 Extended studies of *S. pterophorus* x *S. hypoleucus*

8.3.3.1 Frequency of natural hybridization

8.3.3.2 Likelihood of fertile hybrid formation

8.3.3.3 Evidence of additive gene effects

8.3.4 The rayed gene complex in *Senecio*

8.3.5 Origins of decaploid species

8.3.6 Hybridization and polyploidy in *Senecio* and Senecioneae

8.1 Introduction

Natural hybrids between species of *Senecio* have been noted by many authors. In Britain, Lousley (1946) described a newly discovered hybrid between *S. squalidus* and *S. viscosus* and listed six other previously reported hybrids involving seven parental species. Of these, the realtionship between *S. squalidus* (2N=20), *S. vulgaris* (2N=40), their Fl hybrid *S.* x *baxteri* (2N=30) and allohexaploid derivative *S. cambrensis* (2N=60) has been extensively investigated (Hull 1974a and b, 1975, 1976, Richards 1975, Monaghan and Hull 1976, Stace 1977, Ingram 1977, 1978, Weir and Intram 1980, Ingram et al. 1980). Levyns (1950) suggested that hybrids were also forming between *S. pterophorus*, *S. rosaminifolius*, *S. rigidus* and *S. lanceus* in the Cape Peninsula of South Africa. Putative hybrids between Australian erechthitoid species of *Senecio* were reported by Belcher (1956), who also suspected that introgression might occur between erechthitoid and one or more radiate species of *Senecio*.

All of the above mentioned hybrids occur between different species, but in the strictest sense, a hybrid is any organism produced from a cross between genotypically different parents, so that hybridization is synonymous with outcrossing. The high incidence of interspecific hybridization in *Senecio* suggests that crosses between varieties and forms must also be frequent. There are perhaps two reasons why hybridization is common in *Senecio*. The first is that many species are successful in unstable environments (see Chapter 4) so that two or more species often occur in dense and intermingled populations. In such situation, cross pollination can occur even between facultative inbreeding species with comparatively inconspicuous capitula. The second reason is that the majority of cytologically

investigated species of *Senecio* are polyploid (see Lawrence 1980, copy bound with thesis). Of the 30 species of *Senecio* native to Australia and included in this study, 12 are tetraploid, 15 are hexaploid, 1 is octoploid and 2 are decaploid. Harlan and deWet (1975) commented that "high polyploids can withstand the shock of alien germplasm better than plants at lower ploidy levels and the widest crosses are likely to be most successful at that level." The occurrence of interspecific hybrids in *Senecio* is therefore less surprising than in a genus containing only diploids.

8.2 Materials and Methods

Methods used in the analysis of hybrid plants have been largely described in preceeding chapters. References are:

1) bagging and emasculation of capitula for cross pollination trials - Chapter 4.2.1;
2) preparation and staining of mitotic or meiotic material- Lawrence (198), copy bound with thesis;
3) estimates of 4C nuclear DNA amounts - Chapter 6.2;
4) construction and analysis of karyotypes - Chapter 7.2.

Pollen fertility of hybrid plants was determined by counting 300 grains stained with methyl green and phloxine in a glycerol jelly medium (after Owczarzak, 1952). Seed fertility was not directly determined as seed numbers were usually low. Instead, percentage seed germination was calculated so that progeny could also be studied.

8.3 Results and Discussion

8.3.1 Natural Hybrids

8.3.1.1 Characteristics of hybrid and parent plants.

Nine hybrids between different species of *Senecio* were collected in the field. Each hybrid is designated by the names of the two parents connected by the multiplication sign (x), rather than by a new specific name preceeded by (x). In the case of *S. lautus* x *S. biserratus*, sterile F1 plants occur with sufficient frequency to have been mistakenly recognized as a new taxon - *S. brachyglossus* (=*S. glossanthus*) var. *major* by Bentham (1866) and *S. orarius* by Black (1928). Both are reduced to synonymy (see treatment 39 in Chapter 3).

As most collection sites could not be revisited, studies of natural hybrids depended upon the suitability of material fixed for meiotic preparations and the success of cuttings prepared and potted in the field. However, the locality for hybrids between *S. pterophorus* and *S. hypoleucus* was within 20 kilometers of the laboratory, so that more extensive studies were possible (see part 8.3).

Characteristics of each hybrid and of its parent species are given in the following pages. A range of values is given in most cases and represents the range of mean values calculated from five measurements of each plant.

1. *Senecio pterophorus* DC. x *S. hypoleucus* F.v. Muell. ex Benth. Figure 8.1.

	S. pterophorus	hybrid	*S. hypoleucus*
Chromosome number (2N)	20	40	60
Univalents at MI	0	8-27	0
% seed set	>80	0	>80
% fertile pollen	>80	0	>80
Breeding system	outbreeding	?	outbreeding
Marginal floret type	female ray	female ray	bisexual disc
No plants measured	5	12	5
Leaf length/width	7-9:1	3-5:1	3:1
Pedicel length (mm)	14-17	6-9	3-4
No. involucral bracts	20-21	12-13	8-9
No. bracts in calyculus	14-15	5-9	5-6
Involucre width (mm)	4.0-4.5	3.5	3.0
No. ray florets	12-14	5-8	0
Length ray (mm)	6.0-6.2	1.3-4.7	0
No. disc florets	58-66	17-23	12-14

Collection site: South Australia. ML658-670: dry sclerophyll forest 0.6 km W. of Kangaroo Creek Dam wall, Torrens Gorge; 28.xii.1976.

Frequency: 26 hybrids among 625 parent plants (about 1/10 of total population).

Other species of Senecio present: *S. lautus* subsp. *dissectifolius* (2N=40), *S. quadridentatus* (2N=40).

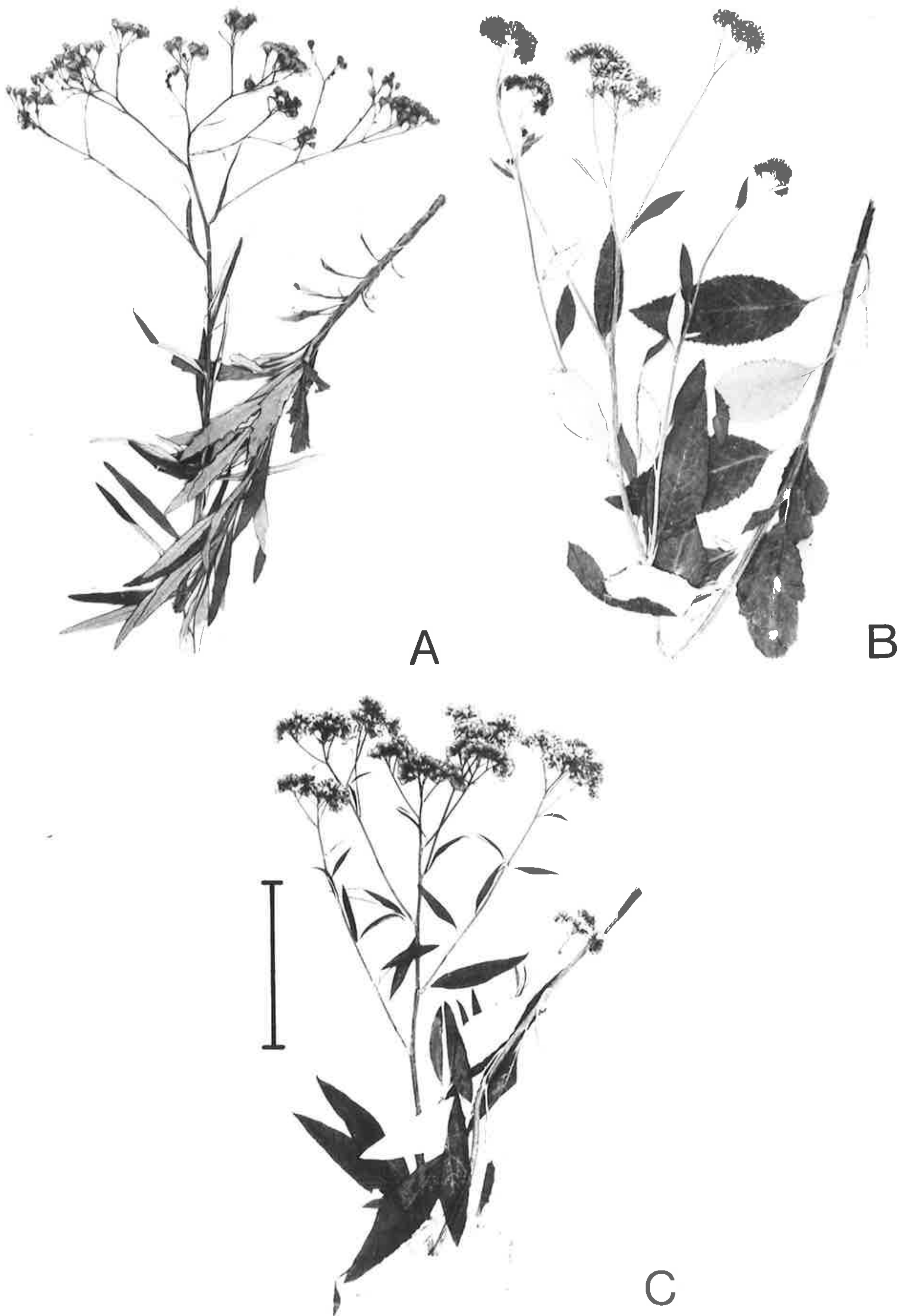


Fig. 8.1 A. Senecio pterophorus. B. S. hypoleucus. C. S. pterophorus x S. hypoleucus. All figures at same magnification. Scale 10 cm.

2. Senecio pterophorus DC. x S. glomeratus Desf. ex Poiret

Figure 8.2.

	S. pterophorus	hybrid	S. glomeratus
Chromosome number (2N)	20	40	60
Univalents at MI	0	c.12	0
% seed set	>80	0	>80
% fertile pollen	>80	0	>80
Breeding system	outbreeding	?	inbreeding
Marginal floret type	female ray	female ray and filiform	female filiform
No. plants measured	5	6	5
Leaf length/width	8-9:1	4-8:1	3-5:1
Pedicel length (mm)	19-23	9-16	8-11
No. involucral bracts	18-19	13-14	11-12
No. bracts in calyculus	16-19	8-12	4-5
No. ray florets	12-13	12-13	0
Length ray (mm)	6.2-6.4	3.0-3.2	0
No. filiform florets	0	8-11	29-34
No. disc florets	71-86	42-50	10-13

Collection site: South Australia. ML675-676, 679-682: sedgeland dominated by Gahnia trifida 5.9 km NE. Coffin Bay township; 23.i.1977.

Frequency: 6 hybrids, 24 S. glomeratus, 41 S. pterophorus.

Other species of Senecio present: S. lautus subsp. dissectifolius (2N=40).

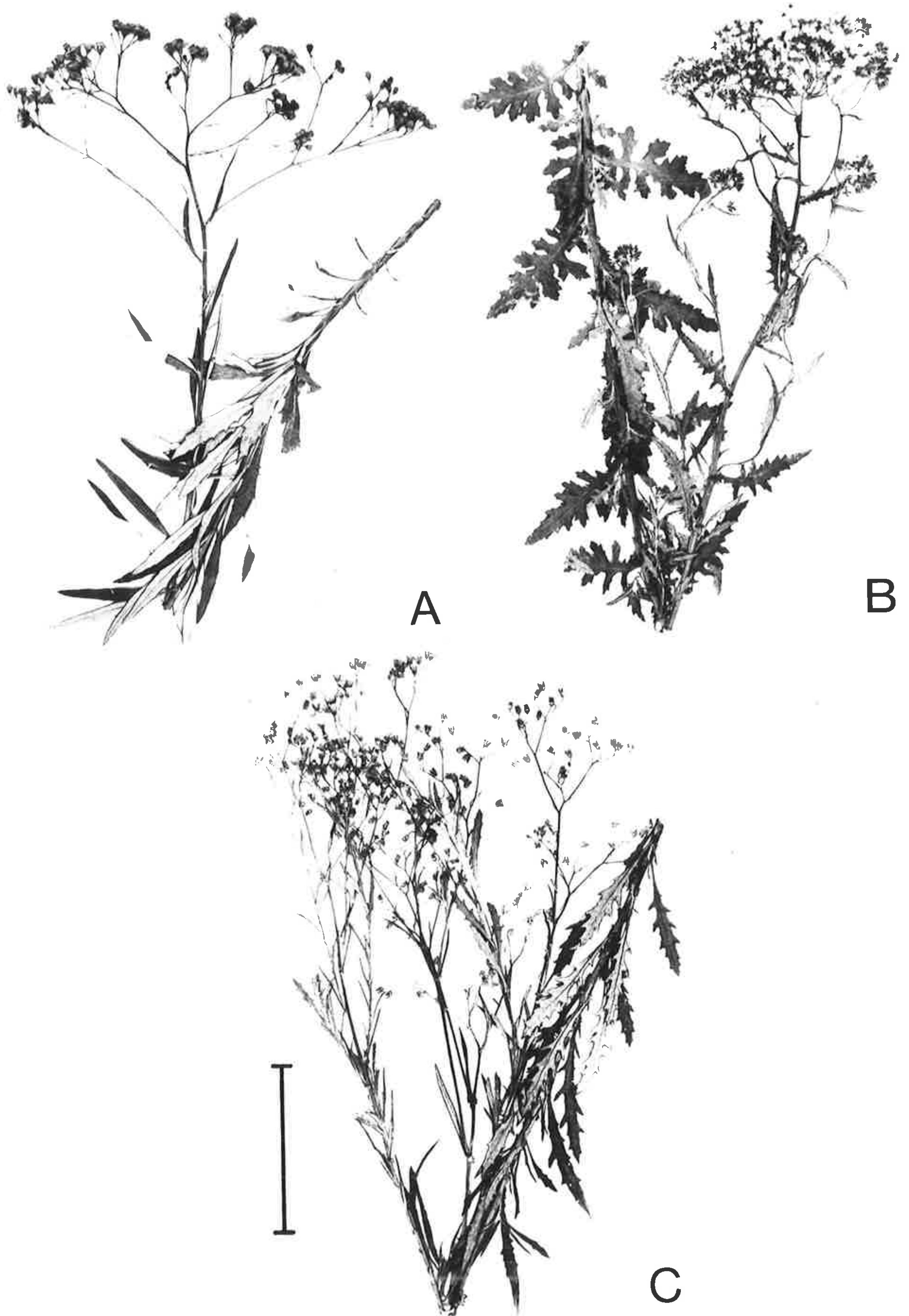


Fig. 8.2 A. Senecio pterophorus. B. S. glomeratus.
C. S. pterophorus x S. glomeratus. All figures at same magnification. Scale 10 cm.

3. Senecio pterophorus DC. x S. picridioides (Turcz.) Lawrence Figure 8.3.

	S. pterophorus	hybrid	S. picridioides
Chromosome number (2N)	20	40	60
Univalents at MI	0	14-19	0
% Seed set	>80	0	>80
% Fertile pollen	>80	0	>80
Breeding system	outbreeding	?	inbreeding
Marginal floret type	female ray	female ray and filiform	female filiform
No. plants measured	5	1	5
Leaf length/width	8-10:1	4:1	2-3:1
Pedicel length (mm)	18-21	8	8-10
No. involucral bracts	19-21	13	8-9
No. bracts in calyculus	14-16	5	3-4
No. ray florets	12-13	8	0
Length ray (mm)	5.8-6.1	2.1	0
No. filiform florets	0	9	15-18
No. disc florets	66-73	18	5-7

Collection site: South Australia: ML914: roadside paddock between North Block and South Block, Eyre Peninsula; 21.x.1977.

Frequency: 1 hybrid only, both parents frequent.

Other species of Senecio present: S. quadridentatus (2N=40).

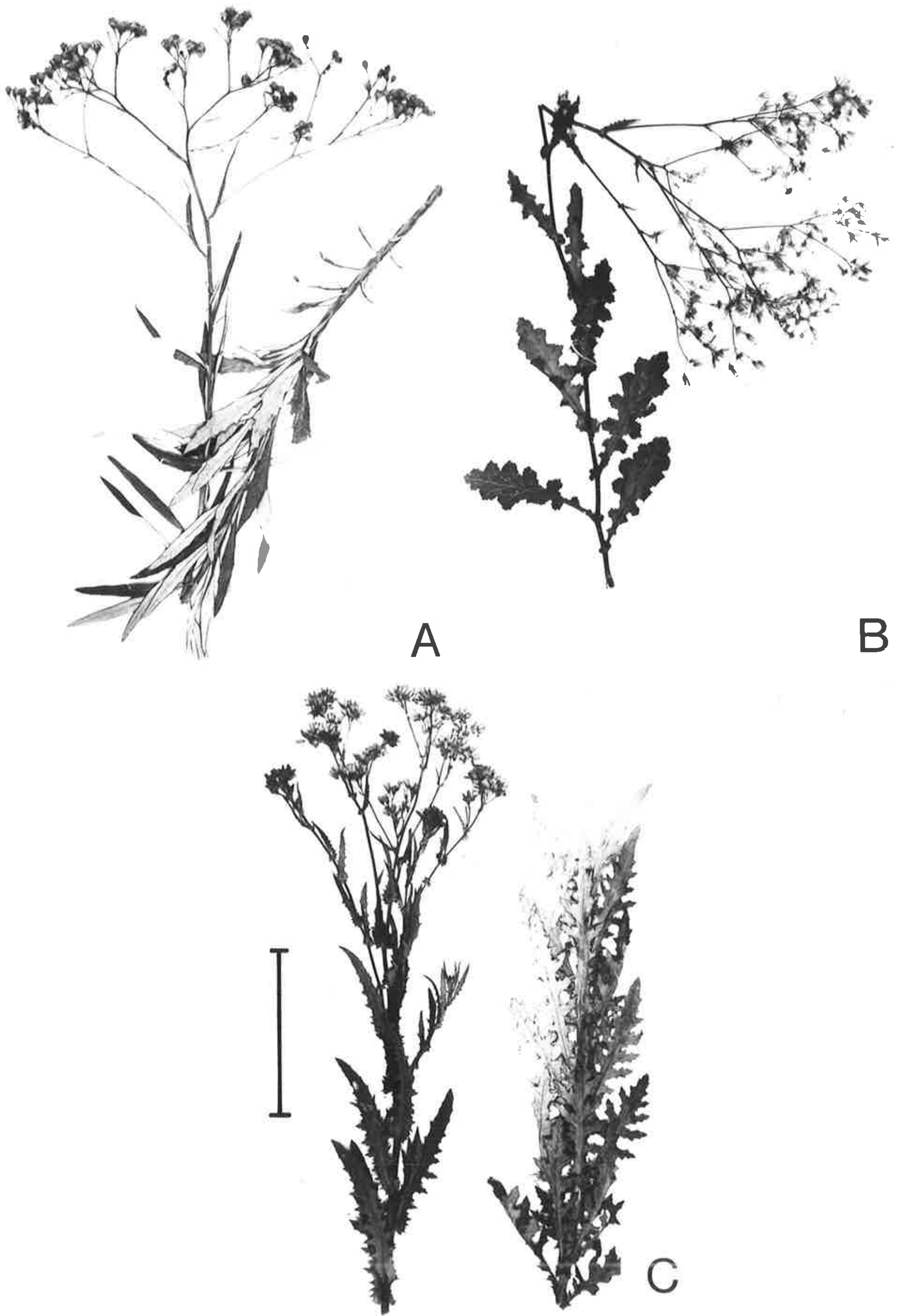


Fig. 8.3 A. Senecio pterophorus. B. S. picridioides. C. S. pterophorus x S. picridioides. All figures at same magnification. Scale 10 cm.

4. Senecio lautus G. Forst. ex Willd. subsp. dissectifolius Ali x S. biserratus Belcher   Figure 8.4.

S. brachyglossus F.v. Muell. var. major Benth Fl. Aust. 3:670 (1866).

S. orarius Black, Trans. Roy. Soc. S. Aust. 52:230 (1928).

	S. lautus	hybrid	S. biserratus
Chromosome number (2N)	40	70	100
Univalents at MI	0	16-47	0
% Seed set	>80	0	>80
% Fertile pollen	>80	0	>80
Breeding system	outbreeding	?	inbreeding
Marginal floret type	female ray	female ray and filiform	female filiform
No. plants measured	5	3	5
Leaf length/width	2-3:1	2-3:1	2:1
Pedicel length (mm)	18-27	9-10	5-7
No. involucral bracts	13-15	12-13	8
No. bracts in calyculus	8-10	5-7	3-4
No. ray florets	10-11	8-10	0
Length ray (mm)	7.7-8.2	2.5-3.0	0
No. filiform florets	0	6-11	14-17
No. disc florets	59-79	24-26	6-8

Collection sites: Victoria. ML 1293-95: narrow divide between Lake Killarny and Lake Victoria, 10 km SSE. Bairnsdale; 14.xii. 1978. - ML 1299: base of coastal cliff on dune 1.5 km from beach, 4 km E. Marlo; 15.xii.1978.

Frequency: SSE. Bairnsdale - 3 plants, both parents frequent. E. Marlo - 1 plant, both parents frequent.

Other species of Senecio present: SSE. Bairnsdale - S. glomeratus (2N=60). E Marlo - none.

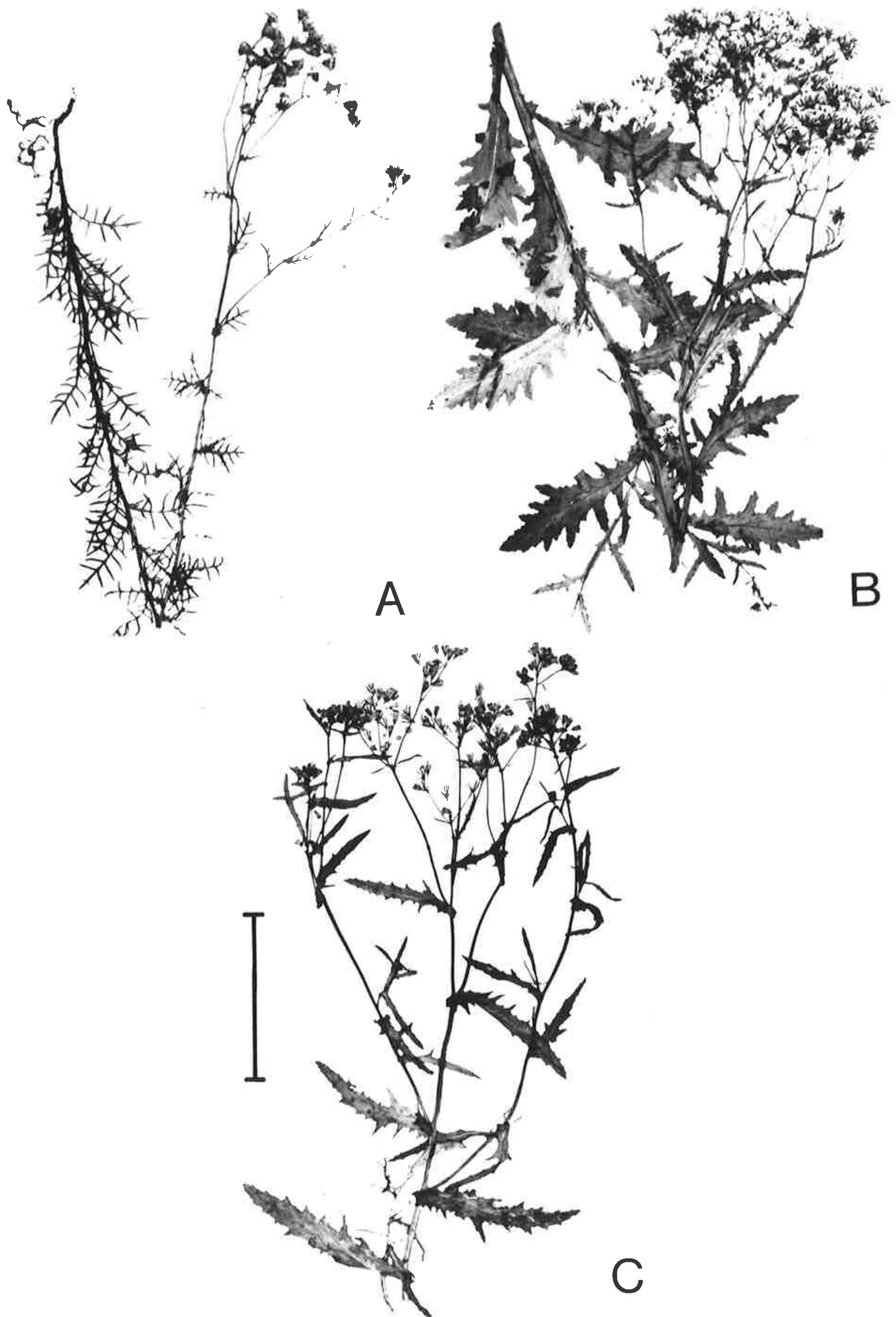


Fig. 8.4 A. Senecio lautus subsp. dissectifolius. B. S. biserratus. C. S. lautus x S. biserratus. All figures at same magnification. Scale 10 cm.

5. *Senecio linearifolius* A. Rich. x *S. bipinnatisectus* Belcher

Figure 8.5.

	S. linearifolius	hybrid	*S. bipinnatisectus*
Chromosome number (2N)	60	60	60
Univalents at MI	0	0-4	0
% Seed set	> 80	0.04	> 80
% Fertile pollen	> 80	2.6	> 80
Breeding system	outbreeding	?	inbreeding
Marginal floret type	female ray	female ray and filiform	female filiform
No. plants measured	5	7	5
Leaf length/width	4-6:1	2:1	1-2:1
Pedicel length (mm)	6-8	6	7
No. involucral bracts	8	13	9
No. bracts in calyculus	3	3	2-3
No. ray florets	5	9	0
Length ray (mm)	4.5-5.0	1.2	0
No. filiform florets	0	0	15-17
No. disc florets	11-12	18	5

Collection site: New South Wales. ML1327: in small quarry near wet sclerophyll forest, 22.9 km. N. Wingham on road to Comboyne; 24.xii.1978.

Frequency: 1 plant only, both parents frequent in forest.

Other species of Senecio present: *S. minimus* (2N=60).

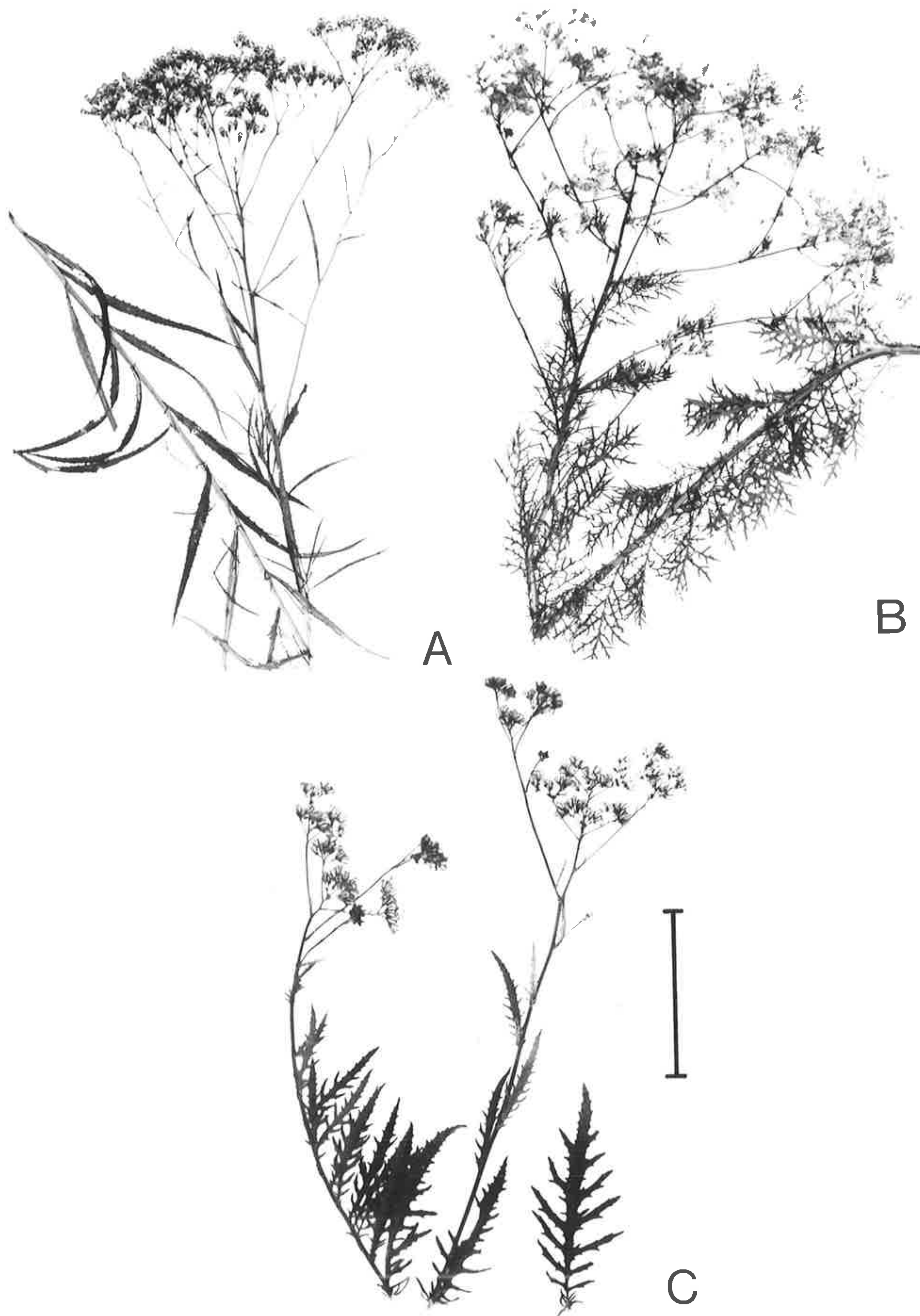


Fig. 8.5 A. *Senecio linearifolius*. B. *S. bipinnatisectus*. C. *S. linearifolius* x *S. bipinnatisectus*. All figures at same magnification. Scale 10 cm.

6. *Senecio linearifolius* A. Rich. x *S.* sp. B. Figure 8.6.

	S. linearifolius	hybrid	*S.* sp. B
Chromosome number (2N)	60	60	60
Univalents at MI	0	0-8	0
% Seed set	> 80	0.12	> 80
% Fertile pollen	> 80	3.0	> 80
Breeding system	outbreeding	?	inbreeding
Marginal floret type	female ray	female ray and filiform	female filiform
No. plants measured	5	1	5
Leaf length/width	4-5:1	6:1	6-7:1
Pedicel length (mm)	6-7	8	7-8
No. involucral bracts	8	13	8-9
No. bracts in calyculus	3	5	3-4
No. ray florets	5	6	0
Length ray (mm)	4.2-4.4	1.3	0
No. filiform florets	0	3	11-13
No. disc florets	10-13	15	6

Collection site: New South Wales: ML1389: roadside in dry sclerophyll forest, 3 km, from Jenolan Caves on road to Oberon; 28.xii.1978.

Frequency: 1 plant only, both parents frequent at roadside.

Other species of Senecio in vicinity: *S. minimus* (2N=60), *S. hispidulus* (2N=60).

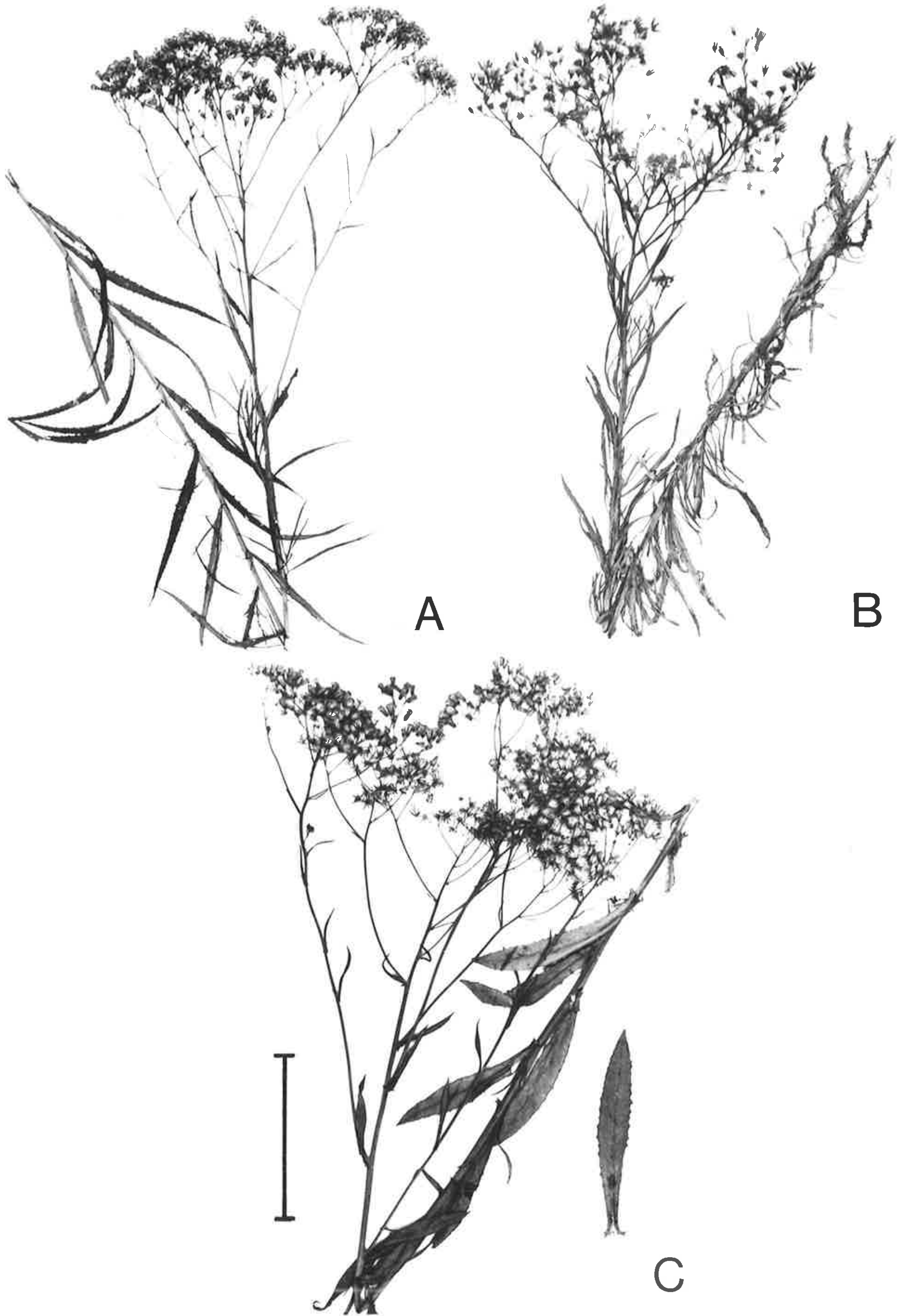


Fig. 8.6 A. Senecio linearifolius. B. S. sp. B.

C. S. linearifolius x S. sp. B. All figures at same magnification.

Scale 10 cm.

7. *Senecio bipinnatisectus* Belcher x *S. minimus* Poiret.

Figure 8.7.

	S. bipinnatisectus	hybrid	*S. minimus*
Chromosome number (2N)	60	60	60
Univalents at MI	0	0-3	0
% Seed set	>80	(in bud)	>80
% Fertile pollen	>80	37.1	>80
Breeding system	inbreeding	?	inbreeding
Marginal floret type	female filiform	female filiform	female filiform
No. plants measured	5	3	5
Leaf length/width	1-2:1	2-3:1	4-5:1
Pedicel length (mm)	7-8	7	7
No. involucral bracts	9	9	9
No. bracts in calyculus	2-4	3	2-3
No. filiform florets	14-17	15	11-14
No. bisexual florets	4-5	5	5

Collection site: New South Wales. ML 1346-48: along roadside 8.4 km. from NE. boundary of Barrington Tops National Park on road to Gloucester Tops; 24.xii.1978.

Frequency: Three plants, both parents frequent along roadside.

Other species of Senecio in vicinity: *S.* sp. B (2N=60).

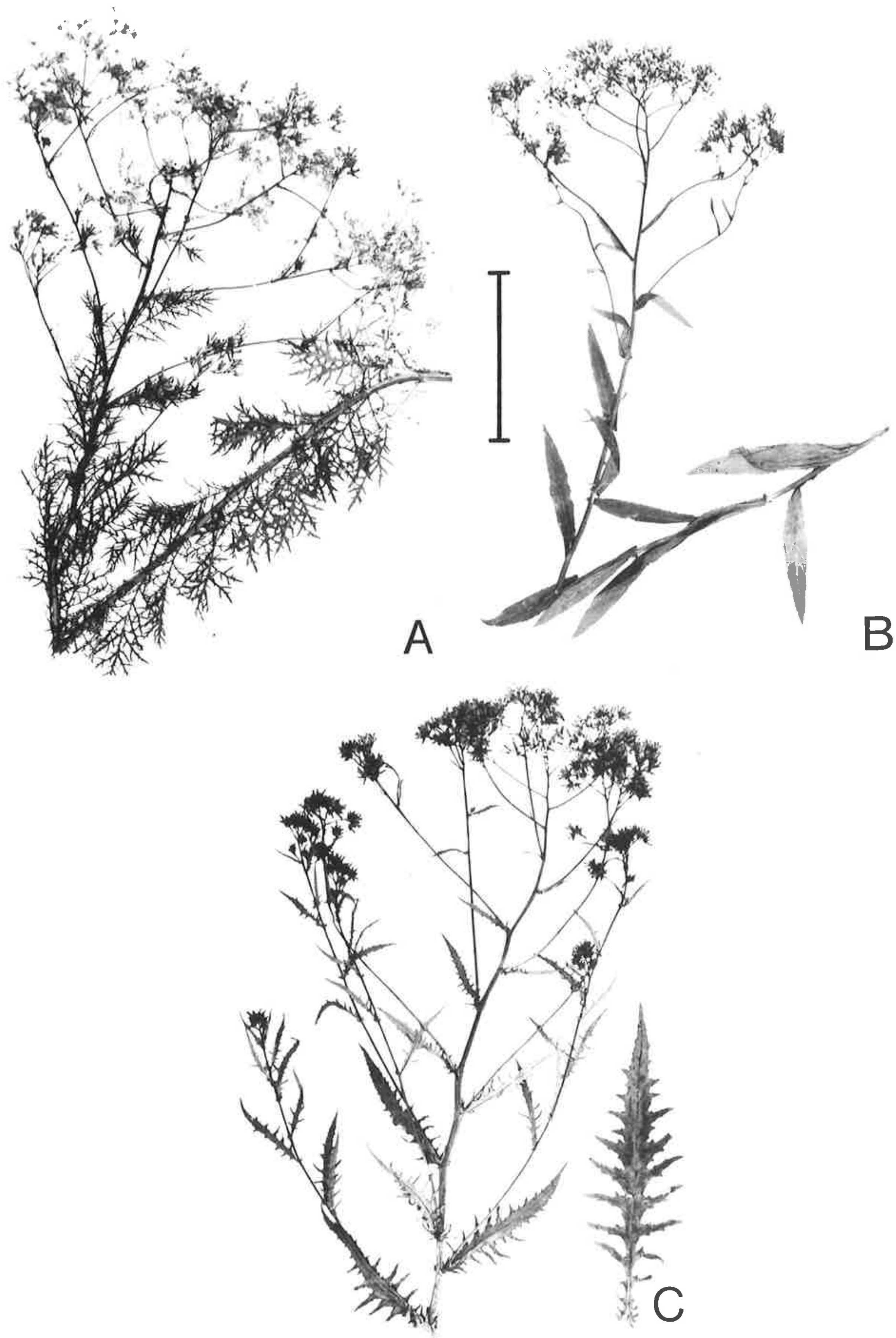


Fig. 8.7 A. Senecio bipinnatisectus. B. S. minimus.

C. S. bipinnatisectus x S. minimus. All figures at same magnification

Scale 10 cm.

8. Senecio glomeratus Desf. ex Poiret x S. hispidulus A. Rich.

Figure 8.8.

	S. glomeratus	hybrid	S. hispidulus
Chromosome number (2N)	60	60	60
Univalents at MI	0	0	0
% Seed set	>80	>80	>80
% Fertile pollen	>80	>80	>80
Breeding system	inbreeding	inbreeding	inbreeding
Marginal floret type	female filiform	female filiform	female filiform
No. plants measured	5	1	5
Leaf length/width	3-4:1	3:1	4-5:1
Pedicel length (mm)	5-8	6	8-10
No. involucral bracts	11-12	12	11-12
No. bracts in calyculus	5-6	5	3
No. filiform florets	19-29	21	13-15
No. bisexual florets	9-11	8	4-6

Collection site: Victoria: ML 1086: among roadside grass 1.3 km SE. Lang Lang on road to Nowra; 3.xii.1978.

Frequency: 1 plant only, parents both locally frequent.

Other species of Senecio in vicinity: S. lautus subsp. lanceolatus (2N=40).

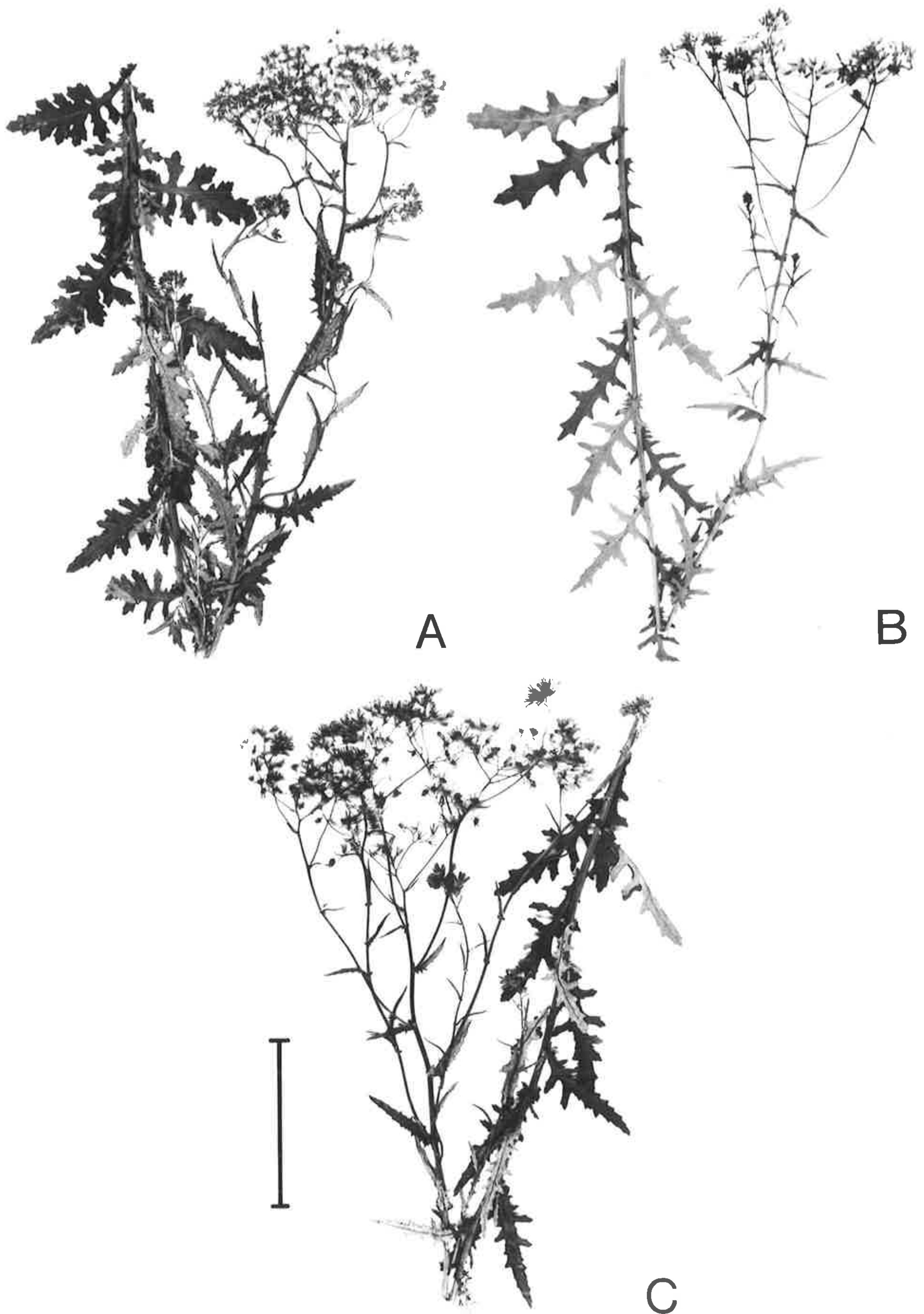


Fig. 8.8 A. Senecio glomeratus. B. S. hispidulus.

C. S. glomeratus x S. hispidulus. All figures at same magnification.

Scale 10 cm.

9. <u>Senecio glomeratus</u> Desf. ex Poiret x <u>S. minimus</u> Poiret.

Figure 8.9.

	<u>S. glomeratus</u>	hybrid	<u>S. minimus</u>
Chromosome number (2N)	60	60	60
Univalents at MI	0	0	0
% Seed set	>80	>80	>80
% Fertile pollen	>80	>80	>80
Breeding system	inbreeding	inbreeding	inbreeding
Marginal floret type	female filiform	female filiform	female filiform
No. plants measured	5	3	5
Leaf length/width	3-4:1	5:1	5:1
Pedicel length (mm)	6-9	7	7-10
No. involucral bracts	11-13	11-12	9
No. bracts in calyculus	5-7	6-7	3-4
No. filiform florets	25-31	28-33	12-16
No. bisexual florets	10-11	7-8	5

<u>Collection site</u>: Victoria. ML 1146-1148: among roadside grass 18 km. NW. Porland; 6.xii.1978.

<u>Frequency</u>: 4 plants, both parents frequent.

<u>Other species of Senecio in vicinity</u>: <u>S. odoratus</u> (2N=60), <u>S. biserratus</u> (2N=100).

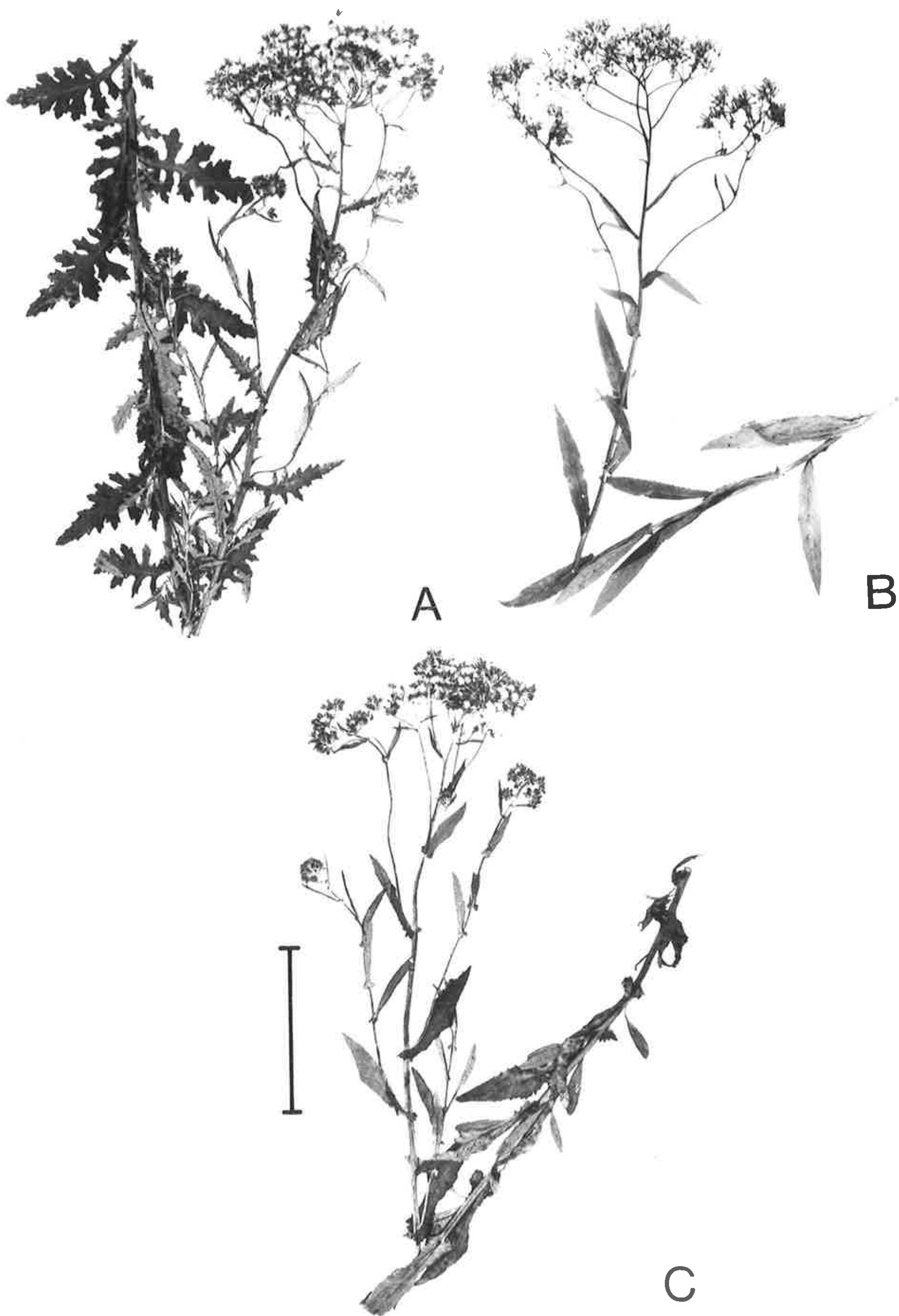


Fig. 8.9 A. Senecio glomeratus. B. S. minimus.

C. S. glomeratus x. S. minimus. All figures at same magnification.

Scale 10 cm.

TABLE 8.1

Characteristics of 9 naturally occurring hybrids of Senecio species

	Hybrid (1 x 2)	marginal florets*	chromosome number (2N)	univalents at MI	% fertile pollen	% seed set	Parent species: marginal florets* 1	Parent species: marginal florets* 2	Parent species: chromosome number (2N) 1	Parent species: chromosome number (2N) 2
1	*S. pterophorus* x *S. hypoleucus*	FR	40	8-27	0	0	FR	BD	20	60
2	*S. pterophorus* x *S. glomeratus*	FR+FF	40	c.12	0	0	FR	FF	20	60
3	*S. pterophorus* x *S. picridioides*	FR+FF	40	14-19	0	0	FR	FF	20	60
4	*S. lautus* x *S. biserratus*	FR+FF	70	16-47	0	0	FR	FF	40	100
5	*S. linearifolius* x *S. bipinnatisectus*	FR+ FF	60	0-4	2.6	0.04	FR	FF	60	60
6	*S. linearifolius* x *S.* sp. B	FR+FF	60	0-8	3.0	0.12	FR	FF	60	60
7	*S. bipinnatisectus* x *S. minimus*	FF	60	0-3	37.1	?	FF	FF	60	60
8	*S. glomeratus* x *S. hispidulus*	FF	60	0	>80	>80	FF	FF	60	60
9	*S. glomeratus* x *S. minimus*	FF	60	0	>80	>80	FF	FF	60	60

* FR = female ray, BD = bisexual disc, FF = female filiform

8.3.1.2 Evidence used to determine parents of hybrids.

Likely parents of all natural hybrids listed in Table 8.1 were first deduced from the morphology of *Senecio* species occurring at the collection site and the morphology of hybrid plants. Mitotic and meiotic preparations were then examined to determine if chromosome numbers gave the correct hybrid combination and if irregularities were visible during meiosis in the hybrid.

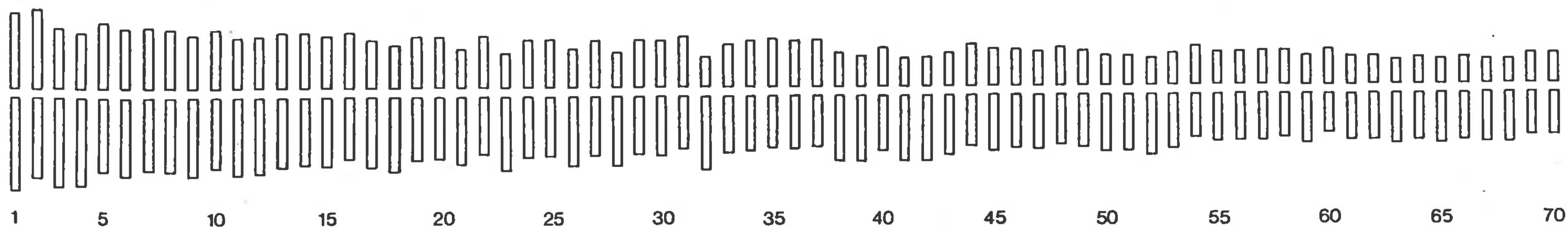
Nuclear DNA amounts vary considerably in *Senecio* (see Table 6.6, page 293) and therefore offered another means of testing parental combinations. Actual hybrid DNA amounts could therefore be compared with expected values obtained by averaging the DNA amounts of putative parents. Results for seven of the nine hybrids are shown in Table 8.2. (As vegetative propagation of *S. pterophorus* x *S. picridioides* and *S. bipinnatisectus* x *S. minimus* was unsuccessful, these plants could not be included). In all cases expected and observed hybrid DNA amounts did not differ significantly ($P > .05$) when compared by a Students t test, and therefore supported the original suspected parents.

A further means of comparing hybrid and parent plants was by karyotype morphology. Because of limited time, this was done in only one case - *S. lautus* x *S. biserratus* - a sterile F1 hybrid occurring with sufficient frequency to have been mistakenly recognised as the species *S. orarius* (see taxonomic treatment 39 in Chapter 3). Karyotype analysis was completed by the method described in Chapter 7, but no attempt was made to pair homologous chromosomes. The karyotype of *S. lautus* x *S. biserratus* (Figure 8.10A) therefore represents the diploid rather than haploid complement. Karyotypes of *S. lautus* subsp. *dissectifolius* (present at both collection sites) and *S. biserratus* are shown in Figure 8.10 B and C. To compare karyotypes a synthetic hybrid (set B) was constructed by combining the karyotypes of parent

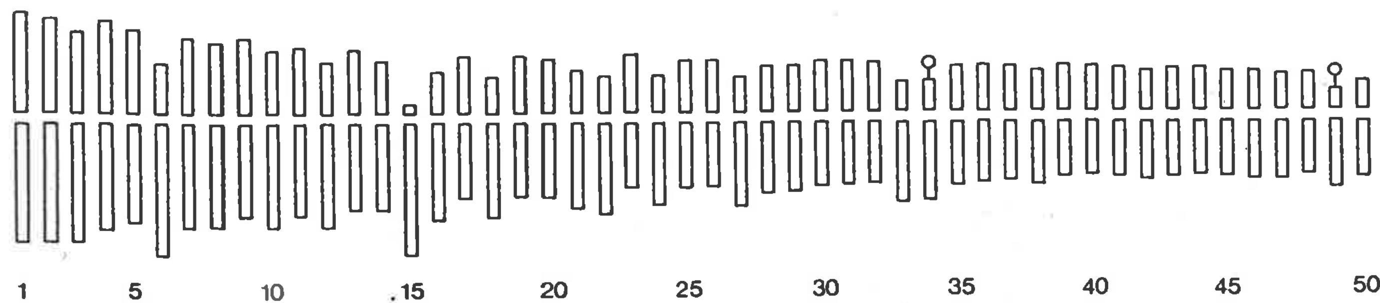
TABLE 8.2

Actual DNA Amounts (picograms/4C nucleus ± s.e.) of Hybrid Plants and Amounts Predicted From Likely Parent Species

Hybrid (parent 1 x parent 2)	Parent Species 1	Parent Species 2	Hybrid expected	Hybrid observed
S. pterophorus x *S. hypoleucus*	4.22 ± 0.05	17.92 ± 0.15	11.02 ± 0.10	11.21 ± 0.09
S. pterophorus x *S. glomeratus*	4.22 ± 0.05	19.81 ± 0.19	11.70 ± 0.12	11.72 ± 0.12
S. lautus x *S. biserratus*	10.63 ± 0.11	25.27 ± 0.15	17.95 ± 0.13	17.79 ± 0.16
S. linearifolius x *S. bipinnatisectus*	18.02 ± 0.15	13.98 ± 0.12	16.00 ± 0.14	16.31 ± 0.11
S. linearifolius x *S.* sp. B	18.02 ± 0.15	19.94 ± 0.11	18.98 ± 0.13	19.27 ± 0.19
S. glomeratus x *S. hispidulus*	19.18 ± 0.19	19.11 ± 0.13	19.15 ± 0.16	18.62 ± 0.13
S. glomeratus x *S. minimus*	19.18 ± 0.19	19.82 ± 0.14	19.50 ± 0.17	19.08 ± 0.18

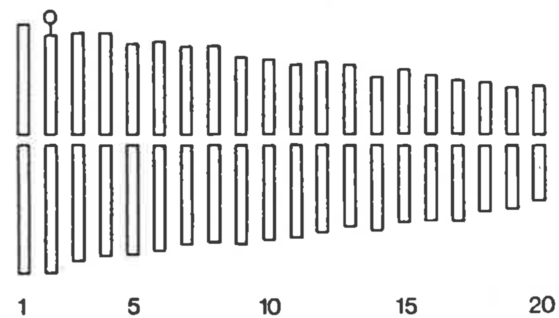


A. S. lautus x S. biserratus (diploid karyotype)



B. S. biserratus

(haploid karyotype)



C. S. lautus

(haploid karyotype)

Fig. 8.10 Karyotypes of the F1 hybrid S. lautus x S. biserratus and of the parent species.

species. Computer matching of the actual (set A) and synthetic hybrid (set B) gave the following results:

94.2% of set A chromosomes have a match in set B

96.7% of set B chromosomes have a match in set A

91.4% of set A and set B chromosomes match uniquely (with a previously unmatched chromosome).

The very high percentage similarity of karyotypes strongly suggests that *S. lautus* and *S. biserratus* are the parents of "*S. orarius*."

8.3.1.3 Pollen and seed development in natural hybrids.

Hybrids listed in Table 8.1 exhibited varying degrees of sterility. In each case pollen mother cell meiosis and subsequent pollen grain development were examined. Embryogenesis was not examined but the appearance of mature achenes was noted.

i) Sterile hybrids: 1. *S. pterophorus* x *S. hypoleucus*, 2. *S. pterophorus* x *S. glomeratus*, 3. *S. pterophorus* x *S. picridioides*, 4. *S. lautus* x *S. biserratus*.

Hybrids 1 to 4 in Table 8.1 (and listed above) formed between parents with different chromosome numbers. In each case pollen was completely infertile and all achenes were white and shrivelled. Meiotic configurations were generally similar. A high but variable number of univalents were always present at metaphase I (Fig. 8.11 A and B), but preparations were not sufficiently clear to determine bivalent and multivalent numbers. Univalents usually remained in the vicinity of the metaphase plate during anaphase I (Fig. 8.11 C) and were then often excluded from the interphase nuclei. Although tetrads were generally produced, small cells that had formed around micronuclei were sometimes

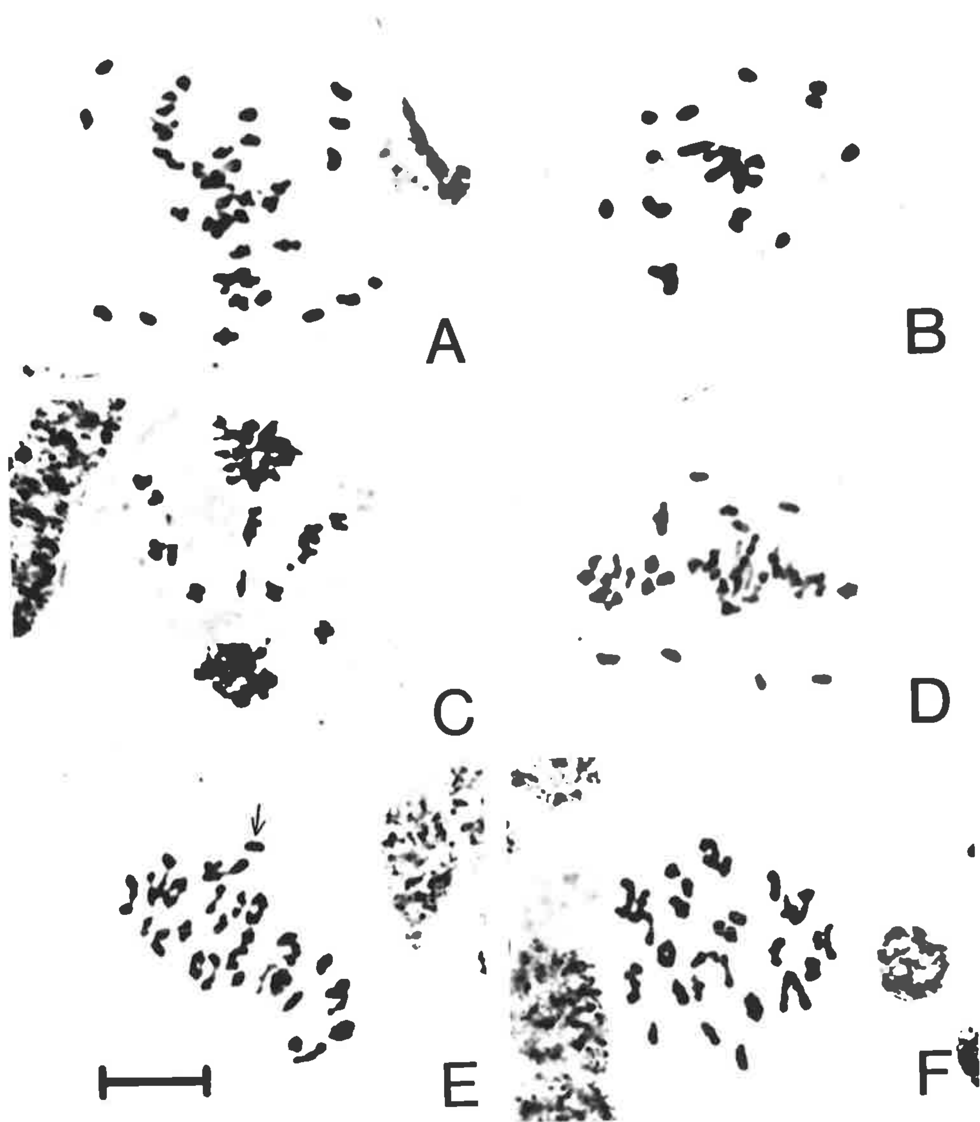


Fig. 8.11 Meiosis in natural hybrids of *Senecio*.
A. *S. lautus* x *S. biserratus*, 2N=70, c. 15 univalents at MI.
B. *S. pterophorus* x *S. hypoleucus*, 2N=40, c. 11 univalents at MI. C. *S. pterophorus* x *S. hypoleucus*, univalents and bivalents lagging at late AI. D. *S. linearifolius* x *S.* sp. B, 2N=60, 8 univalents at MI. E. *S. bipinnatisectus* x *S. minimus*, 2N=60, c. 1 univalent (arrow) at MI.
F. *S. glomeratus* x *S. hispidulus*, 2N=60, apparently normal MI. All figures at same magnification. Scale 10 μm.

observed. In hybrids 2 to 4, cells of the tetrad formed an exine, and although matured grains were shrivelled they were present at anthesis. Similar development occurred in only 1 of 12 plants of hybrid 1 (*S. pterophorus* x *S. hypoleucus*) examined - the remainder failed to develop a well defined exine and mature anthers were empty.

ii) Partially sterile hybrids: 5. *S. linearifolius* x *S. bipinnatisectus*, 6. *S. linearifolius* x *S.* sp B, 7. *S. bipinnatisectus* x *S. minimus*.

Hybrids 5 and 6 in Table 8.1 produced some fertile pollen and some achenes that later germinated in the glasshouse. In both, most metaphase I configurations appeared normal but configurations with up to 8 univalents were also observed (Fig. 8.11D and E). Despite the high proportion of apparently normal configurations, pollen fertility was very low (2.6% and 3.0%). Only one plant of *S. linearifolius* x *S. bipinnatisectus* was found, and although all capitula were examined only 8 apparently normal achenes were detected (approximately 0.04% of the potential seed set). Of the 8 achenes, 1 germinated, and when mature the plant closely resembled the hybrid both in morphology and meiotic behaviour. Seed set in *S. linearifolius* x *S. bipinnatisectus* was therefore interpreted as a low level of successful selfing.

In the case of *S. linearifolius* x *S.* sp B, 5 plants were raised from a collection of 23 achenes. The progeny of the hybrid plant could not be distinguished from *S. linearifolius* either by morphology or by meiotic configurations. Seed set in *S. linearifolius* x *S.* sp B was therefore interpreted as backcrossing to *S. linearifolius*.

The third partially sterile hybrid, *S. bipinnatisectus* x *S. minimus*, was collected at an early stage of anthesis and attempts to propagate plants from cuttings were unsuccessful.

Although seed set is not known, pollen was 37% fertile so that this hybrid may have been able to produce some seed either by selfing or by backcrossing.

iii) Fertile hybrids: 8. S. glomeratus x S. hispidulus, 9. S. glomeratus x S. minimus.

Two collections were classified as hybrids on the basis of their intermediate morphology, but no irregularities were found in either pollen or seed development. The plant designated S. glomeratus x S. hispidulus may have been a variant form of one parent rather than a hybrid. Alternatively, if the plant was a hybrid of S. glomeratus and S. hispidulus then the recognition of separate species is questionable.

In the case of S. glomeratus and S. minimus, there is a clear separation of species both on a morphological basis and by karyotype features. It would therefore seem likely that plants designated S. glomeratus x S. minimus are not F1 hybrids, and instead, may be the products of introgression.

8.3.2 Crossing Programs

Two crossing programs were conducted in an attempt to confirm the parent species of early hybrid collections. As most hybrids and parent species listed in Table 8.1 were collected at a later date they could not be included. Species and hybrids included in each program are listed in Table 8.3. At least 20 capitula were treated in all possible crosses using each species as a pollen donor and a pollen receptor. As neither of the hybrids produced fertile pollen these could only be backcrossed to each parent species.

TABLE 8.3

Senecio Species and Hybrids Included in

Each of Two Crossing Programs

Species or Hybrid		Collection Number	Chromosome Number (2N)
Program 1 (November - December, 1978)			
S. pterophorus		647	20
S. lautus subsp. *dissectifolius*		644	40
S. quadridentatus		790	40
S. hypoleucus		646	60
S. odoratus var. *obtusifolius*		647	60
S. glomeratus		648	60
S. pterophorus x *S. hypoleucus*		658-660	40
S. pterophorus x *S. glomeratus*		679-681	40
Program 2 (August - September, 1979)			
S. lautus subsp. *dissectifolius*		644	40
S. vulgaris		552	40
S. gregorii		1010	40
S. glossanthus	tetraploid	475	40
	octoploid	476	80

8.3.2.1 Program 1.

Achenes of cross pollinated capitula were divided into the following six categories on the basis of visual examination and germination results:

1. white and shrivelled - resembling achenes produced in the absence of fertilization (known from breeding system trials);

2. white and plump but sterile (all species normally produce coloured achenes);

3. coloured and plump - resembling fertile achenes of respective species.

A. ruptured - achene wall split and embryo partially extruded;

B. no germination - achenes possibly sterile;

C. some germination - seedlings with parental chromosome number produced by apomixis;

D. some germination - seedlings with intermediate chromosome numbers produced by hybridization.

Of the 30 different crosses 13 produced some achenes in categories 2 to 3D. Crosses and achene types are listed in Table 8.4 (crosses producing only category 1 achenes are omitted).

The majority of coloured and plump achenes failed to germinate. As achenes were not sown until three weeks after harvesting, it is possible that some were fertile but sensitive to desiccation. *S. glomeratus* and *S. pterophorus*, for example, are known to hybridize naturally but the achenes of this cross failed to germinate. Achenes from three crosses with *S. quadridentatus* (2N=40) as the female parent ruptured during maturation. In each case the male parent was hexaploid (2N=60) with broader achenes than those of *S. quadridentatus*. It is possible that fertilization had occurred in these crosses but that the achene wall of *S. quadridentatus* could not contain the hybrid embryo.

TABLE 8.4

Crosses in Program 1 that Produced Some Seed in Categories 2 to 3D (see text for explanation)

Parent Species		Percent Seed Set in each category				
female	male	2	3A	3B	3C	3D
S. hypoleucus	S. lautus*	31	-	-	-	-
	S. odoratus**	-	10	-	-	32
	S. pterophorus	-	-	-	15	-
S. odoratus**	S. hypoleucus	-	23	-	-	-
	S. pterophorus	-	-	-	11	-
S. pterophorus	S. hypoleucus	-	6	-	-	21
	S. odoratus**	-	6	-	-	28
S. quadridentatus	S. hypoleucus	-	4	10	-	-
	S. odoratus	-	2	13	-	-
	S. pterophorus	-	15	-	-	-
	S. glomeratus	-	5	19	-	-
S. glomeratus	S. pterophorus	-	14	-	-	-
	S. quadridentatus	-	18	-	-	-

* subsp. dissectifolius

** var. obtusifolius

Germination occurred in achenes of five crosses between three parent species - *S. pterophorus*, *S. hypoleucus* and *S. odoratus* var. *obtusifolius*. All seedings were raised to maturity and their external morphology and chromosome numbers compared with that of parent species. Crosses with *S. pterophorus* (2N=20) as the male parent and either *S. hypoleucus* (2N=60) or *S. odoratus* var. *obtusifolius* (2N=60) as the female parent produced progeny with a diploid chromosome number of 2N=60 that were morphologically identical to the female parent. As both *S. hypoleucus* and *S. odoratus* var. *obtusifolius* are self-incompatible, the pollen of *S. pterophorus* must have stimulated apomictic reproduction. According to Nordenstam (1977) apomixis is unknown in Senecioneae. However, apomictic reproduction after stimulation by foreign pollen might not have been considered and could occur in other species of *Senecio*.

Reciprocal crosses with *S. pterophorus* as the female parent and either *S. hypoleucus* or *S. odoratus* var. *obtusifolius* as the male parent produced sterile F1 hybrids. *S. pterophorus* x *S. hypoleucus*.plants were identical to natural hybrids between these parents. *S. pterophorus* x *S. odoratus* var. *obtusifolius* plants could only be distinguished from *S. pterophorus* x *S. hypoleucus* plants by the less pubescent and slightly thicker leaves of the former. As *S. pterophorus* and *S. odoratus* var. *obtusifolius* do not occur in mixed populations in the field, natural hybrids between these parents are unlikely to occur.

S. hypoleucus and *S. odoratus* var. *obtusifolius* are readily distinguished by leaf morphology but their floral morphology is identical. Apparently normal achenes were produced from the two possible crosses between these species. However, germination occurred only in achenes having *S. hypoleucus* as the female parent. Meiosis in the mature progeny was quite normal and leaf

morphology was intermediate between that of the parents. Results therefore suggest a very close realtionship between S. hypoleucus and S. odoratus var. obtusifolius but the reason for failure of the reciprocal cross would need to be known before the taxonomic status of these species is altered.

8.3.2.2 Program 2.

All interspecific crosses in program 2 produced white and shrivelled seed although intraspecific crosses gave normal seed set. Crosses between the tetraploid and octoploid races of S. glossanthus were also unsuccessful, suggesting that the races have diverged from one another despite their close morphology. At the interspecific level, external morphology (Chapter 3) and karyotype morphology (Chapter 4) suggest that tetraploid species of Senecio are not as closely related to one another as are most hexaploid species. Failure of crossing attempts may therefore reflect more distant relationships. However, all species in program 2 were tetraploid (excluding the octoploid race of S. glossanthus) whereas natural and synthetic hybrids all included at least one parent of a higher ploidy level (hexaploid or decaploid). Failure to produce F1 hybrids in program 2 could therefore be related to the lower ploidy level of all parents species rather than to their phylogenetic affinities.

8.3.3 Extended Studies of S. pterophorus x S. hypoleucus

Hybrids of S. pterophorus are of economic as well as biological significance. S. pterophorus is native to South Africa and was first collected in South Australia at Port Lincoln on Eyre Peninsula (Black 1932). Since then S. pterophorus has

spread throughout southern Eyre Peninsula and has extended its range to the Mt. Lofty Ranges of Fleurieu Peninsula. *S. pterophorus* occurs most frequently in disturbed habitats and on nutrient-poor soils. It is not therefore an agressive weed of fertilized pastures. Fertile hybrids of *S. pterophorus* could be troublesome in agriculture, if their nutrient requirements are different, and in natural vegetation stands if they are able to extend their range to undisturbed sites.

The diploid *S. pterophorus* (2N=20) forms natural and sterile F1 hybrids with three native hexaploids (2N=60) - *S. hypoleucus*, *S. glomeratus* and *S. picridioides*. Of these, hybrids between *S. pterophorus* and *S. hypoleucus* occurred at a locality within 20 km of the laboratory and could be examined in greater detail. The major objective was to determine the frequency of hybridization in the field and therefore the likelihood of a fertile allopolyploid forming.

8.3.3.1 Frequency of hybridization.

At the hybrid collection site *S. hypoleucus* formed a dense roadside stand at the top of a rubble embankment whereas *S. pterophorus* was most common on the open woodland slope below. Hybrid plants were most common near the embankment base and were therefore between the greatest concentrations of parent plants. In November when hybrid and parent plants were flowering, all plants were identified in an area 300 m long, 50 m wide and divided lengthwise by the embankment. Of 625 plants, 367 were *S. hypoleucus*, 232 were *S. pterophorus* and 26 (4%) were hybrids.

An attempt was then made to determine the maximum rate of hybrid seed production by a parent plant in the field. An isolated plant of *S. pterophorus* surrounded by *S. hypoleucus* plants and an isolated plant of *S. hypoleucus* surrounded by

S. pterophorus plants were selected and seeds collected from both. Three hundred achenes of each were then sown (10 per 10 cm pot) and maintained in the glasshouse. It was known from the results of crossing program 1 that hybrids occurred only when *S. pterophorus* was the female parent, and from germination trials that the early seedling morphology of both parents differed significantly (see Chapter 4.4.6).

When seeds germinated it was apparent that many seedlings produced by the *S. pterophorus* plant were closer in morphology to those of *S. hypoleucus* whereas all seedlings from the *S. hypoleucus* plant resembled previously measured *S. hypoleucus* seedlings. Fifty seedlings of each parent and 50 intermediates were selected and their hypocotyl length, cotyledon width and cotyledon blade length measured. Results are shown in Figure 8.12 and mean values are summarized in Table 8.5. Although the hybrid seedling morphology overlaps with that of *S. hypoleucus* (Fig. 8.12), hybrids occurred among *S. pterophorus* seedlings and could therefore be readily distinguished.

Ten seedlings of each parent and ten of the intermediate category were raised to maturity. External morphology, chromosome numbers and meiotic behaviour confirmed that the intermediate seedlings were hybrids. In total 39.2% of seedlings from *S. pterophorus* were of hybrid morphology.

TABLE 8.5

Seedling Characteristics of *S. pterophorus*, *S. hypoleucus* and Their F1 Hybrid

	mean of 50 seedlings ± s.e (mm)	
	cotyledon blade length/width	hypocotyl length
S. pterophorus	1.70 ± 0.035	15.27 ± 0.438
F1 hybrid	1.18 ± 0.021	7.76 ± 0.312
S. hypoleucus	1.07 ± 0.017	4.35 ± 0.145

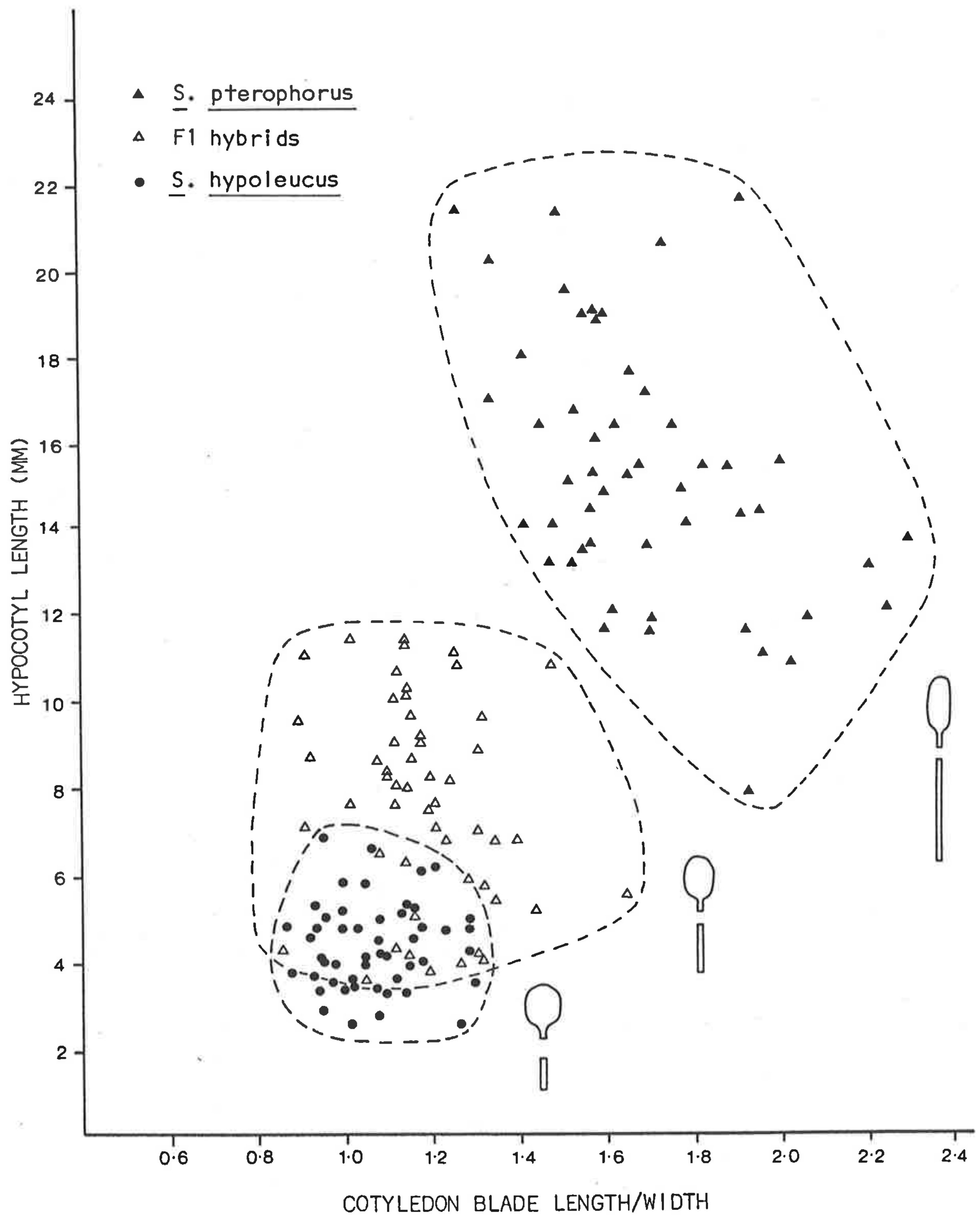


Fig. 8.12 Distribution of seedling characteristics of Senecio hypoleucus, S. pterophorus and their F1 hybrid (50 seedlings of each measured).

8.3.3.2 The likelihood of fertile hybrid formation.

Genes of *S. pterophorus* and *S. hypoleucus* could be combined in a fertile plant either by the fusion of unreduced hybrid gametes or by backcrossing of the hybrid to a parent species. The likelihood of each was considered. At the interface zone between parent populations the maximum rate of hybrid seed formation was 39.1% in an *S. pterophorus* plant surrounded by *S. hypoleucus* plants. The percentage may indicate that at most, 1 in 2 insect visitations lead to a transfer of pollen between species. As plants are more or less evenly distributed within the interface zone (i.e. not generally surrounded by plants of the other species) it was estimated that on average 1 in 12 (rather than the maximum 1 in 2) insect visitations represent an interspecific pollination. On this basis, *S. pterophorus* plants will produce an average of 39.1% ÷ 6 or 6.5% sterile F1 hybrid seed. *S. hypoleucus* does not contribute to the hybrid population as seed is set by apomixis in the presence of *S. pterophorus* pollen. If both parents are equally numerous in the interface zone, then 6.5% of *S. pterophorus* seed and 3.2% of all seed of both parents will be sterile F1 hybrids. Assuming the hybrids are equally likely to reach maturity as both parents, then 32 plants in a mixed population of 1000 will be sterile F1 hybrids.

Meiosis and pollen development were examined in 12 hybrid plants. In 11 of the 12, an exine failed to form and mature anthers were empty. The remaining hybrid was able to produce an exine so that mature anthers contained shrivelled pollen grains. It is therefore possible that of the 32 hybrids mentioned above only three may be able to produce an exine (1/12 of all hybrids). As both parents are self-incompatible, hybrids most probably have the same breeding system. Even if the rate of unreduced gamete formation is quite high, the chance of their coming together is

extremely low if only three hybrid plants in a total population of 1000 are capable of producing an exine.

The chance of backcrossing may be somewhat greater. Hybrid plants produce an average of 1000 capitula, 25 bisexual florets and 2000 grains per florets - in total 50,000,000 pollen grains and 25,000 ovules per plant. No fertile pollen was observed in 200 hybrid grains examined, but if it is assumed that balanced gametes (N=10 or N=30) are produced at a very low frequency - say 1 in 10,000, then 15,000 fertile pollen grains and 7.5 fertile ovules will occur in three hybrid plants. The chance of one of these combining with a parental gamete may be comparatively high.

8.3.3.3 Evidence of additive gene effects.

As *S. pterophorus* is diploid (2N=20) and *S. hypoleucus* is hexaploid (2N=60), hybrid plants contain three *S. hypoleucus* genomes and one *S. pterophorus* genome. It is therefore significant that hybrid seedling morphology is closer to that of *S. hypoleucus* (Figure 8.12). A similar trend is apparent in characteristics of the mature plants. Eight characters that differed in the parent species were selected and mean values were calculated for each of 12 hybrid plants and 5 parent plants. Distributions of mean values are shown in Figure 8.13. In six of the eight chatacters, hybrids were closer to *S. hypoleucus*, but the female floret number and ligule length of hybrids was intermediate. These are the only characters for which *S. hypoleucus* has a zero value. It is therefore possible that most hybrid characters are determined by additive (3 *hypoleucus* + 1 *pterophorus*) gene effect, but that female floret characteristics (being absent in *S. hypoleucus*) are more strongly influenced by the *S. pterophorus* genome.

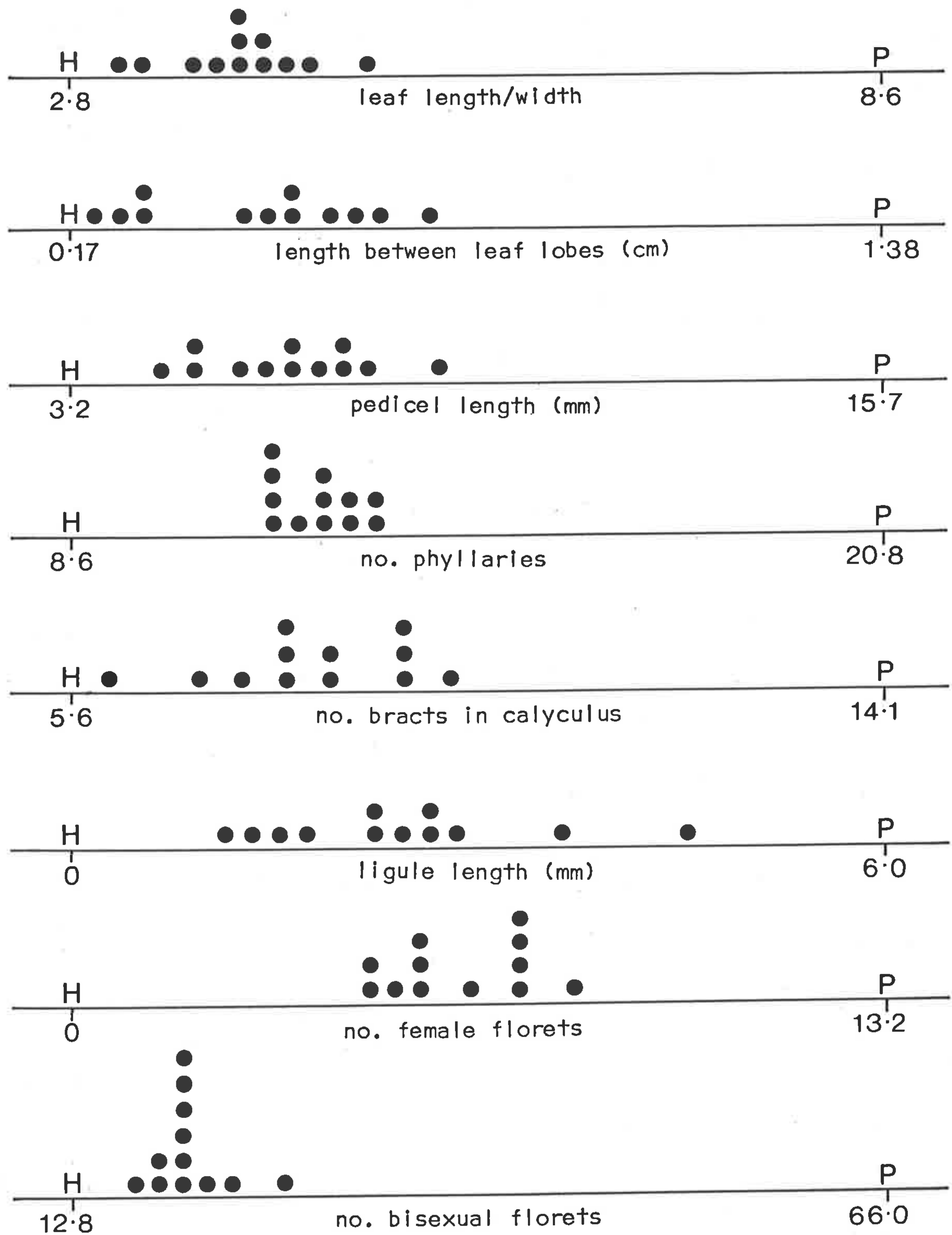


Fig. 8.13 Distribution of mean values of 8 characteristics in F1 hybrids between S. hypoleucus (H) and S. pterophorus (P).

8.3.4 The Rayed Gene Complex in *Senecio*

Belcher (1956) commented that "the relationship between the non-erechthitoid species of *Senecio* (those having female ray or bisexual disc florets in the marginal row) is poorly developed, since it has not passed beyond the crude separation into discoid versus radiate species" and that the "soundness of this separation has been questioned by virtually every competent syntherologist from the time of Linnaeus to the present." Although I agree that the presence or absence of ray florets is by itself a poor indicative of subgenera in *Senecio*, rayed and rayless Australian species of *Senecio* do belong largely to distinct groups based on other morphological and cytogenetic characters. Exceptions are the rayed *S. linearifolius* and *S.* sp A that are otherwise related to truly discoid species such as *S. hypoleucus*. Evidence of intraspecific variation patterns, geographic distribution and karyotype similarities (see Chapters 3 and 7) suggest that *S. linearifolius* and *S.* sp. A may be the result of introgression between a radiate and discoid species.

Of the species included in this study rayed and rayless forms occur only in New Zealand forms of *S. lautus* subsp. *lautus*. All Australian subspecies of *S. lautus* are self-incompatible and have ray florets. As *S. lautus* subsp. *lautus* is self-compatible, the less common rayless forms may represent loss of an insect-attracting floral structure that is redundant in an autogamous plant. Rayed and rayless forms of the European *S. vulgaris* are also known, but in this case, both forms are self-compatible and the rayed forms are less common. Trow (1912) demonstrated that inheritance of the rayed gene in *S. vulgaris* is disomic, and designated rayed forms as RR, half-rayed (with short rays) forms as Rr and rayless forms as rr. Hull (1974a) suggested alternative

designations as dominance of the rayed gene is incomplete, but later authors (Richards 1975, Weir and Ingram 1980) have followed Trow to avoid confusion. In accordance with the latter publications the same symbols are used in this discussion.

Hybrids 1 to 6 in Table 8.1 (pg.360) represent crosses between a rayed and a rayless species of *Senecio*. In each case hybrid plants were rayed, and the rays were shorter than those of the rayed parents. As rayless forms did not occur in the rayed parent species, the parents were assumed to be homozygous for R and similarly, rayless parents were assumed to be homozygous for r. Genotypes of hybrid plants can therefore be represented as follows:

S. pterophorus x *S. hypoleucus*	Rrrr
S. pterophorus x *S. glomeratus*	Rrrr
S. pterophorus x *S. picridioides*	Rrrr
S. lautus x *S. biserratus*	RRrrrrr
S. linearifolius x *S. bipinnatisectus*	RRRrrr
S. linearifolius x *S.* sp. B	RRRrrr

Three of the hybrids were represented by more than one plant. In the case of *S. pterophorus* x *S. glomeratus* and *S. lautus* x *S. biserratus*, the ray number and ray morphology of all hybrid plants was identical. However, variation between hybrid plants was extensive in the case of *S. pterophorus* x *S. hypoleucus* (Fig. 8.14). If the genotype of this cross is Rrrr then it would appear that the recessive rrr condition has a variable effect on the R gene. The different breeding systems of the rayless parents may offer an explanation. *S. glomeratus* and *S. biserratus* are both self-compatible. If mutations occurred at the r locus then it is likely that they would either be eliminated or fixed in any one self-compatible population. However, *S. hypoleucus* is self-incompatible so that elimination or fixation of a mutant form of

r would take longer, hence outcrossing could result in a variety of r genotypes (e.g. $r_1r_2r_3$, $r_1r_3r_3$ etc.) in one population. The different patterns of variation in hybrid ray morphology may therefore reflect different levels of genotypic variation in the rayless parents.

8.3.5 Origins of Decaploid Species

Two Australian species, S. vagus (2N=98) and S. biserratus (2N=100) are decaploid. Other species native to Australia are either tetraploid (2N=40), hexaploid (2N=60) or octoploid (2N=80). As diploids are present only by recent introduction, it would seem likely that both decaploids are the product of hybridization between a tetraploid and hexaploid species, either by direct combination of unreduced gametes (60 + 40 = 100) or by the combination of unreduced gametes in a pentaploid hybrid (60 x 40 = 50, 50 + 50 = 100). Likely parents of S. vagus and S. biserratus were searched for by comparing external morphology, karyotype morphology and DNA amounts of all other species.

S. vagus has 42.90 pg of DNA per 4C nucleus. As the majority of hexaploids have between 18 and 19 pg a likely tetraploid parent must have about 25 pg per 4C nucleus. Such a value occurs only in S. amygdalifolius (26.87 pg) - all other tetraploids differing by at least 5 pg. However, percentage similarity values of karyotypes do not support such an ancestry for S. vagus as most of its chromosomes are smaller than those of S. amygdalifolius and larger than those of hexaploid species. In Chapter 6 it was concluded that species with high DNA amounts per genome (such as S. amygdalifolius) were derived from species with smaller DNA amounts per genome, and that high ploidy levels appeared to retard further DNA increases. S. vagus might there-

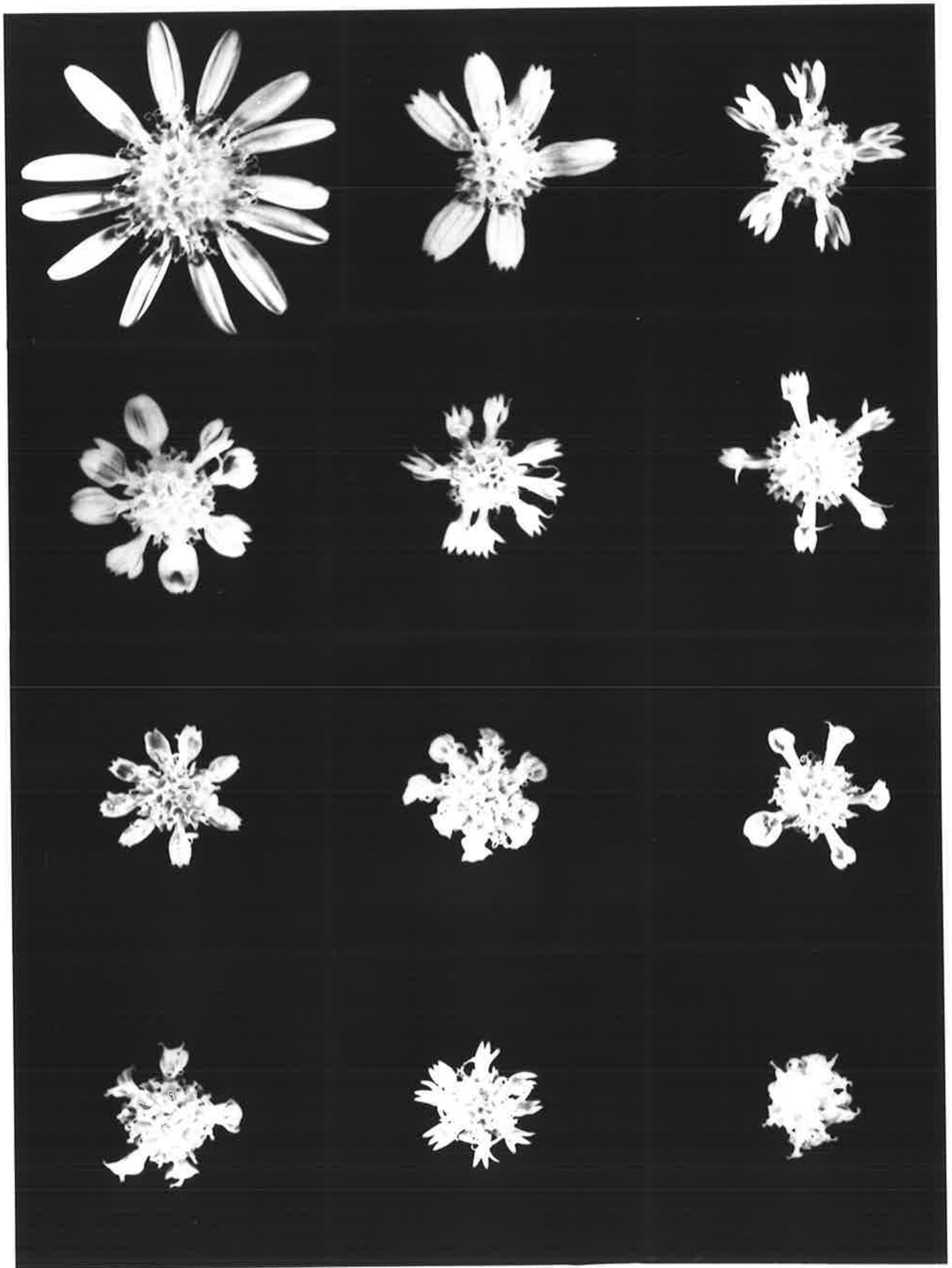


Fig. 8.14 Capitula of natural hybrids between Senecio pterophorus (upper left) and S. hypoleucus (lower right). All magnified 3.5X.

fore have formed when all genomes were comparatively small. If S. vagus was later subjected to a similar selective pressure for increased DNA amount as S. amygdalifolius, then in view of the ploidy levels (10x and 4x) the genome size of S. vagus would increase by a much smaller amount. Such a sequence of evolutionary events would explain why the karyotype of S. vagus does not represent a combination of karyotypes found in extant Senecio species.

The external morphology of S. biserratus is typical of other inbreeding erechthitoid species (Group 3). It is therefore likely that the parent species of S. biserratus also belong to Group 3. If DNA amounts per genome are compared (Table 6.6, pg.293), then it is apparent that S. biserratus has less DNA per genome than all species of Group 3B with the exception of S. bipinnatisectus. S. biserratus and S. bipinnatisectus are also related by one very acrocentric chromosome found in no other species. If S. bipinnatisectus is the hexaploid parent of S. biserratus then the most likely tetraploid parent on the basis of DNA amounts in S. quadridentatus. DNA amounts per 4C nucleus are 12.75 pg (S. quadridentatus), 13.98 pg (S. bipinnatisectus) and 25.27 pg (S. biserratus), which are in close agreement if S. biserratus is an allopolyploid of S. quadridentatus and S. bipinnatisectus. To further test the prediction, a karyotype of S. biserratus was synthesized by adding together karyotypes of S. quadridentatus and S. bipinnatisectus. Synthetic and actual karyotypes were then compared by the method described in Chapter 7. Total matches of one set with the other were 96% for S. biserratus and 82% for S. quadridentatus + S. bipinnatisectus. Of the 50 chromosomes in each karyotype 76% matched uniquely. Other combinations were attempted but the next highest unique match with S. biserratus was 58% using the combination of S. quadridentatus

and *S. anethifolius*. Karyotypes therefore support *S. quadridentatus* and *S. bipinnatisectus* as likely parents of *S. biserratus*, but indicated that some structural changes have occurred in karyotypes of either the parents or their allopolyploid.

8.3.6 Hybridization and Polyploidy in *Senecio* and Senecioneae

When viewed as a whole, speciation in *Senecio* has occurred mainly at the tetraploid level (Lawrence 1980), but in Australia, tetraploids and hexaploids are almost equally abundant. Hexaploids could have arisen either by the combination of unreduced gametes in a triploid hybrid (which assumes the presence of a diploid), or by the combination of an unreduced and a reduced tetraploid gamete. As Australian hexaploids are more or less confined to this continent, and no diploids are known to occur in the area, the latter mode of hexaploid origin would seem most likely. The reduced and unreduced tetraploid gametes might have come from the same or very similar plants, or from different species provided that the two genomes in the reduced gamete were homologous or homeologous. For example, the unreduced gamete might be AABB but the reduced gamete could not then be AB (giving AAABBB) and instead must be of the form A'A' or C'C'. Alternatively the hexaploids may have formed as autopolyploids (ie. AAAA plus AA) but the adaptive inferiority of raw autopolyploids (discussed by Stebbins (1971)) suggests this pathway is less likely.

Australian hexaploid species can be subdivided into three groups as follows (groups in Chapter 3 shown in parentheses):

1) Perennial self-incompatible species with discoid capitula (Group 2A)

2) Perennial self-incompatible species with radiate capitula (Group 2B)

3) Annual self-compatible species with erechthitoid[1] capitula (Group 3B).

Species within each group are closely related, and although superficially diverse, the three groups are related by their distinctive achene morphology. I therefore believe it is likely that Australian hexaploid species evolved from one or at most two ancestral hexaploids, rather than by repeated and independent hexaploid formation.

A similar pattern is apparent at the tribal level. Sixty two of the 100 genera of Senecioneae have been cytologically investigated (Nordenstam, 1977). Of these, 28 are hexaploid and 31 are diploid or tetraploid (or obvious aneuploid derivatives of each). In the majority of cases hexaploid genera (such as the Australian Bedfordia) correspond to "cacalioid" genera. These are related by a continuous stigmatic surface, polarized endothecal tissue and a cylindrical filament collar (Nordenstam 1977). Diploid and tetraploid genera are largely "senecioid" with a divided stigmatic surface, radial endothecal tissue and a swollen filament collar. The morphological affinity between hexaploid genera again suggests that they may have arisen from a common ancestral hexaploid, rather than by repeated hexaploid formation.

Although repeated allopolyploid formation does not appear likely in the formation of hexaploid groups, it is likely that events involving hybridization are important for subsequent speciation within the groups. As discussed in Chapter 5, polyploidy buffers the formation of heterozygous gametes at the

---

[1] Marginal florets female and filiform, central florets bisexual and discoid.

expense of homozygous ones. An extreme case is the formation of fixed heterozygotes which may be polymorphic at a gene locus (e.g. AA A^1A^1 A^2A^2) but incapable of forming recombinant gametes. Hexaploid species are therefore likely to respond very slowly to directional selection, whereas changes at lower ploidy levels may be more rapid. A possible means of recombinant genotype formation at the hexaploid level, and in turn, of a rapid response to selective pressures is by introgressive hybridization between different species. Significantly, five of the nine natural hybrids listed in Table 8.1 have two hexaploid parents, and in each case some pollen was fertile. None of the remaining four hybrids occurred between two tetraploids. Harlan and deWet's (1975) suggestion that wide crosses are most likely to be successful at the highest ploidy levels is therefore supported in _Senecio_. Evolution of hexaploid species may therefore be related to two opposing aspects of polyploidy - the reduced likelihood of an intraspecific response to directional selection but the increased likelihood of successful interspecific hybridization.

If this is the case, then continued evolution of hexaploid species groups will depend on the geographic range of existing species. In this respect the Australian hexaploid groups previously mentioned differ. The majority of Group 2A species (self-incompatible, discoid perennials) are geographically isolated from one another in hilly refuges of the drier parts of Australia. Continued evolution of these species would therefore seem unlikely unless environmental changes allow interspecific contact. Alternatively, introgressive hybridization could occur with a more widely distributed tetraploid. Such an event may have occurred in the evolution of Group 2B hexaploids - _S. linearifolius_ and _S._ sp. A - that are most closely related

to Group 2A hexaploids, but having ray florets, are also linked to radiate tetraploids.

The majority of Group 3B species (self-compatible, erechthitoid annuals) are widely distributed in the wetter parts of the eastern states and frequently occur in mixed populations. Although self-compatible, the occurrence of three hybrids between Group 3B species and two hybrids between a Group 2B and a Group 3B species (see Table 8.1, pg.360) indicates that cross-pollination does occur. Continued hybridization between populations and occasional introgressive hybridization between species may therefore maintain a higher level of genotypic variation than expected in a self-compatible species, and may also permit a more rapid response to directional selection.

The arguments above are supported by the patterns of morphological variation within hexaploid groups, but contradict the general view that self-compatible species are usually less polymorphic than self-incompatible ones. However, such a view is based on intraspecific recombination at the diploid level, whereas the present discussion is of interspecific recombination within hexaploid groups of species that differ in their pattern of geographic distribution.

CHAPTER 9

General Conclusions

9.1 Systematics of Senecioneae in Australia

9.1.1 The generic status of species examined

9.1.2 Subdivisions of Australian species of _Senecio_

9.2 The application of current evolutionary theories

9.2.1 r- and K-selection

9.2.2 Recombination systems

9.2.3 The C-value paradox

9.2.4 Karyotype evolution

9.3 Polyploid evolution in Senecioneae and _Senecio_

9.4 The size of _Senecio_

9.1 Systematics of Senecioneae in Australia

9.1.1 The Generic Status of Species Examined

Although Senecioneae has been the subject of a number of recent reviews, there is still no published description of *Senecio* that includes microcharacters investigated during the last century. As a guide in the present study the following characteristics of a typical *Senecio* species were deduced from treatments of Senecioneae by Nordenstam (1977, 1978), Jeffrey et al. (1977) and Jeffrey (1979).

1. Stigmatic surface of two marginal lines
2. Style apices truncate, without a sterile appendage or median fascicle
3. Endothecal tissue "radial" (thickenings on side and end walls)
4. Filament collar basally swollen
5. Involucre uniseriate with a basal calyculus
6. Receptacle naked
7. Pappus bristles usually caducous and often dimorphic
8. Gametic chromosome numbers of N = 10, 20 or obvious aneuploid derivatives.

It was then apparent that a number of Australian species classified as *Senecio* in current floras possess characteristics that are not typical of the genus. Conclusions concerning the systematic position of these taxa were withheld until other aspects (i.e. reproductive biology, DNA amounts, and karyotypes) had been investigated, and are given below.

A few of the atypical characteristics may represent secondary or parallel evolution, so that their presence in *Senecio* is of little or dubious systematic significance at the generic level.

Such characteristics are (1) the occurrence of gametic chromosome numbers of N = 30 among homogamous discoid species and two closely related heterogamous radiate species (groups 2A and 2B in Chapter 3.6) which are otherwise typical of Senecio (2) the connate involucre of S. gregorii which might be an adaptation to allow the maturation of many large seeds and (3) the persistent pappus of S. magnificus, S. spathulatus and S. gregorii (three very different species) which may facilitate the distribution of large seeds by allowing them to be blown along the ground.

Other characteristics not typical of Senecio and found in some Australian species have no obvious adaptive significance. Secondary evolution in the genus would therefore seem to be unlikely. The characters and their occurrence in seven Australian species are given in Table 9.1. Perhaps the single most important character of the five listed is a continuous stigmatic surface, found by Nordenstam (1977, 1978) to be characteristic of "cacalioid" genera when combined with a cylindrical filament collar, "polarized" endothecal tissue and a gametic chromosome number of N = 30. Although six of the seven species in Table 9.1 have a continuous or superficially continuous stigmatic surface, they are otherwise "senecioid" with a basally swollen filament collar, "radial" endothecal tissue and gametic chromosome numbers of N = 19, 20, 40 or 49.

New and revised genera described and illustrated by Nordenstam (1978) were examined to see if any were recognised by a combination of characters similar to those found in Australian species. There are several examples, although no direct relationship with Australian species is implied. Odontodine (Jamaica), Phaneroglossa (South Africa), Dendrosenecio (Tropical Africa), Pladaroxylon and Lachanodes (both of St. Helena) each have a

TABLE 9.1

Occurrence of Characteristics Not Typical* of Senecio in Seven Australian Species Presently Assigned to the Genus

Characteristic	*S. gregorii*	*S. magnificus*	*S. velleioides*	*S. macranthus*	*S. pectinatus*	*S. vagus*	*S. amygdalifolius*
Stigmatic surface continuous or superficially continuous	-	+	+	+	+	+	+
Style apices domed or rounded	+	+	+	+	±	+	+
Style apices with a median fascicle	+	-	-	-	-	±	-
Filament collar very short and scarcely swollen	-	-	+	-	-	-	+
Calyculus absent	+	+	+	-	-	-	-

* typical characteristics of Senecio listed on page 384.

continuous or narrowly divided stigmatic surface but otherwise "senecioid" characteristics. A similar combination occurs in six of the seven species listed in Table 9.1. The remaining Australian species, Senecio gregorii, can be compared with the South American Iocenes as both are distinguished by a median fascicle at the style apices, an ecalyculate involucre but otherwise "senecioid" characteristics.

If an approach comparable with that of Nordenstam (1977,1978) is adopted for Australian species presently treated as Senecio, then those species listed in Table 9.1 will have to be treated as separate genera. As Jeffrey (1979) expressed some doubt as to the validity of Nordenstam's treatment of Senecioneae, species listed in Table 9.1 have been treated as Senecio throughout the present study and should perhaps retain that status until Senecio sensu stricto is better understood. However, it is my opinion that when the latter is achieved, the seven listed species will have to be transferred to new or other existing genera of Senecioneae.

9.1.2 Subdivisions of Australian Species of Senecio

The majority of species examined in the present study are in all respects typical of Senecio (as defined in the previous section of this chapter). In Chapter 3.6 all species were assigned to one of three groups on the basis of external morphology, and in subsequent chapters the validity of the grouping was strengthened by additional evidence of reproductive biology, chromosome numbers, DNA amounts and karyotype symmetry. Characteristics of each group are summarized in Table 9.2, but in view of opinions expressed in the preceeding part of this chapter, seven of the ten species originally assigned to group 1 are omitted (see Table 9.1).

TABLE 9.2

Characteristics of Subdivisions of Australian Species of *Senecio*

Group and Species	Capitulum Type	Growth Form	Breeding System	N
GROUP 1				
S. lautus *S. spathulatus*	heterogamous radiate	perennial herbs	outbreeding	20
S. glossanthus		ephemeral	inbreeding	20,40
GROUP 2A				
S. hypoleucus *S. odoratus* *S. cunninghamii* *S. anethifolius* *S. gawlerensis*	homogamous discoid	perennial shrubs	outbreeding	30
GROUP 2B				
S. linearifolius *S.* sp. A	heterogamous radiate	perennial shrubs	outbreeding	30
GROUP 3A				
S. quadridentatus *S. gunnii* *S. runcinifolius* *S.* aff. *apargiaefolius*	heterogamous discoid	perennial herbs	inbreeding	20
GROUP 3B				
S. glomeratus *S. hispidulus* *S.* sp. B *S.* sp. C *S. minimus* *S. picridioides* *S. bipinnatisectus* *S. squarrosus*	heterogamous discoid	annual herbs	inbreeding	30
S. biserratus				50

Belcher (1956) suggested that there are some erechthitoid species (group 3) which "clearly intergrade into the discoid group (group 2A) and others which intergrade into the radiate group (groups 1 and 2B)." Although introgressive hybridization appears possible between hexaploid groups (N = 30), the only plants with a morphology intermediate between that of major groups were F1 hybrids with a very low fertility. Fully fertile intermediates were not detected. I therefore believe that recognition of at least three sections, corresponding with the groups listed in Table 9.2, would be taxonomically useful.

9.2 The Application of Current Evolutionary Theories

In the introductory chapter it was suggested that the size of a genus might be indicative of its ability to adapt to a range of selective pressures - smaller genera being generally less adaptable than larger ones. A further suggestion was that current evolutionary theories might be restricted in their application, as they are usually deduced from small genera (less than 100 species) that are amenable to study. Observations of Australian species of Senecio were compared with predictions of a number of theories and models with the following conclusions.

9.2.1 r- and K-selection

The r- and K-selection model (see Chapter 4) suggests that in stable density-dependent environments K-selection will favour late reproductive maturity, few large young (seeds) a long life and a small reproductive effort. In unstable, density-independent environments r-selection will favour early reproductive maturity, many small young, a short life and a large reproductive effort.

At first the model did not appear to be supported by *Senecio* as annuals and perennials are equally successful in unstable environments, although the few species occurring in comparatively stable environments are perennials. However, the r- and K-selection model was based largely on zoological examples and therefore does not include differences in breeding systems that occur in plants. All annual Australian species of *Senecio* are inbreeders whereas all perennials are outcrossers. Previous authors have suggested that the primitive Compositae were likely to be outbreeding perennial shrubs. It was therefore concluded that a predominance of r-selection resulted in a reduction in the age of reproductive maturity among perennial species of *Senecio* so that they would be able to compete in unstable environments. The later evolution of inbreeding in some species would ensure at least some seed set so that these species (annuals) senesced after reproducing. However, as the age at reproductive maturity, the size and number of seeds and the reproductive effort of inbreeding annuals and outbreeding perennials is similar, both are equally well adapted to unstable environments. Longevity may therefore only be important in a stable density-dependent environment, provided all other factors are equal.

9.2.2 Recombination Systems

Grant (1958, 1975) listed nine factors thought to regulate recombination of genotypes in plants. All factors are considered in Chapters 4 to 8, but the four most frequently discussed in the literature - namely chromosome number, chiasma frequency and position, breeding system and generation length - were discussed in detail in Chapter 5. Although *Senecio* does generally support Grant's hypotheses concerning recombination systems (that in unstable environments recombination systems will be "restricted"

rather than "open" or "closed") it was again necessary to clarify the suggested effects of regulatory factors.

Grant used the term "generation length" in his list of factors and the term has most commonly been equated with longevity (i.e. annual versus perennial) by other authors. Australian species of *Senecio* are either ephemeral, annual or perennial, but to use the terminology of Bennett (1972) the perennials are facultative - being able to flower in the first season. The number of recombinant gametes produced will therefore depend on the age at reproductive maturity. Generation length or longevity will affect the number of recombinant progeny expressed, but only in stable environments in which population replacement times are important.

A major difference in interpretation is that Grant discussed only changes in basic chromosome numbers whereas variation in *Senecio* is the result of polyploidy. Grant suggested that recombination is increased as chromosome numbers increase, but in the case of polyploidy, the reverse applies as buffering of intermediate heterozygous genotypes will restrict the formation of recombinant genotypes. Darlington's recombination index (the sum of the haploid chromosome number and the number of chiasmata per cell) therefore has little meaning in cases involving polyploidy.

The extent of variation in factors regulating recombination in *Senecio* also illustrated that generalizations based on a limited number of factors can lead to difficulties. For example, suggested correlations between breeding systems, longevity and environmental stability do not apply in the case of *Senecio* as recombination is "balanced" by a wide range of additional factors.

9.2.3 The C-value Paradox

Variation in DNA amounts of *Senecio* were found to be comparatively large (see Chapter 6). Among species native in Australia there is a 6.4-fold difference in DNA amounts per nucleus and a 5.6-fold difference in amounts per chromosome. Differences increase to 10.2-fold and 8.5-fold, respectively, if the four exotic taxa examined are included.

Opinions differ as to why related species may have very different DNA amounts per nucleus. Some argue that part of the genome is "selfish" or "parasitic" as it is not determined by natural selection acting on the phenotype. Others suggest that the known nucleotypic effects of DNA amounts on the phenotype are sufficient evidence that DNA amounts are selected for.

DNA amounts were compared with cell size, cell cycle times (inferred) minimum generation times and environmental conditions. Although the evidence is not conclusive, general correlations observed are best explained in terms of natural selection - that is, that plants in different environments actually require different genome sizes. The likely direction of evolutionary changes in genome sizes was also examined. It was thought that further changes in genome size might be restricted at higher ploidy levels and that high polyploids might therefore indicate earlier genome sizes. On this basis the primitive or basic genome size of *Senecio* is thought to lie between 1.5 and 1.8 picograms of DNA (per genome of 10 chromosomes). If this is the case then the genome size of specialized ephemerals has decreased whereas the genome size of some morphologically primitive species has increased. In the latter case, most of the species now occur in the comparatively stable but congested environments of warm

wet sclerophyll forests. The larger genome size and associated large cell size may therefore be an adaptation which allows a rapid differentiation of mass in a very competitive environment.

9.2.4 Karyotype Evolution

Changes in ploidy level and in genome size are both responsible for major karyotypic differences among *Senecio* species. However, the mean chromosome arm ratio and the ratio of the longest divided by the shortest chromosome are characteristics independent of chromosome number and absolute size. Ratios indicated that each morphologically defined group of species can also be defined by its karyotype symmetry (see Chapter 7). It is generally thought that karyotype symmetry decreases in the most specialized groups. Outcrossing radiate shrubs are thought to be the most primitive members of *Senecio*. Although some of these have the most symmetrical karyotypes others have asymmetrical karyotypes apparently caused by unequal increases in arm lengths with increasing genome size. Evidence from *Senecio* also contradicts the suggestion that species with lower chromosome numbers have the most asymmetrical karyotypes, but again, the contradiction is due to changes by polyploidy rather than by aneuploidy in *Senecio*. Two factors may contribute to the greater asymmetry of high polyploid karyotypes - (1) as each chromosome is represented several times structural changes may be more readily tolerated and (2) as high polyploids are likely to have formed by events involving hybridization, differences in parental karyotypes might contribute to the karyotype asymmetry of their derivative allopolyploid.

9.3 Polyploid Evolution in Senecioneae and *Senecio*

The taxonomic treatment of Senecioneae by Nordenstam (1977, 1978) and the obvious significance of polyploidy in the development of both *Senecio* and Senecioneae can be combined in a speculative evolutionary scheme. It is then possible to suggest likely origins and development of the elements of Senecioneae occurring in Australasia.

As circumscribed by Nordenstam (1977, 1978) Senecioneae contains two major groups of genera that are connected by a number of intermediates. The groups differ in distribution, morphological development and ploidy level as follows:

1. Truly "cacalioid" genera (see Chapter 2.12) are especially rich in the Mexican region but are well developed in the New World and in eastern Asia. All have comparatively simple style and stamen morphology (i.e. continuous stigmatic surfaces and a cylindrical filament collar) and a gametic chromosome number of N = 30 (e.g. *Cacalia*, c. 50 spp.; *Cremanthodium*, c. 60 spp.; *Roldana*, c. 50 spp.)

2. Truly "senecioid" genera are apparently centred in Africa but are well developed in all parts of the world. All have a more specialized style and stamen morphology (i.e. divided stigmatic surfaces and a basally swollen filament collar) and gametic chromosome numbers of N = 10, 20 or obvious aneuploid derivatives (e.g. *Senecio*, c. 1500 spp.; *Othonna*, c. 150 spp; *Europs*, 98 spp.).

3. Intermediate genera have no obvious phytogeographic center, mixed style and stamen morphology and a gametic chromosome number frequently of N = 20 (e.g. *Odontocline*, 6 spp; *Dendrosenecio*, 3 spp; *Scrobicaria*, 2 spp.).

My view of a likely series of events in the evolution of Senecio and Senecioneae is based on two general premises. The first is that the progenitor of Senecioneae had "cacalioid" style and stamen morphology as these characteristics appear to be most primitive in the tribe (see Chapter 3.6). However, unlike extant "cacalioid" genera which are hexaploid (N = 30) the progenitor of Senecioneae would be diploid (N = 10). Furthermore, as extant "cacalioid" genera are best developed in the Mexican region (Nordenstam 1977) a similar locality would seem likely for the origin of Senecioneae.

The second premise is that polyploidy has a differential effect on evolutionary rates (see discussions in Chapter 5 and Stebbins (1980). In other words, the buffering effects of polyploidy on recombinant genotype formation will slow the rate with which evolutionary changes can occur. Polyploids formed at an early stage in the evolutionary history of a group may therefore retain a more primitive morphology than polyploids derived at a later stage from morphologically advanced diploids. It is thought (see Chapter 5.3.1.2) that allopolyploids of related taxa will be most likely to succeed, rather than strict autopolyploids or allopolyploids of widely separated taxa. The former allopolyploid type may later hybridize with other allopolyploids so that very different genotypes are combined. High polyploids of hybrid origin are likely to have the advantages of a buffered heterozygous condition as well as the potential to produce novel heteromeric enzymes. Such advantages may account for the initial extension of polyploid distributions beyond that of their diploid progenitors.

The evolution of Senecioneae is therefore seen as follows (see also Fig. 9.1).

1. The diploid (N = 10) progenitor of Senecioneae most probably had a "cacalioid" style and stamen morphology, and arose in the Mexican region (see notes above).

2. An early evolutionary event was then the formation of hexaploids (N = 30) with "cacalioid" morphology that extended their range of distribution beyond that of diploids. However, because of their higher ploidy levels morphological evolution was restricted, so that extant "cacalioid" genera (all hexaploid) retain comparatively primitive style and stamen characteristics. Other characteristics of greater adaptive significance may have been subjected to greater selective pressures, so that with time, localized differentiation of "cacalioid" hexaploids occurred.

3. A second evolutionary event may then have been the formation of some diploids with partially "senecioid" morphology. Present distributions of intermediate genera suggest that intermediate progenitors must have been widely distributed, but they were not very successful in the long term. Nearly all cytologically known genera with intermediate morphology are tetraploid, so that polyploidy may again have preserved some morphological characteristics.

4. Changing environmental conditions may then have led to the extinction of less successful "cacalioid" diploids and tetraploids and most intermediate diploids, while at the same time favouring diversification of truly "senecioid" diploids in Africa. In the case of Senecio, tetraploid species are now more numerous and more widely distributed than diploids (Lawrence 1980). The distribution may indicate that polyploidy in Senecio is a comparatively recent development, and that adaptive radiation of polyploids is perhaps still occurring. However, the numbers and

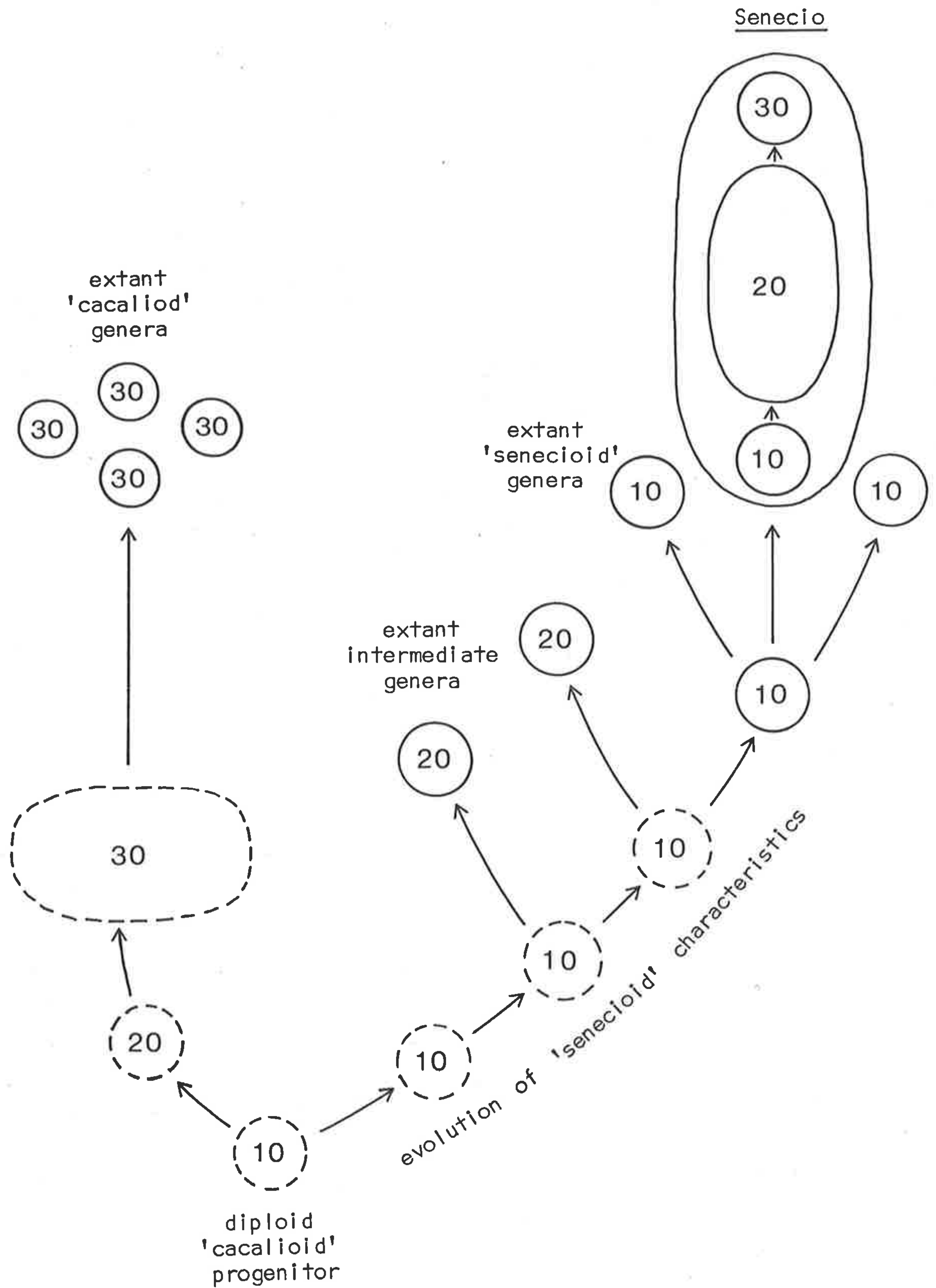


Fig. 9.1 Postulated scheme of evolution of Senecioneae (see text for explanation). Broken circles indicate extinct progenitors; gametic chromosome numbers shown within circles.

general success of polyploid species may also indicate that the original diploid stock was comparatively large and diverse.

5. Although all "cacalioid" genera are hexaploid (N = 30), hexaploids are generally rare in *Senecio* (Nordenstam 1977) and comprise the greater proportion of species only in Australia (Lawrence 1980). A possible explanation is that in most parts of the world, competition from extant diploids or tetraploids prevented the successful long-term establishment of hexaploid *Senecio* species. However, if hexaploids were formed from a small number of migrant tetraploid species in Australia, then initial radiation at the hexaploid level may have met with less competition. The present distribution of Australian hexaploid species of *Senecio* with homogamous discoid capitula does suggest that the group was once widely distributed, but the present relict distributions may be further evidence of the general inability of hexaploids to respond rapidly to changing selective pressures.

A final point is the migration route by which each element of Senecioneae reached Australia. If the evolutionary scheme proposed above is correct, then it would seem likely that "cacalioid" hexaploid taxa were first to arrive. These are represented by *Bedfordia* and *Brachyglottis* in Tasmania, and by *Brachyglottis*, *Dolichoglottis*, *Urostemon* and *Traversia* in New Zealand (all endemic to Australasia). If "cacalioid" progenitors migrated from Asia through New Guinea and across Australia, then it is difficult to explain why no relict populations occur in the latter two regions. A more reasonable explanation in view of present distributions is that "cacalioid" genera migrated to southern Australia from South America at a time when both continents were linked. Such a suggestion presupposes a much earlier ancestry for Compositae than is generally accepted, but

supports the suggestion by Turner (1977) that the origins of Compositae date back to at least the Cretaceous. If such a migratory pathway was possible, Australian taxa with an intermediate morphology (i.e. those discussed in part 1.1 of this chapter) might also have arrived at a similar time.

In the scheme suggested *Senecio* is thought to have evolved at a later point in time, so that migration is likely to have occurred across continents positioned much as they are today. Progenitors of Australian species of *Senecio* would therefore have first reached the most northerly parts of Australia via Asia - a pathway supported by the distribution of diploid and tetraploid species on a world-wide basis (see Lawrence 1980).

9.4 The Size of *Senecio*

A final question is the reason why *Senecio* is the largest angiosperm genus. Part of the answer must be that *Senecio* belongs to Compositae - the general characteristics of which have produced the largest and most widespread angiosperm family. However, *Senecio* (and most Senecioneae) differ from other members of Compositae in two respects. The first is the specialized uniseriate involucre of interlocking bracts which might have resulted in more efficient seed dispersal. The second and perhaps more important difference is that evolution in *Senecio* occurred predominantly at the tetraploid level. Polyploidy does occur in other genera of Compositae, but it would appear (from reviews of the tribes edited by Heywood, Harbourne and Turner 1977) that aneuploidy is generally more important. A large proportion of Compositae species occur in unstable environments so that maintenance of the diploid condition or reduction by aneuploidy would contribute to restricted

recombination systems thought to be advantageous in such environments. However, as suggested in Chapter 5, polyploidy may be an alternative means of restricting recombination that has the added advantage of a greater potential for gene polymorphisms. This advantage, combined with introgressive hybridization, structural rearrangements of chromosomes and occasional gene mutations might account for the extensive radiation and subsequent speciation in *Senecio*.

REFERENCES

Ali, S. I. (1964a). *Senecio lautus* complex in Australia. I. Taxonomic considerations and discussion of some of the related taxa from New Zealand. *Aust. J. Bot.* 12, 282-91.

Ali, S. I. (1964b). *Senecio lautus* complex in Australia. II. Cultural studies of populations. *Aust. J. Bot.* 12, 292-316.

Ali, S. I. (1964c). The effect of gibberellic acid on flowering in the montane race of *Senecio lautus*. *Biologia (Lahore)* 10, 39-45.

Ali, S. I. (1966). *Senecio lautus* complex in Australia. III. The genetic system. *Aust. J. Bot.* 14, 317-27.

Ali, S. I. (1968). *Senecio lautus* complex in Australia. IV. The biology of the complex. *Phyton* 13, 53-62.

Ali, S. I. (1969). *Senecio lautus* complex in Australia. V. Taxonomic interpretations. *Aust. J. Bot.* 17, 161-76.

Antonovics, J. (1968). Evolution in closely adjacent plant populations. V. Evolution of self-fertility. *Heredity* 23, 219-38.

Arroyo, M. T. K. (1973). Chiasma frequency evidence on the evolution of autogamy in *Limnanthes floccosa* (Limnanthaceae). *Evolution* 27, 679-88.

Baggøe, J. (1977). Microcharacters in the ligules of the Compositae. *In* 'The Biology and Chemistry of the Compositae', ed. V. H. Heywood, J. B. Harborne and B. L. Turner. Vol. 1, pp. 119-40. (Academic Press: London.)

Bachmann, K., Goin, O. B., and Goin, C. J. (1974). Nuclear DNA amounts in vertebrates. *Brookhaven Symp. Biol.* 23, 419-50.

Bailey, F. M. (1900). 'The Queensland Flora', Pt. 3. (Government Printer: Brisbane.)

Baker, H. G. (1955). Self-incompatibility and establishment after 'long distance' dispersal. Evolution 9, 347-49.

Baker, H. G. (1959). Reproductive methods as factors in speciation in flowering plants. Cold Spring Harbor Symp. Quant. Biol. 24, 177-91.

Beadle, N. C. W., Evans, O. E., and Carolin, R. C. (1976). 'Flora of the Sydney Region.' (Reed: London.)

Belcher, R. O. (1956). A revision of the genus Erechtites (Compositae), with inquiries into Senecio and Arrhenechtites. Ann. Mo. Bot. Gard. 43, 1-85.

Bennett, M. D. (1971). The duration of meiosis. Proc. R. Soc. Lond. B 178, 277-99.

Bennett, M. D. (1972). Nuclear DNA content and minimum generation time in herbaceous plants. Proc. R. Soc. Lond. B 181, 109-35.

Bennett, M. D., and Smith, J. B. (1972). The effect of polyploidy on meiotic duration and pollen development in cereal anthers. Proc. R. Soc. Lond. B 181, 81-107.

Bennett, M. D., and Smith, J. B. (1976). Nuclear DNA amounts in angiosperms. Philos. Trans. R. Soc. Lond. B 274, 227-74.

Bentham, G. (1866). 'Flora Australiensis.' Vol. 3. (Lovell, Reeve, and Co.: London.)

Bentham, G. (1873a). Compositae. In 'Genera Plantarum'. ed. G. Bentham and J. D. Hooker. Vol. 2, pp. 163-533.

Bentham. G. (1873b). Notes on the classification, history and geographic distribution of the Compositae. J. Linn. Soc. Lond., Bot. 13, 335-577.

Black, J. M. (1928). Additions to the flora of South Australia. No. 26. _Trans. R. Soc. S. Aust._ 52, 225-30.

Black, J. M. (1929). 'Flora of South Australia'. (Government Printer: Adelaide.)

Black, J. M. (1932). Additions to the flora of South Australia No. 30. _Trans. R. Soc. S. Aust._ 56, 47.

Black, J. M. (1965). 'Flora of South Australia'. Second edition. (Government Printer: Adelaide.)

Boivin, A., Vendrely, R., and Vendrely, C. (1948). L'acide désoxyribonucléique du noyau cellulaire, dé positaire des caractéres héréditaires; arguments d'ordre analytique. _C. R. Acad. Sci. (Paris)_ 226, 1061-63.

Brown, H. S. (1961). Differential chiasma frequencies in self-pollinating and cross-pollinating species of the genus _Gilia_. _Aliso_ 5, 67-81.

Cabrera, A. L., and Ré, R. R. (1965). Sobre un _Senecio_ adventicio en la provincia de Buenos Aires. _Revista de la Facultad de Agronomia, La Plata_. 41, 43-50.

Campbell, J. M., and Abbott, R. J. (1976). Variability of outcrossing frequency in _Senecio vulgaris_ L. _Heredity_ 36, 267-74.

Carlquist, S. (1976). Tribal interrelationships and phylogeny of the Asteraceae. _Aliso_ 8, 465-92.

Cassini, H. (1826). 'Opuscules phytologique'. Vols. 1 and 2. (Paris.)

Cassini, H. (1829). Tableau synoptique des Synanthérées. _Ann. Sci. Nat., Paris_. 17, 387-423.

Cassini, H. (1834). 'Opuscules phytologique'. Vol. 3. (Paris.)

Cavalier-Smith, T. (1977). Visualising jumping genes. Nature (Lond.) 270, 10-12.

Cavalier-Smith, T. (1978). Nuclear volume control by nucleo-skeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J. Cell. Sci. 34, 247-78.

Cavalier-Smith, T. (1980). How selfish is DNA? Nature (Lond.) 285, 617-18.

Chooi, W. Y. (1971). Variation in nuclear DNA content in the genus Vicia. Genetics 68, 195-211.

Colwell, R. N. (1951). The use of radioactive isotopes in determining spore distribution patterns. Am. J. Bot. 38, 511-23.

Crisci, J. V., and Stuessy, T. F. (1980). Determining primitive character states for phylogenetic reconstruction. Syst. Bot. 5, 112-35.

Cronquist, A. (1955). Phylogeny and Taxonomy of the Compositae. Am. Midl. Nat. 53, 478-511.

Cronquist, A. (1977). The Compositae revisited. Brittonia 29, 137-53.

Cruden, R. W. (1977). Pollen-ovule ratios: A conservative indicator of breeding system in flowering plants. Evolution 31, 21-46.

Curtis, W. M. (1963). 'A Student's Flora of Tasmania'. Pt. 2. (Government Printer: Tasmania.)

Darlington, C. D. (1939). 'The Evolution of Genetic Systems'. Cambridge University Press: Cambridge.)

Darlington, C. D. (1965). 'Cytology'. (J. and A. Churchill: London.)

Darlington, C. D., and LaCour, L. F. (1976). 'The Handling of Chromosomes.' Sixth edition, revised by L. F. LaCour. (George Allen and Unwin: London.)

Davis, P. H., and Heywood, V. H. (1963). 'Principles of Angiosperm Taxonomy'. (Oliver and Boyd: London.)

DeCandolle, A. P. (1836-37). 'Prodromus Systematis Naturalis Regni Vegetabilis'. Vols. 5 and 6. (Paris.)

Decosse, J. J., and Aiello, N. (1966). Feulgen hydrolysis: effect of acid and temperature. J. Histochem. Cytochem. 14, 601-604.

Deeley, E. M. (1955). An integrating microdensitometer for biological cells. J. Sci. Instrum. 32, 263-67.

Demetrius, L. (1975). Reproductive strategies and natural selection. Am. Nat. 109, 243-49.

deWet, J. M. J. (1968). Diploid-tetraploid-haploid cycles and the origin of variability in Dichanthium agamospecies. Evolution 22, 394-97.

deWet, J. M. J. (1971). Reversible tetraploidy as an evolutionary mechanism. Evolution 25, 545-48.

Dhir, N. K., and Miksche, J. P. (1974). Intraspecific variation of nuclear DNA content in Pinus resinosa Ait. Can. J. Genet. Cytol. 16, 77-83.

Doolittle, W. F., and Sapienza, C. (1980). Selfish genes, the phenotype paradigm and genome evolution. Nature (Lond.) 284, 601-03.

Dormer, K. J. (1961). The crystals in the ovaries of certain Compositae. Ann. Bot. (Lond.) 25, 241-54.

Dormer, K. J. (1962). The fibrous layer in the anthers of Compositae. New Phytol. 61, 150-153.

Drury, D. G. (1973a). Nodes and leaf structure in the classification of some Australian shrubby Senecioneae - Compositae. N. Z. J. Bot. 11, 525-54.

Drury, D. G. (1973b). Annotated key to the New Zealand shrubby Senecioneae - Compositae and their wild and garden hybrids. N. Z. J. Bot. 11, 731-84.

Drury, D. G., and Watson, L. (1965). Anatomy and the taxonomic significance of gross vegetative morphology in Senecio. New Phytol. 64, 307-314.

Drury, D. G., and Watson, L. (1966). A bizarre pappus form in Senecio. Taxon 15, 309-311.

Durrant, A. (1962). The environmental induction of heritable changes in Linum. Heredity 17, 27-61.

Eames, A. J. (1961). 'Morphology of the angiosperms'. (McGraw-Hill, New York.)

Evans, G. M. (1968). Nuclear changes in flax. Heredity 23,25-38.

Evans, G. M., Rees, H., Snell, C. L., and Sun, S. (1972). The relationship between nuclear DNA amount and the duration of the mitotic cycle. Chromosomes Today 3, 24-31.

Feulgen, R., and Rössenbeck, H. (1924). Mikroskopisch-chemischer Nachweiss einer Nucleinsäure von Typus der Thymonucleinsäure und die darauf beruhende elective Färbung von Zellkernen in mikroskopischen präparaten. Hoppe-Seyler's Z. physiol. Chem. 135, 203-48.

Fox, D. P. (1969). Some characteristics of the cold hydrolysis technique for staining plant tissues by the Feulgen reaction. J. Histochem. Cytochem. 17, 266-72.

Friedburg, S. H., and Davidson, D. (1970). Duration of S-phase and cell cycles in diploid and tetraploid cells in mixoploid meristems. Expl. Cell Res. 61, 216-18.

Fryxell, P. A. (1957). Mode of reproduction of higher plants. Bot. Rev. 23, 135-233.

Furuta, Y. (1970). DNA content per nucleus in Aegilops species. Wheat Inf. Serv. 30, 20-22.

Garber, E. D. (1950). Cytotaxonomic studies in the genus Sorghum. Univ. Calif. Publ. Bot. 23, 283-62.

Gibbs, P. E., Milne, C., and Vargas Carrillo, M. (1975). Correlation between the breeding system and recombination index in five species of Senecio. New Phytol. 75, 619-26.

Gierer, A. (1974). Molecular models and combinatorial principles in cell differentiation and morphogenesis. Cold Spring Harbor Symp. Quant. Biol. 38, 951-61.

Goepfert, D. (1974). Karyotypes and DNA content in species of Ranunculus L. and related genera. Bot. Not. 127, 467-89.

Gottlieb, L. D. (1971). Gel electrophoresis: New approach to the study of evolution. Bioscience 21, 939-43.

Gottlieb, L. D. (1976). Biochemical consequences of speciation in plants. In 'Molecular Evolution', ed. F. J. Ayala. pp. 123-40. (Sinauer: Sunderland, Massachusetts.)

Grant, V. (1958). The regulation of recombination in plants. Cold Spring Harbor Symp. Quant. Biol. 23, 337-63.

Grant, V. (1975). 'Genetics of Flowering Plants'. (Columbia University Press: New York.)

Grant, W. F. (1969). Decreased DNA content of Birch (Betula) chromosomes at high ploidy as determined by cytophotometry. Chromosoma (Berl.) 26, 326-36.

Grieve, B. J., and Blackall, W. E. (1975). "How to Know Western Australian Wildflowers'. (University of Western Australia Press: Perth.)

Gunge, N., and Nakatomi, Y. (1972). Genetic mechanisms of rare matings of the yeast _Saccharomyces cerevisiae_ heterozygous for mating type. _Genetics_ 70, 41-58.

Gupta, S. B. (1969). Duration of mitotic cycle and regulation of DNA replication in _Nicotiana plumbaginifolia_ and a hybrid derivative of _N. tabaccum_ showing chromosome instability. _Can. J. Genet. Cytol._ 11, 133-42.

Hamrick, J. L., Linhart, Y. B., and Mitton, J. B. (1979). Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. _Annu. Rev. Ecol. Syst._ 10, 173-200.

Harlan, J. R., and deWet, J. M. J. (1975). On Ö. Winge and a prayer: the origins of polyploidy. _Bot. Rev._ 41, 361-390.

Harper, J. L., Lovell, P. H., and Moore, K. G. (1970). The shape and size of seeds. _Annu. Rev. Ecol. Syst._ 1, 327-56.

Hayinarayana, G., and Murty, B. R. (1971). Cytological regulation of recombination in _Pennisetum_ and _Brassica_. _Cytologia_ 36, 435-48.

Hereford, L. M., and Rosbash, M. (1977). Number and distribution of polyadenylated RNA sequences in yeast. _Cell_ 10, 453-62.

Heywood, V. H., Harborne, J. B., and Turner, B. L. (Eds.) (1977a). 'The Biology and Chemistry of the Compositae'. (Academic Press: London.)

Heywood, V. H., Harborne, J. B., and Turner, B. L. (1977b). An overture to the Compositae. _In_ 'The Biology and Chemistry of the Compositae' ed. V. H. Heywood, J. B. Harbourne and B. L. Turner. Vol. 1, pp. 1-20. (Academic Press: London.)

Hinergardner, R. (1976). Evolution of genome size. In 'Molecular Evolution', ed. F. J. Ayala. pp. 179-99. (Sinauer: Sunderland, Massachusetts.)

Hoffman, O. (1894). Compositae. In 'Die naturlichen Pflanzenfamilien.' ed. A. Engler and K. Prantl. Vol 4, part 5, pp. 87-394.

Holm-Hansen, O. (1969). Algae: amounts of DNA and organic carbon in single cells. Science, 163, 87-88.

Hull, P. (1974a). Self fertilization and the distribution of the radiate form of Senecio vulgaris L., in Central Scotland. Watsonia 10, 69-75.

Hull, P. (1974b). Differences in esterase distribution detected by electrophoresis as evidence of continuing interspecific hybridization in the genus Senecio. Ann. Bot. (Lond.) 38, 697-700.

Hull, P. (1975). Selection and hybridization as possible causes of changes in the frequency of alleles controlling capitulum-type in Senecio vulgaris L. Watsonia 10, 395-402.

Hull, P. (1976). The influences of different degrees of interspecific hybridization with Senecio squalidus on the frequency of two morphs of Senecio vulgaris. Heredity 36, 67-72.

Ingram, R. (1977). Synthesis of the hybrid Senecio squalidus L. x S. vulgaris L. f. radiatus Hegi. Heredity 39, 171-73.

Ingram, R. (1978). The genomic relationship of Senecio squalidus L. and Senecio vulgaris L. and the significance of genomic balance in their hybrid S. x baxteri Druce. Heredity 40, 459-62.

Ingram, R., Weir, J., and Abbott, R. J. (1980). New evidence concerning the origin of inland radiate groundsel, S. vulgaris L. var. hibernicus Syme. New Phytol. 84, 543-46.

Jackson, R. C. (1971). The karyotype in systematics. Annu. Rev. Ecol.Syst. 2, 327-68.

Jackson, R. C. (1976). Evolution and systematic significance of polyploidy. Annu. Rev. Ecol. Syst. 7, 209-234.

Jain, S. K. (1976). The evolution of inbreeding in plants. Annu. Rev. Ecol. Syst. 7, 469-95.

Jeffrey, C. (1977). Corolla forms in Compositae - some evolutionary and taxonomic speculations. In 'The Biology and Chemistry of the Compositae', ed. V. H. Heywood, J. B Harborne and B. L. Turner. Vol. 1, pp. 111-18. (Academic Press: London.)

Jeffrey, C. (1979). Generic and sectional limits in Senecio (Compositae): II. Evaluation of some recent studies. Kew Bull. 34, 49-58.

Jeffrey, C., Halliday, P., Wilmot-Dear, M., and Jones, S. W. (1977). Generic and sectional limits in Senecio (Compositae) I. Progress report. Kew Bull. 32, 47-67.

Jones, K. (1978). Aspects of chromosome evolution in higher plants. Adv. Bot. Res. 6, 119-194.

Jones, R. N., and Brown, L. M. (1976). Chromosome evolution and DNA variation in Crepis. Heredity 36, 91-104.

Jones, R. N., and Rees, H. (1968). Nuclear DNA variation in Allium. Heredity 23, 591-605.

Kadir, Z. B. A. (1974). DNA values in the genus Phalaris (Gramineae). Chromosoma (Berl.) 45, 379-86.

Keyl, H. G. (1965). Demonstrable local and geometric increase in the chromosomal DNA of *Chironomus*. *Experientia* (Basel.) 21. 191-93.

Kiper, M., Bartels, D., and Kochel, H. (1979). Gene number estimates in plant tissues and cells. *Plant Syst. Evol., Suppl.* 2, 129-49.

Lawrence, M. E. (1980). *Senecio* L. (Asteraceae) in Australia: chromosome numbers and the occurrence of polyploidy. *Aust. J. Bot.* 28, 151-65.

Levitsky, G. A. (1931a). The morphology of chromosomes. *Bull. Appl. Bot. Genet. Plant Breed.* 27, 19-174.

Levitsky, G. A. (1931b). The karyotype in systematics. *Bull. Appl. Bot. Genet. Plant Breed.* 27, 220-40.

Levyns, R. (1950). *Senecio*. *In* 'Flora of the Cape Peninsula', ed. R. S. Adamson and T. M. Salter. pp. 808-818. (Juta: Cape Town.)

Lewis, D., (1954). Comparative incompatibility in angiosperms and fungi. *Adv. Genetics* 6, 235-85.

Lewis, W. H. (1980). Polyploidy in angiosperms: dicotyledons. *In* 'Polyploidy, biological relevance', ed. W. H. Lewis. pp. 241-68. (Plenum Press: New York.)

Linnaeus, C. (1754). 'Genera Plantarum'. Edition 5, pp. 375.

Lloyd, D. G. (1965). Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). *Contrib. Gray Herb. Harv. Univ.* 195, 3-134.

Lloyd, D. G. (1979). Parental strategies of angiosperms. *N. Z. J. Bot.* 17, 595-606.

Lloyd, D. G. (1980). Demographic factors and mating patterns in angiosperms. In 'Demography and Evolution in Plant Populations ed. O. T. Solbrig. pp. 67-88. (Blackwell Scientific Publications: London.)

Lousley, J. E. (1946). A new hybrid *Senecio* from the London area. *Rep. Bot. Exch. Club Brit. Is. 1943-4* 12, 869-74.

MacArthur, R. H., and Wilson, E. O. (1967). 'The Theory of Island Biogeography'. (Princeton University Press: Princeton.)

Maiden, J. H., and Betche, E. (1916). 'A Census of New South Wales Plants'. (Government Printer: Sydney.)

Martin, N. G. (1974). Nuclear DNA content of the Emu. *Chromosoma* (*Berl*.) 47, 71-74.

Martin, P. G., and Hayman, D. L. (1965). A quantitative method for comparing the karyotypes of related species. *Evolution* 19, 157-61.

Matérn, B. and Simark, M. (1969). On some statistical problems connected with the identification of chromosomes. *Inst. Skoglig Matemat. Stat., Stockholm. Research Notes* 11, 1-81.

Mather, K. (1943). Polygenic inheritance and natural selection. *Biol. Rev.* 18, 32-64.

Mather, K. (1973). 'Genetical Structure of Populations'. (Chapman and Hall: London.)

Maslin, P. P. (1952). Morphological criteria of phylogenetic relationships. *Syst. Zool.* 1, 49-70.

Maynard Smith, J. (1964). Group selection and kin selection. *Nature* (*Lond*.) 201, 1145-47.

McClintock, B. (1934). The relation of a particular chromosomal element to the development of nucleoli in *Zea mays*. *Z. Zellforsch. Mikrosk. Anat.* 21, 294-328.

Michener, C. D. (1970). Apoidea. In 'The Insects of Australia'. Sponsored by C.S.I.R.O., Canberra. pp. 943-51. (Melbourne University Press: Melbourne.)

Miksche, J. P. (1968). Quantitative study of intraspecific variation of DNA per cell in Picea glauca and Pinus banksiana. Can. J. Genet. Cytol. 10, 590-600.

Miksche, J. P. (1971). Intraspecific variation of DNA per cell between Picea sitchensis (Bong.) Carr. provenances. Chromosoma (Berl.) 32, 343-52.

Mirsky, A. E., and Ris, H. (1949). Variable and constant components of chromosomes. Nature (Lond.) 163, 666-67.

Monaghan, J. L., and Hull, P. (1976). Differences in vegetative characteristics among four populations of Senecio vulgaris L. possibly due to interspecific hybridization. Ann. Bot. (Lond.) 40, 125-28.

Moore, P. D. (1976). r, K and evolution. Nature (Lond.) 262, 351-52.

Nagl, W. (1974). Mitotic cycle time in seedling roots of perennial and annual Anthemideae (Asteraceae. Dev. Biol. 39, 342-46.

Nagl, W. (1979). Differential DNA replication in plants: a critical review. Z. Pflanzenphysiol. 95, 283-314.

Nagl, W., and Ehrendorfer, F. (1974). DNA content, heterochromatin, mitotic index and growth in perennial and annual Anthemideae (Asteraceae). Plant Syst. Evol. 123, 35-54.

Nagl, W., Frisch, B., and Frölich, E. (1979). Extra-DNA during floral induction? Plant Syst. Evol., Suppl. 2, 111-118.

Nordenstam, B. (1977). Senecioneae and Liabeae - systematic review. In 'The Biology and Chemistry of the Compositae', ed. V. H. Heywood, J. B. Harborne and B. L. Turner. Vol. 2, pp. 799-830. (Academic Press: London.)

Nordenstam, B. (1978). Taxonomic studies in the tribe Senecioneae (Compositae). Opera Bot. 44, 1-84.

Ohata, T., and Kimura, M. (1971). Functional organization of genetic material as a product of molecular evolution. Nature (Lond.) 233, 118-19.

Ohno, S. (1972). An argument for the genetic simplicity of man and other mammals. J. Hum. Evol. 1, 651-62.

Olorode, O. (1973). Identification of the genomic complement of Emilia praetermissa (Senecioneae - Compositae). Am. J. Bot. 60, 55-60.

Orgel, L. E., and Crick, F. H. C. (1980). Selfish DNA: the ultimate parasite. Nature (Lond.) 284, 604-607.

Ornduff, R. (1960). An interpretation of the Senecio lautus complex in New Zealand. Trans. R. Soc. N. Z. 88, 63-77.

Ornduff, R. (1964). Evolutionary pathways of the Senecio lautus alliance in New Zealand and Australia. Evolution 18, 349-60.

Ornduff, R. (1969). Reproductive biology in relation to systematics. Taxon 18, 121-33.

Ornduff, R., Mosquin, T., Kyhos, D. W., and Raven, P. H. (1967). Chromosome numbers in Compositae. VI. Senecioneae. II. Am. J. Bot. 54, 205-13.

Ornduff, R., Raven, P. H., Kyhos, D. W., and Kruckeberg, A. R. (1963). Chromosome numbers in Compositae. III. Senecioneae. Am. J. Bot. 50, 131-39.

Owczarzak, A. (1952). A rapid method for mounting pollen grains, with special regard to sterility studies. Stain. Technol. 27, 249-51.

Pai, M. D., Upadhya, S., Bhaskaran, S., and Swaminathan, M. S. (1961). Chromosome diminuation and evolution of polyploid species in Triticum. Chromosoma (Berl.) 12, 398.

Porter, C. L. (1967). 'Taxonomy of Flowering Plants'. Second Edition. (Freeman and Company: San Francisco.)

Price, H. J. (1976). Evolution of DNA content in higher plants. Bot. Rev. 42, 27-52.

Price, H. J., and Backmann, K. (1976). Mitotic cycle time and DNA content in annual and perennial Microseridinae (Compositae, Cichoriaceae). Plant Syst. Evol. 126, 323-30.

Price, H. J., Backmann, K., Chambers, K. L., and Riggs, J. (1980). Detection of intraspecific variation in nuclear DNA content in Microseris douglasii. Bot. Gaz. 141, 195-98.

Price, H. J., Sparrow, A. H., and Nauman, A. F. (1973). Correlations between nuclear volume, cell volume and DNA content in meristematic cells of herbaceous angiosperms. Experientia (Basel.) 29, 1028-29.

Price, H. J. Sparrow, A. H., and Nauman, A. F. (1974). Evolutionary and developmental considerations of the variability of nuclear parameters in higher plants. I. Genome volume, interphase chromosome volume, and estimated DNA content of 236 gymnosperms. Brookhaven Symp. Biol. 25, 390-421.

Reed, E. M. (1970). Thysanoptera. In 'The Insects of Australia'. Sponsored by C.S.I.R.O., Canberra. pp. 458-64. (Melbourne University Press: Melbourne.)

Rees, H. (1972). DNA in higher plants. Brookhaven Symp. Biol. 23, 394-418.

Rees, H., and Dale, P. J. (1974). Chiasmata and variability in Lolium and Festuca populations. Chromosoma (Berl.) 47, 335-51.

Rees, H., and Hazarika, M. H. (1969). Chromosome evolution in Lathyrus. Chromosomes Today 2, 158-65.

Rees, H., and Jones, G. H. (1967). Chromosome evolution in Lolium. Heredity 22, 1-18.

Rees, H., and Jones, R. N. (1972). The origin of the wide species variation in nuclear DNA content. Int. Rev. Cytol. 32, 53-92.

Rees, H., and Thompson, J. B. (1956). Genotypic control of chromosome behavior in rye. III. Chiasma frequency in homozygotes and heterozygotes. Heredity 10, 409-424.

Richards, A. J. (1975). The inheritance and behavior of the rayed gene complex in Senecio vulgaris. Heredity 34, 95-104.

Robinson, H. and Brettell, R. D. (1973). Tribal revisions in the Asteraceae. III. A new tribe, Liabeae. Phytologia 25, 404-407.

Rothfels, K., Sexsmith, E., Heimburger, M., and Krause, M. O. (1966). Chromosome size and DNA content of species of Anemone L. and related genera (Ranunculaceae). Chromosoma (Berl.) 20, 54-74.

Rydberg, P. A. (1927). Carduaceae, Liabeae, Neurolaeneae, Senecioneae. North American Flora 34, 289-360.

Salisbury, E. J. (1942). 'The Reproductive Capacity of Plants'. (Bell: London.)

Salisbury, E. J. (1961). 'Weeds and Aliens'. Second edition (Collins: London.)

Sheldon, J. C., and Burrows, F. M. (1973). The dispersal effectiveness of the achene-pappus units of selected compositae in steady winds with convection. New Phytol. 72, 665-75.

Skvarla, J. J., Turner, B. L., Patel, V. C., and Tomb, A. S. (1977). Pollen morphology in the Compositae and in morphologically related families. In 'The Biology and Chemistry of the Compositae', ed. V. H. Heywood, J. B. Harborne and B. L. Turner. Vol. 1, pp. 141-248. (Academic Press: London.)

Sokurova, E. N. (1973). The content of nucleic acids in the cells of yeasts belonging to various taxonomic groups. Mikrobiologiya 42, 1020-24.

Solbrig, O. T. (1977). Chromosomal cytology and evolution in the family Compositae. In 'The Biology and Chemistry of the Compositae,' ed. V. H. Heywood, J. B. Harborne and B. L. Turner. Vol. 1, pp. 267-82. (Academic Press: London.)

Sparrow, A. H., Price, H. J., and Underbrink, A. G. (1972). A survey of DNA content per cell and per chromosome of prokaryotic and eukaryotic organisms: some evolutionary considerations. Brookhaven Symp. Biol. 23:451-94.

Stace, C. A. (1977). The origin of radiate *Senecio vulgaris* L. Heredity 39, 383-88.

Stearn, W. T. (1966). 'Botanical Latin'. (David and Charles, Devon.)

Stearns, S. C. (1976). Life-history tactics: A review of the ideas. Q. Rev. Biol. 51, 3-47.

Stebbins, G. L. (1950). 'Variation and Evolution in Plants'. (Columbia University Press: New York.)

Stebbins, G. L. (1957). Self fertilization and population variability in the higher plants. Am. Nat. 41, 337-54.

Stebbins, G. L. (1958). Longevity, habitat, and release of genetic variability in higher plants. Cold Spring Harbor Symp. Quant. Biol. 23, 365-78.

Stebbins, G. L. (1971). 'Chromosomal Evolution in Higher Plants'. (Edward Arnold: London.)

Stebbins, G. L. (1971b). Adaptive radiation of reproductive characteristics in angiosperms, II: Seeds and Seedlings. Annu. Rev. Ecol. Syst. 2, 237-60.

Stebbins, G. L. (1974). 'Flowering Plants: Evolution above the Species Level'. (Harvard University Press: Massachusetts.)

Stebbins, G. L. (1980). Polyploidy in plants: Unsolved problems and prospects. In 'Polyploidy, biological relevance', ed. W. H. Lewis. pp. 495-520. (Plenum Press, New York.)

Stephens, S. G., and Finkner, M. D. (1953). Natural crossing in cotton. Econ. Bot. 7, 257-69.

Stergios, B. G. (1976). Achene production, dispersal, seed germination, and seedling establishment of Hieracium aurantiacum in an abandoned field community. Can. J. Bot. 54, 1189-97.

Swift, H. (1950). The constancy of desoxyribose nucleic acid in plant nuclei. Proc. Nat. Acad. Sci. U.S.A. 36, 643-54.

Sybenga, J. (1972). 'General Cytogenetics'. (North-Holland: Amsterdam.)

Teoh, S. B., and Rees, H. (1976). Nuclear DNA amounts in populations of Picea and Pinus species. Heredity 36, 123-37.

Thomas, C. A. (1971). The genetic organization of chromosomes. Annu. Rev. Genet. 5, 237-56.

Trow, A. H. (1912). On the inheritance of certain characters in the common groundsel, *Senecio vulgaris* L., and its segregates. *J. Genet.* 2, 239-76.

Turner, B. L. (1977). Fossil history and geography. *In* 'The Biology and Chemistry of the Compositae', ed. V. H. Heywood, J. B. Harborne and B. L. Turner. Vol. 1, pp. 21-40. (Academic Press: London.)

Turner, B. L., and Lewis, W. H. (1965). Chromosome numbers in the Compositae. IX. African species. *J. S. Afr. Bot.* 31, 207-17.

Turner, B. L., and Powell, A. M. (1977). Helenieae - systematic review. *In* 'The Biology and Chemistry of the Compositae' ed. V. H. Heywood, J. B. Harborne and B. L. Turner. Vol. 2, pp. 699-738. (Academic Press: London.)

Van't Hof, J. (1965). Relationships between mitotic cycle duration, S period duration and the average rate of DNA synthesis in the root meristem cells of several plants. *Expl. Cell. Res.* 39, 48-58.

Weir, J., and Ingram, R. (1980). Ray morphology and cytological investigations of *Senecio cambrensis* Rosser. *New Phytol.* 86, 237-41.

Williams, G. C. (1966). 'Adaptation and Natural Selection: A Critique of Some Current Evolutionary Thought'. (Princeton University Press: Princeton.)

Willis, J. H. (1957). Vascular flora of Victoria and South Australia. *Victorian Nat.* 73, 201-202.

Willis, J. H. (1972). 'A Handbook to Plants in Victoria. Vol. 2. Dicotyledons'. (University Press: Melbourne.)

Yang, D. P., and Dodson, E. O. (1970). The amounts of nuclear DNA and the duration of DNA synthesis period (S) in related diploid and autotetraploid species of oats. Chromosoma (Berl.) 31, 309-20.

APPENDIX 1

Chromosome Arm Measurements of Karyotypes Discussed in Chapter 7 and of *S. lautus* x *S. biserratus* (Chapter 8)

The method of karyotype construction is fully described in Chapter 7.2.1. In brief, the method is as follows. The total length of each replicate complement was determined and the original measurements converted to a percentage of the total. As absolute chromosome sizes were required, percent lengths were multiplied by the 4C nuclear DNA amount. Values for homologous chromosomes were then pooled and mean chromosome arm sizes calculated. Measurements given in the following pages are in picograms x 10^{-3}, and the sum of all chromosome arms in a complement equals the 2C nuclear DNA amount of that taxon.

Satellite chromosomes are indicated by parentheses around the appropriate chromosome number. If satellites were large enough to be measured, their value is also enclosed in parentheses and positioned next to the arm to which the satellite was attached as follows:

(14)	15	16	(17)	18	chromosome number, ( ) = satellite
			(21)		satellite attached to short arm
86	138	139	85	82	short arm
287	184	182	105	107	long arm
				(15)	satellite attached to long arm

List of Karyotypes (numbers 1 - 39 correspond to illustrations in Figure 7.1, number 40 is illustrated in Figure 8.)

GROUP 1A

1. Senecio magnificus
2. S. velleioides
3. S. amygdalifolius
4. S. macranthus
5. S. vagus subsp. eglandulosus

GROUP 1B

6 - 10 S. lautus subspp.
11. S. spathulatus
12. S. glossanthus
13. S. gregorii

GROUP 2A

14. S. hypoleucus
15. S. odoratus
16. S. cunninghamii
17. S. anethifolius
18. S. gawlerensis

GROUP 2B

19. S. linearifolius
20. S. sp. A

GROUP 3A

21. S. quadridentatus
22. S. gunnii
23. S. aff. apargiaefolius
24. S. runcinifolius

GROUP 3B

25. S. sp. B
26. S. squarrosus
27. S. bipinnatisectus
28. S. minimus
29. S. picridioides
30. S. glomeratus
31 - 32 S. hispidulus vars.
33. S. sp C
34. S. biserratus

EXOTIC SPECIES

35. S. vulgaris
36. S. pterophorus
37. S. mikanioides
38. S. discifolius

OTHER GENERA

39. Erechtites valerianaefolia

40. S. lautus x S. biserratus

1. *S. magnificus* N = 20 4C DNA = 31.7 pg.

1	2	3	4	5	6	7	8	9	10
555	508	356	375	402	392	319	317	298	309
658	598	626	563	501	471	519	485	493	463

11	12	13	(14)	15	(16)	17	18	19	20
360	271	306	269	304	243	279	228	284	264
405	443	390	147	367	215	368	397	329	323
			(256)		(192)				

2. *S. velleioides* N = 19 4C DNA = 33.1 pg.

1	2	3	4	5	6	(7)	8	9	(10)
525	396	381	329	158	400	284	300	197	181
569	672	643	655	810	558	633	605	680	674

11	12	13	14	15	16	17	18	19
153	141	348	334	84	135	311	137	128
694	697	471	475	719	620	426	530	472

3. *S. amygdalifolius* N = 19 4C DNA = 26.9 pg.

1	2	3	4	5	6	7	8	9	10
426	328	371	360	353	353	270	241	304	269
515	530	485	460	446	423	502	504	439	446

11	12	13	14	15	16	17	18	19
324	249	258	267	246	278	252	210	218
372	443	410	379	376	330	343	350	238

4. *S. macranthus* N = 20 4C DNA = 37.48 pg.

(1)	2	3	4	5	6	7	8	9	10
(413)									
180	473	559	481	536	315	433	320	351	404
802	833	622	651	587	792	589	639	567	512

11	12	13	14	15	16	17	18	19	20
316	385	311	376	361	305	302	280	274	150
542	445	495	428	428	469	433	441	424	515

5. *S. vagus* subsp. *eglandulosus* N = 49 4C DNA = 42.90 pg.

1	2	3	4	5	6	7	8	9	10
267	256	246	230	231	207	207	190	216	200
382	359	359	348	336	347	343	345	318	313

11	12	13	14	15	16	17	18	19	20
182	230	208	223	180	142	170	210	135	137
326	273	284	269	302	338	304	256	320	312

21	22	23	24	25	26	27	28	29	30
185	186	188	123	145	154	163	152	179	120
239	235	241	304	280	269	258	265	228	286

31	32	33	34	35	36	37	38	39	40
131	184	106	158	157	161	164	156	167	88
274	214	291	228	223	213	208	204	190	261

41	42	43	44	45	46	47	48	49
114	111	145	137	26	145	105	124	65
232	234	191	197	304	173	212	177	234

6. *S. lautus* subsp. *lautus* N = 20 4C DNA = 10.81 pg.

(1)	2	3	(4)	5	6	7	8	9	10
130	130	140	114	127	131	128	114	94	114
218	196	169	186	166	158	160	160	178	157

11	12	13	14	15	16	17	18	19	20
129	120	95	106	102	103	112	96	83	85
134	140	162	147	151	142	125	132	144	130

7. *S. lautus* subsp. *dissectifolius* N = 20 4C DNA = 10.63 pg.

1	2	(3)	4	5	6	(7)	8	9	10
148	138	116	130	135	131	106	126	119	108
176	162	177	167	151	141	171	148	151	161

11	(12)	13	14	15	16	17	18	19	20
128	102	114	99	113	101	110	88	92	84
140	164	140	154	136	147	125	147	138	124

8. *S. lautus* subsp. *maritimus* N = 20 4C DNA = 10.19 pg.

1	2	3	4	(5)	6	7	(8)	9	10
142	137	116	130	96	123	123	94	123	101
159	153	164	147	173	146	142	168	139	153

(11)	12	(13)	14	15	(16)	17	18	19	20
103	119	107	112	100	92	104	93	81	75
150	132	142	133	142	145	127	138	140	130

9. *S. lautus* subsp. *alpinus* N = 20 4C DNA = 10.74 pg.

1	(2)	3	4	5	6	7	8	9	10
138	115	130	119	131	123	117	120	121	118
174	189	170	174	159	164	168	155	152	154

11	12	13	14	15	16	17	18	19	20
116	120	106	107	112	104	117	85	98	87
154	146	159	151	136	141	125	147	134	134

10. *S. lautus* subsp. *lanceolatus* N = 20 4C DNA = 9.79 pg.

1	2	(3)	(4)	5	6	7	8	9	10
135	124	105	99	121	117	119	115	110	97
156	144	159	165	142	140	137	138	137	147

11	12	13	(14)	15	(16)	17	18	19	20
111	112	104	94	89	90	104	86	81	68
132	127	134	143	143	132	117	133	130	130

11. *S. spathulatus* N = 20 4C DNA = 12.28 pg.

1	(2)	3	4	5	6	7	8	(9)	10
164	139	151	128	145	127	125	144	129	132
196	203	187	209	187	200	194	175	190	185

11	12	13	14	15	16	17	18	19	20
141	137	127	127	118	127	105	106	106	87
174	171	178	171	171	154	165	158	152	155

12. S. glossanthus N = 20 4C DNA = 6.71 pg.

1	(2)	3	4	5	6	7	8	9	10
81 107	62 119	73 107	79 99	67 109	75 98	59 109	58 110	72 95	71 93
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
74 90	62 101	68 89	63 92	62 91	60 88	59 89	65 82	59 85	51 80

13. S. gregorii N = 20 4C DNA = 12.55 pg.

(1)	2	3	4	(5)	6	7	8	9	10
(153) 34 251	197 221	143 228	164 187	(128) 21 202	149 191	149 171	138 172	135 166	125 170
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
136 159	117 171	123 158	122 156	118 153	129 141	123 140	116 136	112 130	113 122

14. S. hypoleucus N = 30 4C DNA = 17.92 pg.

1	2	3	(4)	5	6	7	8	9	(10)
191 227	184 193	161 189	108 239	161 172	117 211	150 167	94 219	111 195	92 211
11	**12**	**13**	**(14)**	**15**	**16**	**(17)**	**(18)**	**19**	**20**
138 159	100 196	106 189	86 207	138 154	139 152	85 205	82 207	131 149	130 149
21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
134 143	83 191	105 163	123 139	120 141	118 141	114 132	102 144	90 152	109 119

15. *S. odoratus* N = 30 4C DNA = 18.14 pg.

1	2	(3)	4	5	6	7	8	9	10
214	182	108	159	92	149	108	157	99	100
236	210	267	192	256	191	222	172	212	209

11	12	(13)	14	(15)	16	17	(18)	19	20
92	133	86	110	85	135	95	89	134	134
213	172	217	193	213	162	198	200	152	149

21	22	23	24	25	26	27	28	29	30
129	124	122	125	120	92	123	99	109	106
145	147	149	143	144	170	138	146	130	118

16. *S. cunninghamii* N = 30 4C DNA = 18.90 pg.

1	2	3	4	5	6	7	(8)	9	10
236	214	190	122	151	157	126	106	112	133
254	229	215	270	226	215	221	231	224	196

11	12	(13)	14	15	16	17	(18)	19	20
139	126	120	126	122	128	128	90	131	122
184	182	186	179	179	170	169	203	160	166

21	22	23	24	25	26	(27)	28	29	30
124	115	116	123	119	96	93	97	110	101
163	163	162	142	142	165	165	157	139	125

17. *S. anethifolius* N = 30 4C DNA = 17.35 pg.

1	2	3	4	5	6	7	8	9	10
207	155	170	112	159	107	149	113	110	128
222	222	168	243	188	228	180	213	201	171

11	(12)	13	14	15	16	17	18	(19)	20
128	88	113	128	130	97	119	112	87	99
165	202	172	156	153	181	155	156	180	167

21	22	23	24	25	26	27	28	29	30
118	115	110	82	106	82	85	106	103	86
144	142	144	170	142	170	149	125	125	126

18. S. gawlerensis N = 30 4C DNA = 20.39 pg.

1	2	3	4	5	6	7	8	9	(10)
249	217	208	139	183	144	144	120	119	122
280	250	236	281	227	258	233	245	238	227

11	12	13	(14)	15	16	17	18	19	20
160	133	145	112	136	128	98	137	126	130
189	208	195	216	188	189	216	174	183	178

21	22	23	24	25	26	27	28	29	30
134	134	127	100	129	126	99	124	90	114
167	166	166	188	157	159	182	153	176	137

19. S. linearifolius N = 30 4C DNA = 18.02 pg.

1	2	(3) (106)	4	5	6	7	8	9	10
196	177	50	169	152	75	88	105	130	82
301	252	266	203	190	260	239	215	185	229

11	12	13	14	15	16	(17)	18	19	20
136	132	127	121	132	134	83	79	72	102
174	169	164	169	156	153	202	204	201	162

21	22	23	24	25	26	27	28	29	30
125	109	92	111	104	86	95	104	47	96
139	150	167	147	153	166	148	131	171	117

20. S. sp. A N = 30 4C DNA = 18.03 pg.

1	(2) (126)	3	4	5	6	7	8	9	(10)
230	28	183	163	79	148	155	100	132	77
254	265	221	240	266	195	169	222	186	234

(11)	(12)	13	14	15	16	17	18	19	(20)
75	123	82	73	129	133	85	128	124	78
233	180	215	218	161	153	198	154	156	201

21	22	23	24	25	26	27	28	29	30
127	118	80	113	114	122	116	111	83	77
146	151	183	147	145	132	137	128	153	159

21. S. quadridentatus N = 20 4C DNA = 12.75 pg.

1	(2)	3	4	5	6	7	8	9	10
207	187	188	188	170	174	167	169	148	141
240	239	216	209	206	197	186	181	185	179

11	12	13	14	15	16	17	18	19	20
132	140	133	112	125	116	108	102	93	98
172	163	152	160	146	141	142	129	123	110

22. S. gunnii N = 20 4C DNA = 14.04 pg.

1	2	(3)	4	5	6	7	8	9	10
222	189	210	199	197	178	159	175	163	168
279	271	248	232	222	217	226	199	195	185

11	12	13	14	15	16	17	18	19	20
156	95	152	145	104	117	111	114	110	105
187	246	178	169	208	156	158	132	127	118

23. S. aff. apargiaefolius N = 20 4C DNA = 14.10 pg.

1	2	3	(4)	5	6	7	8	9	10
218	220	191	163	177	169	161	171	171	165
277	254	252	274	238	235	233	215	199	191

11	12	13	14	15	16	17	18	19	20
160	146	146	132	135	122	96	107	107	107
193	182	172	182	167	155	167	142	135	124

24. S. runcinifolius N = 20 4C DNA = 16.15 pg.

1	2	(3)	4	5	(6)	7	8	9	10
261	240	212	239	229	206	216	195	217	193
313	299	305	271	257	275	251	229	246	210

11	12	13	14	15	16	17	18	19	20
179	173	164	165	150	151	147	130	132	119
209	197	187	181	183	166	157	174	145	141

25. *S.* sp. B N = 30 4C DNA = 19.94 pg.

1	2	3	4	5	6	7	8	9	10
253	239	214	211	192	194	197	185	184	180
277	254	243	226	238	224	212	212	203	200

11	12	13	14	15	(16)	17	18	19	20
172	149	150	157	148	96	135	130	133	121
194	210	198	176	180	207	165	169	158	156

21	(22)	23	24	25	26	27	28	29	30
100	91	88	119	111	105	103	99	92	95
167	174	175	141	135	135	132	132	136	112

26. *S. squarrosus* N = 30 4C DNA = 19.81 pg.

1	2	3	4	5	6	7	8	9	10
239	228	217	193	201	200	192	177	177	179
275	260	249	272	238	225	219	214	208	202

11	12	13	14	15	16	17	(18)	(19)	(20)
173	171	169	155	149	134	108	94	91	87
199	190	188	183	171	162	181	188	185	177

21	(22)	23	24	25	26	27	28	29	30
116	83	106	103	116	97	93	97	98	92
146	171	146	144	129	148	143	125	117	115

27. *S. bipinnatisectus* N = 30 4C DNA = 13.98 pg.

1	2	3	4	5	(6)	7	8	9	10
147	141	92	111	122	93	105	107	84	69
200	187	219	192	173	199	178	165	179	183

11	12	(13)	14	15	16	17	18	19	20
87	89	21	85	85	94	63	94	70	91
163	158	225	158	144	128	150	113	135	108

21	22	23	24	25	26	27	28	29	30
72	89	84	85	84	78	76	77	73	66
126	108	106	101	102	99	95	90	91	84

28. S. minimus N = 30 4C DNA = 19.82 pg.

(1)	2	3	4	5	6	7	8	9	10
181 323	189 293	175 285	209 245	191 261	127 290	168 241	175 221	120 267	185 197
11	**(12)**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
157 209	99 246	146 191	124 209	157 174	142 170	97 208	102 186	95 182	119 152
21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
92 177	92 175	109 148	87 167	108 140	104 140	105 130	106 123	97 113	89 111

29. S. picridioides N = 30 4C DNA = 19.68 pg.

1	2	3	4	5	6	7	8	9	10
205 295	193 262	174 276	197 252	145 275	167 242	122 280	168 228	173 208	164 201
(11)	**12**	**13**	**(14)**	**15**	**16**	**17**	**18**	**19**	**20**
85 271	112 242	159 191	78 271	143 191	125 185	129 162	104 180	108 176	120 163
21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
114 156	116 148	117 144	113 137	78 171	93 144	106 130	101 127	100 118	95 109

30. S. glomeratus N = 30 4C DNA = 19.18 pg.

1	2	3	4	5	6	7	8	9	10
231 257	218 246	206 239	199 232	197 227	189 216	184 202	181 203	177 201	170 200
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**(19)**	**20**
170 185	152 184	154 181	144 178	141 168	124 169	119 159	120 157	87 185	114 156
21	**22**	**(23)**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
111 151	111 147	79 172	109 141	108 136	107 130	108 123	100 122	96 117	92 104

31. *S. hispidulus* var. *hispidulus* N = 30 4C DNA = 19.11 pg.

1	2	3	4	5	6	7	8	9	10
227	190	204	200	177	189	174	177	172	161
247	250	235	219	233	214	216	197	200	211

11	12	13	14	15	16	(17)	18	(19)	20
159	157	152	152	139	126	94	121	84	114
210	193	189	175	161	168	196	162	187	156

21	22	23	24	25	26	27	28	29	30
119	103	109	113	106	87	105	103	94	92
140	155	140	129	135	150	129	129	124	109

32. *S. hispidulus* var. *dissectus* N = 30 4C DNA - 19.41 pg.

1	2	3	4	5	6	7	8	9	10
216	208	201	196	186	179	182	172	157	166
245	246	237	216	222	214	202	199	212	194

11	12	13	14	15	16	17	18	(19)	20
155	144	144	156	149	144	111	84	119	129
194	201	195	178	174	167	193	211	171	153

21	22	23	24	25	26	27	28	29	30
123	120	117	85	113	105	99	110	107	95
149	143	144	176	143	145	151	131	121	107

33. *S.* sp. C N = 30 4C DNA = 20.12 pg.

1	2	3	4	5	6	7	8	9	10
242	226	219	225	209	192	195	184	185	162
267	263	254	248	240	231	228	217	204	214

11	12	13	14	15	16	17	(18)	19	20
153	159	154	152	139	144	121	86	122	112
194	190	184	182	179	171	190	208	168	166

21	(22)	23	24	25	26	27	28	29	30
121	76	116	119	89	105	100	82	97	91
156	192	147	140	167	146	150	154	123	115

34. S. biserratus N = 50 4C DNA = 25.27 pg.

1	2	3	4	5	6	7	8	9	10
190	180	158	177	162	100	146	137	148	121
226	226	221	200	188	247	195	196	173	195
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
130	103	127	105	22	86	112	73	111	108
171	193	164	165	247	182	142	179	140	141
21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
87	74	111	76	105	106	71	92	95	103
161	171	129	157	127	125	160	136	132	123
31	**32**	**33**	**(34)**	**35**	**36**	**37**	**38**	**39**	**40**
101	100	64	66	92	96	91	83	92	91
121	120	153	150	124	119	115	121	110	109
41	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**(49)**	**50**
89	81	86	89	81	73	73	78	49	61
110	115	110	105	110	117	115	108	130	111

35. S. vulgaris N = 20 4C DNA = 7.82 pg.

(1)	(2)	3	4	5	6	7	8	9	10
144	121	91	82	95	95	73	98	96	55
156	134	157	160	126	116	138	110	112	148
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
55	85	79	54	76	56	71	58	36	44
139	105	95	120	97	107	84	81	99	75

36. S. pterophorus N = 10 4C DNA = 4.22 pg.

1	(2)	3	4	(5)	6	7	8	9	10
103	64	88	94	70	97	85	86	68	77
154	175	140	122	139	111	115	99	109	95

37. S. mikanioides N = 10 4C DNA = 11.78 pg.

1	2	3	4	5	6	(7)	8	9	10
296	313	307	271	244	259	269	226	204	200
404	382	346	355	357	337	317	323	249	204

38. *S. discifolius* N = 5 4C DNA = 14.27 pg.

1	2	(3)	4	5
596	503	426	480	297
1120	967	1015	889	841

39. *Erechtites valerianaefolia* N = 20 4C DNA = 25.02 pg.

1	2	3	4	5	(6)	7	8	9	10
259	264	194	157	164	281	171	161	176	154
570	502	542	547	528	400	500	484	464	474

11	12	13	14	15	16	17	18	19	20
92	162	184	171	165	158	226	145	201	153
531	452	407	408	391	396	317	375	294	341

40. *S. lautus* subsp. *dissectifolius* x *S. biserratus* 2N = 70
4C DNA = 17.79 pg.

(Diploid karyotype)

1	2	3	4	5	6	7	8	9	10
179	185	147	132	157	143	148	142	129	140
216	187	204	203	173	182	170	173	184	168

11	12	13	14	15	16	17	18	19	20
121	122	134	137	126	134	117	104	124	122
181	180	165	160	161	148	164	172	150	147

21	22	23	24	25	26	27	28	29	30
97	125	88	114	117	96	115	89	115	112
160	138	174	147	144	164	143	165	139	141

31	32	33	34	35	36	37	38	39	40
121	77	105	111	117	112	113	83	79	99
129	172	139	131	125	126	124	152	156	133

41	42	43	44	45	46	47	48	49	50
71	72	83	102	91	90	85	95	88	78
157	155	141	124	134	129	130	120	126	116

51	52	53	54	55	56	57	58	59	60
77	70	80	98	83	83	85	88	72	89
136	142	130	105	117	116	112	107	120	99

61	62	63	64	65	66	67	68	69	70
72	72	65	70	65	70	65	65	78	78
115	115	121	116	121	116	120	120	106	106

APPENDIX 2

Total Percent Match (TPS), Unique Percent Match (UPS) And Duplicate Genome Values for Comparisons of all Karyotypes Discussed in Chapter 7

A The total percent match value of a comparison between karyotypes A and B is the sum of (1) the percentage of all chromosomes in A matching with any chromosome in B and (2) the percentage of all chromosomes in B matching with any chromosome in A.

B The unique percent match value of the same comparison is the sum of the percentage of uniquely matching chromosomes in A and uniquely matching chromosomes in B, - where a unique match involves chromosomes not previously matched to a chromosome in the other set.

C Duplicate genome values represent the number of chromosomes (expressed as a fraction of the genome of ten chromosomes) that do not match uniquely - in other words, that part of the complement that is a duplicate of the unique match. Duplicate genome values are calculated as TPS - UPS/(100/number of genomes in the haploid complement).

Eight species with very large chromosomes had zero TPS and UPS values when compared with other species. Values for these species are therefore given in separate tables. Numbers preceeding species names correspond to karyotype illustrations in Figure 7.1.

Species With a Mean Chromosome Size Greater than 0.20 pg

A. Total percentage match values

	1	2	3	4	5	37	38
1. Senecio magnificus							
2. S. velleioides	72						
3. S. amygdalifolius	107	38					
4. S. macranthus	110	77	67				
5. S. vagus	23	0	47	0			
37. S. discifolius	0	0	0	47	0		
38. S. mikanioides	75	31	76	0	7	0	
39. Erechtites valerianaefolia	30	41	36	16	26	0	30

B. Unique percentage match values

	1	2	3	4	5	37	38
1. Senecio magnificus							
2. S. velleioides	51						
3. S. amygdalifolius	92	34					
4. S. macranthus	100	62	62				
5. S. vagus	23	0	36	0			
37. S. discifolius	0	0	0	25	0		
38. S. mikanioides	60	15	61	0	7	0	
39. Erechtites valerianaefolia	20	40	10	10	21	0	30

C. Duplicate genome values of species A compared to species B

Species A	Species B 1	2	3	4	5	37	38	39
1. *Senecio magnificus*	0	.3	.3	.1	0	0	.1	.1
2. *S. velleioides*	.1	0	0	.1	0	0	.1	.1
3. *S. amygdalifolius*	0	.1	0	.1	.2	0	.1	.2
4. *S. macranthus*	.1	.2	0	0	0	0	0	.1
5. *S. vagus*	0	0	0	0	0	0	0	0
37. *S. discifolius*	0	0	0	0	0	0	0	0
38. *S. mikanioides*	.1	.1	.1	0	0	0	0	0
39. *Erechtites valerianaefolia*	.1	0	.1	0	.1	0	0	0

Species with a Mean Chromosome Size of Less Than 0.22 pg. (note *S. vagus* included in both sections).

A. Total percentage match values

	2N	5	6	7	8	9	10	11	12
5. vagus	98								
6. lautus	40	21							
7. ssp. dissectifolius	40	36	190						
8. ssp. lanceolatus	40	14	180	185					
9. ssp. alpinus	40	58	190	200	155				
10. ssp. maritimus	40	21	195	170	170	175			
11. spathulatus	40	102	160	140	70	170	70		
12. glossanthus	40	0	0	0	0	0	0	0	
13. gregorii	40	103	145	135	120	155	115	140	0
14. hypoleucus	60	64	160	138	127	142	132	97	0
15. odoratus	60	91	165	152	133	155	128	143	0
16. cunninghamii	60	116	177	153	128	162	120	155	0
17. anethifolius	60	76	173	160	148	158	142	160	0
18. gawlerensis	60	133	150	80	77	123	77	127	0
19. linearifolius	60	86	158	145	137	148	105	143	0
20. sp. A	60	86	158	133	115	153	130	113	0
21. quandridentatus	40	113	100	90	130	60	70	130	0
22. gunnii	40	129	105	105	90	120	65	100	0
23. aff. apargiaefolius	40	105	110	95	90	120	70	120	0
24. runcinifolius	40	113	90	80	65	85	55	85	0
25. sp. B	60	95	137	150	142	148	142	127	0
26. squarrosus	60	106	132	120	132	137	118	117	0
27. bipinnatisectus	60	46	120	87	82	87	83	123	100
28. minimus	60	130	152	137	112	138	103	143	0
29. picridioides	60	125	143	130	130	138	103	125	0
30. glomeratus	60	90	137	145	103	128	72	130	0
31. hispidulus	60	102	146	157	150	157	127	147	0
32. var. dissectus	60	104	160	145	132	160	147	163	0
33. sp. C	60	112	152	148	122	128	131	157	6
34. biserratus	100	71	171	155	150	154	140	144	69
35. pterophorus	20	0	60	75	100	85	55	40	0
36. vulgaris	40	0	85	90	110	85	100	30	125

	13	14	15	16	17	18	19	20	21	22	23	24
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												
13.												
14.	133											
15.	142	190										
16.	157	147	177									
17.	142	177	156	174								
18.	130	130	170	170	146							
19.	122	143	163	157	150	127						
20.	143	153	170	156	140	133	170					
21.	170	93	148	142	142	127	132	110				
22.	160	135	143	127	135	115	127	92	150			
23.	135	113	117	110	118	103	138	88	155	155		
24.	135	88	103	82	71	105	73	82	145	120	130	
25.	157	173	174	170	166	140	137	147	168	168	133	118
26.	132	127	133	140	153	127	123	90	155	155	152	118
27.	60	87	100	117	110	70	117	80	58	53	33	17
28.	148	150	157	173	163	157	156	133	158	157	145	108
29.	137	103	150	160	150	154	177	130	123	135	142	107
30.	142	106	133	133	130	116	127	113	180	162	155	103
31.	170	177	176	174	177	144	163	153	187	163	157	142
32.	177	180	174	173	167	170	163	173	182	182	153	147
33.	162	153	170	184	163	130	153	163	167	157	143	142
34.	130	147	154	155	133	139	164	133	150	140	119	83
35.	0	13	50	63	63	0	60	39	40	20	20	0
36.	45	53	80	58	57	8	80	50	65	35	30	25

	25	26	27	28	29	30	31	32	33	34	35	36
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												
13.												
14.												
15.												
16.												
17.												
18.												
19.												
20.												
21.												
22.												
23.												
24.												
25.												
26.	190											
27.	104	96										
28.	163	170	130									
29.	140	154	103	184								
30.	184	177	80	153	130							
31.	194	186	130	177	160	187						
32.	193	194	120	190	180	197	197					
33.	197	187	126	160	143	173	190	190				
34.	165	167	169	174	164	163	181	181	171			
35.	73	77	147	63	77	50	110	73	80	158		
36.	103	82	133	67	65	60	93	82	52	152	115	

B. Unique percentage match values

	2N	5	6	7	8	9	10	11	12
5. *vagus*	98								
6. *lautus* ssp. *lautus*	40	21							
7. ssp. *dissectifolius*	40	21	180						
8. ssp. *lanceolatus*	40	14	120	130					
9. ssp. *alpinus*	40	35	180	200	130				
10. ssp. *maritimus*	40	21	170	150	150	150			
11. *spathulatus*	40	70	120	90	60	60	60		
12. *glossanthus*	40	0	0	0	0	0	0	0	
13. *gregorii*	40	61	140	130	80	140	100	120	0
14. *hypoleucus*	60	43	133	117	92	133	92	67	0
15. *odoratus*	60	53	142	125	92	133	120	83	0
16. *cunninghamii*	60	70	150	125	67	150	75	142	0
17. *anethifolius*	60	53	150	142	108	142	108	117	0
18. *gawlerensis*	60	86	92	67	43	100	58	110	0
19. *linearifolius*	60	59	150	125	100	133	108	108	0
20. sp. A	60	60	142	108	92	133	100	58	0
21. *quandridentatus*	40	85	60	70	90	40	70	80	0
22. *gunnii*	40	113	70	70	60	50	40	70	0
23. aff. *apargiaefolius*	40	99	70	70	50	60	50	80	0
24. *runcinifolius*	40	106	40	50	50	40	30	50	0
25. sp. B	60	86	142	117	83	108	117	92	0
26. *squarrosus*	60	86	117	92	92	92	83	75	0
27. *bipinnatisectus*	60	27	100	67	67	67	67	67	58
28. *minimus*	60	97	133	108	83	100	75	83	0
29. *picridioides*	60	86	133	92	87	108	75	92	0
30. *glomeratus*	60	81	125	125	67	117	68	83	0
31. *hispidulus*	60	97	133	133	125	125	100	100	0
32. var. *dissectus*	60	97	142	125	108	117	117	113	0
33. sp. C	60	102	142	108	85	100	92	125	8
34. *biserratus*	100	52	133	119	140	126	116	98	0
35. *pterophorus*	20	0	45	45	75	45	45	30	0
36. *vulgaris*	40	0	60	50	80	60	60	20	80

	13	14	15	16	17	18	19	20	21	22	23	24
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												
13.												
14.	117											
15.	125	174										
16.	133	114	140									
17.	142	134	146	146								
18.	108	80	120	154	120							
19.	108	120	166	146	144	94						
20.	133	134	160	126	126	94	166					
21.	120	67	100	92	117	92	108	92				
22.	120	92	100	92	83	83	92	67	140			
23.	110	83	83	83	83	67	92	58	150	140		
24.	80	50	67	50	50	67	50	50	120	100	120	
25.	125	114	120	120	134	106	120	120	150	142	117	108
26.	92	94	86	100	120	80	100	60	133	142	133	108
27.	42	54	74	86	86	64	94	43	50	42	33	17
28.	125	126	134	126	120	94	126	114	135	135	108	83
29.	108	94	120	120	120	104	140	117	108	117	83	120
30.	117	86	94	94	114	86	106	142	125	125	75	75
31.	150	120	126	126	134	86	126	126	150	133	125	108
32.	150	134	140	140	120	123	123	126	158	142	133	117
33.	125	94	126	126	134	120	126	120	142	133	117	108
34.	112	96	96	107	91	85	117	106	112	105	98	63
35.	0	13	40	40	53	0	40	27	40	15	15	0
36.	20	33	50	33	33	8	50	33	50	30	30	20

	25	26	27	28	29	30	31	32	33	34	35	36
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												
13.												
14.												
15.												
16.												
17.												
18.												
19.												
20.												
21.												
22.												
23.												
24.												
25.												
26.	160											
27.	66	86										
28.	120	134	100									
29.	120	126	80	160								
30.	166	160	60	106	106							
31.	186	174	86	146	140	174						
32.	174	166	66	140	154	194	194					
33.	180	160	100	140	126	146	180	166				
34.	117	123	139	123	117	123	133	128	128			
35.	53	53	120	53	53	40	80	40	53	120		
36.	52	67	108	58	50	42	58	50	37	112	105	

C. Duplicate genomes of species A compared with species B

Species A	Species B 5	6	7	8	9	10	11	12	13	14	15	16	17	18
5. vagus	0	0	0	0	.2	0	.1	0	1.0	.3	.3	.3	.8	.6
6. lautus	0	0	0	.5	0	.2	.3	0	0	.2	.2	0	0	.5
7. ssp. dissectifolius	.3	.2	0	.5	0	.2	.5	0	0	.3	.4	.3	.3	.1
8. ssp. lanceolatus	0	.7	.6	0	.5	.3	.1	0	.4	.5	.7	.9	.6	.6
9. ssp. alpinus	.4	.2	0	0	0	.2	.3	0	.3	.1	.3	.1	.2	.4
10. ssp. maritimus	0	.3	.2	.1	.3	0	.1	0	.1	.6	.1	.7	.6	.3
11. spathulatus	.6	.5	.5	.1	.5	.1	0	0	.3	.4	.8	.2	.2	.2
12. glossanthus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13. gregorii	.4	.1	.1	.4	0	.2	.1	0	0	.2	.2	.2	0	.3
14. hypoleucus	.6	.5	.6	.3	.1	.3	.3	0	.2	0	.3	.6	.6	.9
15. odoratus	.8	.7	.2	.2	.2	.1	.6	0	.2	.2	0	.5	0	.7
16. cunninghamii	.9	.8	.4	.5	.2	.3	.1	0	.4	.4	.6	0	.4	.4
17. anethifolius	.3	.7	.1	.3	.2	.1	.7	0	0	.7	.3	.4	0	.4
18. gawlerensis	.7	1.0	.1	.4	.1	.1	.2	0	.2	.6	.8	.1	.2	0
19. linearifolius	.3	.1	0	.2	0	.3	.3	0	.1	.3	0	.1	.0	.4
20. sp. A	.3	.2	.3	.1	0	0	.6	0	0	.1	.1	.3	.3	.5
21. quadridentatus	.1	0	.3	.1	.2	0	.3	0	.4	.3	.7	.4	.1	.3
22. gunnii	0	.1	.6	0	.2	0	0	0	.2	.4	.2	.3	.3	.3
23. aff. apargiaefolius	.1	0	.1	.1	.1	0	.2	0	.4	.4	.4	.2	.3	.4
24. runcinifolius	0	.6	0	0	.2	0	.2	0	.3	.3	.2	.1	.1	.3
25. sp. B	.2	0	.1	.4	.3	0	.3	0	.5	.7	.5	.4	.2	.5
26. squarrosus	.3	0	.1	0	0	0	.5	.2	.3	.7	1.1	.7	.3	.5
27. bipinnatisectus	.3	0	0	0	0	.2	.2	0	.1	.4	.3	.2	.3	.5
28. minimus	0	.1	.1	.1	.1	.1	.3	0	.1	.1	.3	.5	.1	.9
29. picridioides	.4	0	.1	.1	0	.1	.1	0	.4	.2	.6	.4	.6	.6
30. glomeratus	.2	.2	.2	.5	.2	.4	.5	0	.6	.6	1.1	.7	.3	.3
31. hispidulus	.1	0	.1	0	.2	.1	.2	0	.3	.6	.6	.7	.3	.6
32. var. dissectus	.1	.1	0	.1	.4	0	.3	0	.5	.6	.5	.4	.4	.7
33. sp. C	.1	0	.3	.1	.1	.6	.2	.1	.5	.7	.4	1.0	.2	.6
34. biserratus	.1	1.4	1.3	.5	.9	.7	.8	.3	.4	.7	1.4	1.1	.9	1.0
35. pterophorus	0	0	.1	0	.1	.1	0	0	0	0	.1	.1	0	0
36. vulgaris	0	0	.1	0	0	0	0	0	0	0	.2	.1	0	0

19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1.1	.8	1.2	.8	.1	.4	.2	.5	.5	1.7	1.3	.1	.1	.2	.3	.9	0	0
.1	.2	.8	.8	.8	.4	.1	.3	.4	.3	.2	.1	.3	.3	.2	0	.3	.5
.4	.3	.1	.1	.4	.6	.6	.5	.4	.5	.7	.3	.4	.4	.6	.2	.3	.8
.6	.4	.7	.6	.7	.3	.9	.8	.3	.5	.7	.4	.5	.4	.7	0	.5	.5
.3	.4	.2	1.2	1.1	.7	.6	.9	.4	.7	.5	.1	.5	.6	.5	.2	.6	.5
0	.6	0	.5	.4	.5	.5	.7	.2	.5	.5	0	.8	.6	.5	0	0	.8
.5	.7	.7	.6	.6	.5	.5	.5	1.0	1.0	.6	.6	.8	.4	.5	.6	.2	.2
0	0	0	0	0	0	0	.7	0	0	0	0	0	0	.1	.1	0	.9
.2	.2	.6	.6	.5	.4	.3	.6	.3	.4	.3	.1	.2	.2	.4	.2	0	.5
.4	.5	.5	.6	.4	.7	.8	.3	.6	.6	.1	0	1.1	.8	1.1	1.1	0	.6
0	.2	.4	1.0	.4	.8	.8	.3	.5	.4	.3	.1	.9	.5	.9	.9	0	.6
.2	.6	.9	.6	.5	.8	.8	.5	.7	.9	.8	.5	.7	.6	.7	.8	.4	.3
.5	.1	.6	1.1	.6	.5	.8	.7	.6	.7	.6	.2	.9	.9	.7	.8	.3	.7
.6	.7	.6	.5	.5	.7	.5	.5	.5	1.3	.9	.3	1.0	.7	.1	1.0	0	0
0	0	.7	.6	.5	.3	.3	.2	.6	.6	.3	.2	.5	.7	.5	.5	.3	.6
.1	0	.4	.6	.6	.8	.6	.5	.4	.4	.4	.2	.2	.6	.7	.3	.1	.5
.2	.1	0	.2	.1	.4	.1	.1	.1	.4	0	.1	.2	0	.1	.2	0	.3
.3	.1	0	0	.1	.4	0	0	.1	.3	0	0	0	0	0	.3	.1	.1
.6	.2	0	.2	0	.1	0	.1	0	.6	.1	0	.1	0	0	.1	.1	0
.3	.1	.1	.1	.1	0	0	0	0	.3	0	.1	.2	0	0	.2	0	.1
.2	.2	.4	.8	.5	.3	0	.5	.4	.9	.4	.1	.1	.2	.2	.4	0	1.0
.3	.4	.4	.4	.4	.3	.4	0	0	.9	.4	.2	.2	.4	.3	.3	.4	.3
.1	1.2	.1	.2	0	0	1.0	.3	0	.6	.6	.4	.9	1.1	.4	0	.8	.6
.3	.2	.1	.2	.2	.3	.4	.2	.3	0	.2	.3	.2	.6	0	.1	.3	.1
.8	.2	.2	.7	.6	.7	.2	.4	.1	.5	0	0	.3	.3	.2	.2	.1	.3
.4	0	1.0	1.1	.9	.7	.4	.3	.2	1.1	.7	0	.4	.1	.3	.5	.3	.4
.6	.8	.7	.9	.7	.7	.1	.2	.4	.7	.3	0	0	0	.1	.3	.6	.6
.6	.7	.7	1.2	.6	.9	.4	.4	.5	.9	.5	0	.1	0	.4	.5	.4	.5
.3	.7	.6	.7	.8	1.0	.3	.5	.4	.2	.3	.5	.2	.3	0	.2	.5	.3
1.5	.8	1.4	1.0	.8	.5	1.7	1.8	1.5	2.4	2.0	1.6	1.9	1.8	1.8	0	1.9	2.0
0	.1	0	0	0	0	.2	.1	0	0	.2	0	.1	.2	.1	0	0	0
.2	0	.1	0	0	0	.4	.1	.1	.1	.1	.1	.3	.3	.1	0	.2	0

Appendix 3

Listings of Program MPL and Program KARYO used to Construct And Compare Karyotypes (see Methods, Chapter 7)

```
      PROGRAM MPL (INPUT,TAPE2=INPUT,OUTPUT,TAPE3=OUTPUT)
************************************************************************
C
C   MPL CALCULATES THE MEAN PERCENT LENGTH AND STANDARD ERROR FOR EACH ARMOF EACH
C                      CHROMOSOME PAIR IN A KARYOTYPE
C
C   TWO DATA SETS ARE REQUIRED
C     1 CHROMOSOME ARM LENGTHS
C     2 CORRECT ORDER OF CHROMOSOMES
C
C     MPL CALCULATES 1 TOTAL ARM LENGTH FOR EACH CHROMOSOME SPREAD
C                    2 TAKES CHROMOSOMES FROM DATA SET 1 IN THE ORDER
C                       SPECIFIED BY DATA SET 2
C                    3 CALCULATES PERCENT LENGTH OF EACH ARM
C                    4 CALCULATES MEAN PERCENT LENGTH AND STANDARD ERROR
C
C   DATA - THE FIRST DATA CARD SPECIFIES THE NUMBER OF DATA BLOCKS TO BE
C          ANALYSED
C          FORMAT IS I4  I.E. ---6 FOR SIX BLOCKS OF DATA
C
C   DATA BLOCKS (ONE TAXON EACH) ARE THEN ADDED. EACH BLOCK IS AS FOLLOWS:-
C     CARD 1  SPECIES NAME (STARTING IN COLUMN 1)
C     CARD 2  J,K,KA,KB,D  FORMAT(I2,I4,I3,I2,F6.1)
C         WHERE J = NUMBER OF SETS OF CHROMOSOMES (UP TO 6)
C               K = NUMBER OF CHROMOSOME ARMS (UP TO 200)
C               KA = K/20 ROUNDED UP IF FRACTIONAL
C               KB = K/40 ROUNDED UP IF FRACTIONAL
C                E.G. K=76  KA=(3.8)=4  KB=(1.9)=2
C               D = DNA VALUE IN PICOGRAMS X 10 (IF VALUE IS NOT KNOWN - USE D=100)
C     CARDS 3 ONWARDS  DATA SET 1 - CHROMOSOME ARM LENGTHS
C         FORMAT IS (20F4.1) I.E. -237-391-231-394--61-102--ETC.
C         (NOTE: -- = 2 BLANKS)
C          WHERE 237 IS THE SHORT ARM OF CHROMOSOME A
C                391 IS THE LONG ARM OF CHROMOSOME A AND A = 1 TO K/2
C          E.G. FOR 2N=38 AND 6 SPREADS MEASURED -
C               DATA SET 1 = 4X6 = 24 CARDS
C               CARD 3 = 20 ARMS OF FIRST 10 CHROMOSOMES OF SPREAD 1
C               CARD 4 = 20 ARMS OF NEXT 10 CHROMOSOMES OF SPREAD 1
C               CARD 5 = 20 ARMS OF NEXT 10 CHROMOSOMES OF SPREAD 1
C               CARD 6 = 16 ARMS OF LAST 8 CHROMOSOMES OF SPREAD 1
C            .. CARD 26= 16 ARMS OF LAST 8 CHROMOSOMES OF SPREAD 6
C     CARDS FOLLOWING DATA SET 1 = DATA SET 2 =CORRECT ORDER OF CHROMOSOMES
C         FORMAT IS (20I4)  E.G. CARD 27 --11--23--34---6--21--
C                                CARD 28 ---3--14--21--28---8--
C                                CARD 29 --12--15--25---9---7--
C            WHERE CHROMOSOMES 27 AND 11 OF SPREAD 1 AND 12 AND 15 OF
C            SPREAD 2 ETC. REPRESENT THE SAME CHROMOSOME PAIR OF THE
C            KARYOTYPE.
C         FOR 2N=38 AND 6 SPREADS MEASURED -
C          DATA SET 2 = 2X6 = 12 CARDS
************************************************************************
      DIMENSION HA(20),ORIG(200,6),I(100,6),II(100,6),TOTS(6),PL(100,14)
      IF(EOF(2))3000,4000
 4000 CONTINUE
      READ(2,100)IJK
  100 FORMAT(I3)
      DO 101 JKL=1,IJK
      READ(2,24)(HA(IX),IX=1,20)
   24 FORMAT(20A4)
      WRITE(3,102)(HA(IX),IX=1,20)
  102 FORMAT(1H1,1X,20A4)
      READ(2,4)J,K,KA,KB,D
    4 FORMAT(I2,I4,I3,I2,F6.1)
      WRITE(3,31)
   31 FORMAT(//,1X,*J*,2X,*K*,2X,*KA*,1X,*KB*,3X,*D*,/,1X,20(1H-))
      WRITE(3,83)J,K,KA,KB,D
   83 FORMAT(//,I2,I4,I3,I2,F6.1)
C READ ARM LENGTHS INTO MATRIX ORIG
      DO 3 M=1,J
      DO 3 LA=1,KA
      LC=LA*20
      LE=(LA-1)*20+1
      READ(2,81)(ORIG(LD,M),LD=LE,LC)
   81 FORMAT(20F4.1)
    3 CONTINUE
      WRITE(3,34)
   34 FORMAT(///,1X, *UNORDERED CHROMOSOME ARM MEASUREMENTS (MM)*)
```

```
      WRITE(3,39)
   39 FORMAT(1X,120(1H-))
      DO 42 L=1,K
      WRITE(3,32)L,(ORIG(L,M),M=1,J)
   32 FORMAT(I4,6F10.1)
   42 CONTINUE
C CHECK DATA ELEMENTS DO NOT EXCEED 100.0
      DO 6 L=1,J
      DO 7 M=1,K
      IF(ORIG(M,L).LE.100.0)7,8
    8 WRITE(3,9)M,L
    9 FORMAT(,*DATA ERROR AT POSITION*,2I5)
    7 CONTINUE
    6 CONTINUE
C READ CORRECT ORDER OF CHROMOSOMES INTO MATRIX I
      KK=K/2
      DO 5 M=1,J
      DO 5 LA=1,KB
      LC=LA*20
      LE=(LA-1)*20+1
      READ(2,82)(I(LD,M),LD=LE,LC)
   82 FORMAT(20I4)
    5 CONTINUE
      WRITE(3,35)
   35 FORMAT(///,1X,*CORRECT ORDER OF CHROMOSOMES*)
      WRITE(3,56)
   56 FORMAT(1X,120(1H-))
      DO 43 LL=1,KK
      WRITE(3,33)LL,(I(LL,MM),MM=1,J)
   33 FORMAT(I4,6I6)
   43 CONTINUE
C CHECK FOR DUPLICATIONS IN MATRIX (I)
      DO 200 IJ=1,J
      DO 200 IK=1,KK
      II(IK,IJ)=0
  200 CONTINUE
      IAB=0
      DO 201 IJ=1,J
      DO 209 IK=1,KK
      IL=I(IK,IJ)
      IF(II(IL,IJ).EQ.0)202,203
  202 II(IL,IJ)=IL $GO TO 209
  203 IAB=1
      WRITE(3,204)IL,IJ
  204 FORMAT(1X,I3,* IS DUPLICATED IN MATRIX I COLUMN*,I2,1X)
  209 CONTINUE
      DO 205 IA=1,KK
      IF(II(IA,IJ).EQ.0)207,205
  207 WRITE(3,208)IA,IJ
  208 FORMAT(1X,I3,* IS MISSING IN MATRIX I COLUMN*,I2,1X)
  205 CONTINUE
  201 CONTINUE
      IF(IAB.EQ.1)101,210
  210 CONTINUE
C CALCULATE TOTAL ARM LENGTH AND STORE IN ARRAY TOTS
      DO 28 N=1,J
      TOTS(N)=0.0
   28 CONTINUE
      DO 11 N=1,J
      DO 12 IK=1,K
      TOTS(N)=TOTS(N)+ORIG(IK,N)
   12 CONTINUE
   11 CONTINUE
C CALCULATE % LENGTHS OF DNA VALUE AND STORE IN CORRECT ORDER IN PL
      DO 71 L=1,100
      DO 71 M=1,14
      PL(L,M)=0.0
   71 CONTINUE
      KKK=K/4
      IP=2
      DO 15 MM=1,J
      DO 14 MN=1,KKK
      DO 13 IB=1,IP
      N=I(2*MN-1,MM)
      IF(IB.EQ.2) N=I(2*MN,MM)
      IA=2*MM-1
      IF(IB.EQ.2) IA=2*MM
```

```
      NP=(2*N)-1
      O=ORIG(NP,MM)
      R=(O/TOTS(MM))*D
      PL(2*MN-1,IA)=R
      IE=2*MN-1
      NP=2*N
      O=ORIG(NP,MM)
      R=(O/TOTS(MM))*D
      PL(2*MN,IA)=R
   13 CONTINUE
   14 CONTINUE
   15 CONTINUE
C CALCULATE MEAN AND STANDARD ERROR FOR EACH SET OF ARMS I.E. EACH ROW OF PL
      IR=2*J
      DO 18 IO=1,KK
      B=0.0
      DO 16 IL=1,IR
      B=PL(IO,IL)+B
   16 CONTINUE
      C=B/IR
      PL(IO,13)=C
      PL(IO,14)=0.0
      DO 17 IM=1,IR
      A=PL(IO,IM)
      E=A-C
      F=E*E
      PL(IO,14)=PL(IO,14)+F
   17 CONTINUE
      G=PL(IO,14)/(IR*(IR-1))
      H=SQRT(G)
      PL(IO,14)=H
   18 CONTINUE
C PRINT MATRIX PL
      WRITE(3,19)(HA(IX),IX=1,20)
   19 FORMAT(//,45X,20A4,/,1X,120(1H-))
    · WRITE(3,25)
   25 FORMAT(//,40X,*MEAN PERCENT LENGTHS OF CHROMOSOME ARMS*)
      WRITE(3,44)
   44 FORMAT(1X,50X,*(% OF DNA VALUE X 10)*)
      WRITE(3,26)
   26 FORMAT(//,106X,*MEAN*,2X,*ST. ERR.*)
      WRITE(3,38)
   38 FORMAT(1X,120(1H-))
      KK=KK/2
      NS=0
      DO 20 LN=1,KK
      WRITE(3,21)2*LN-1,((PL(2*LN-1,LU),LU=1,14))
   21 FORMAT(1X,I3,6(2X,2F7.2),3X,F7.2,F10.4)
      WRITE(3,22)2*LN,((PL(2*LN,LU),LU=1,14))
   22 FORMAT(1X,I3,6(2X,2F7.2),3X,F7.2,F10.4,//)
   20 CONTINUE
      WRITE(3,23)
   23 FORMAT(1X,120(1H-))
  101 CONTINUE
 3000 CONTINUE
      END
```

```
      PROGRAM KARYO (INPUT,TAPE2=INPUT,OUTPUT,TAPE3=OUTPUT)
      DIMENSION TTEST(32),HA(50,20),IPAR(50,3),DATA(180,20),STERR(180,
     C20),IPAIR(60,21)
**********************************************************************
* KARYO CALCULATES AND LISTS ALL CHROMOSOMES IN KARYOTYPE A THAT MATCH
*   WITH ONE OR MORE CHROMOSOMES IN KARYOTYPE B
*
*   THE SHORT ARMS OF ANY TWO CHROMOSOMES ARE COMPARED FIRST USING A
*   T TEST (AT THE 5% PROBABILITY LEVEL). IF THE SHORT ARMS MATCH,THEN THE
*   LONG ARMS ARE COMPARED. IF BOTH ARMS ARE FOUND TO MATCH THEN THE
*   CHROMOSOMES BEING COMPARED ARE LISTED AS EQUIVALENT.
*
*   FOR EACH EXECUTION OF KARYO THE LAST CHROMOSOME SET IS COMPARED WITH
*   ALL PRECEEDING SETS.
*   TO COMPARE ALL KARYOTYPES, KARYO MUST THEREFORE BE RUN AFTER THE
*   ADDITION OF EACH DATA SET(KARYOTYPE).
*
*  ADDING DATA
*    LINES 1 AND 2 ARE VALUES OF T - DO NOT ALTER
*    DATA SETS START AT LINE 3. ONE DATA SET IS AS FOLLOWS:-
*      LINE 1 - SPECIES NAME AND CHROMOSOME NUMBER
*               E.G. SENECIO LAUTUS  N=20
*      LINE 2 - VARIABLES USED IN CALCULATIONS (FORMAT 3I4)
*                 FIRST-NUMBER OF LINES FOR DATA MATRIX (N/10 ROUNDED UP IF FRACTIONAL)
*                       E.G. FOR N=12 12/10=2 LINES
*                 SECOND-NUMBER OF ORIGINAL MEASUREMENTS OF EACH CHROMOSOME
*                 THIRD-NUMBER OF CHROMOSOMES
*             FOR S.LAUTUS THIS LINE BECOMES (-=BLANK) ---2--12--20
*      LINE 3 ONWARD - AVERAGED ARM LENGTH MEASUREMENTS FROM PROGRAM MPL
*                SHORT ARM VALUES APPEAR FIRST I.E. 1 ROW IS AS FOLLOWS:-
*                SHORT 1, LONG 1, SHORT 2, LONG 2, SHORT 3 ....ETC.
*                FORMAT IS 20F5.2  E.G.  --213--549---98--341--UP TO 20
*                2.13 AND 5.49 REPRESENT 1 CHROMOSOME
*      NEXT LINES -STANDARD ERRORS OF THE ARM LENGTHS
*                FORMAT IS 20F4.3  E.G.  --41--89--27--23--UP TO 20
*                WHERE THE ORIGINAL VALUES WERE 0.041,0.089,0.027..ETC.
*    ADDITIONAL DATA SETS ARE THEN ADDED
*
*  ***NOTE***   THE NUMBER OF DATA SET (NUM) MUST BE SPECIFIED BEFORE RUNNING KARYO
*                 (SEE 3RD LINE AFTER COMMENTS SECTION)
*
**********************************************************************
      IF(EOF(2))3000,4000
 4000 CONTINUE
C SPECIFY NUMBER OF DATA SETS
      NUM=35
      M=0 $MM=0
C ZERO FILL MATRIX DATA AND STERR
      DO 5 J=1,20
      DO 5 I=1,180
      DATA(I,J)=0.0
      STERR(I,J)=0.0
    5 CONTINUE
C READ IN DATA
      DO 18 I=1,2
      IA=I*16-15
      IB=I*16
      READ(2,19)(TTEST(K),K=IA,IB)
   19 FORMAT(16F5.3)
   18 CONTINUE
      DO 1 I=1,NUM
      READ(2,2)(HA(I,J),J=1,20)
    2 FORMAT(20A4)
      READ(2,3)(IPAR(I,K),K=1,3)
    3 FORMAT(3I4)
      KA=IPAR(I,1)
      DO 4 L=1,KA
      M=M+1
      READ(2,6)(DATA(M,N),N=1,20)
    6 FORMAT(20F5.2)
    4 CONTINUE
      DO 22 LL=1,KA
      MM=MM+1
      READ(2,23)(STERR(MM,N),N=1,20)
   23 FORMAT(20F4.3)
   22 CONTINUE
      KB=M-KA+1
```

```
      DO 7 II=1,20
      DO 7 J=KB,M
      IF(DATA(J,II).GT.100.0)8,7
    8 WRITE(3,9)J,II
    9 FORMAT(1X,*ERROR IN MATRIX DATA AT POSITION*,2I4)
    7 CONTINUE
      DO 39 J=I,20
      DO 39 K=KB,M
      IF(STERR(K,J).GT.5.0)40,39
   40 WRITE(3,41)K,J
   41 FORMAT(1X,*ERROR IN MATRIX STERR AT POSITION*,2I4)
   39 CONTINUE
    1 CONTINUE
C SELECT NEXT TWO DATA SETS AND DETERMINE WHICH IS SMALLEST
      NUMA=NUM-1
      NUMB=NUM
      DO 10 I=1,NUMA
      NUMB=NUMB-1
      IF(IPAR(NUM,3).GT.IPAR(NUMB,3))11,12
   11 K=IPAR(NUMB,3) $L=NUMB $KA=IPAR(NUM,3) $LA=NUM
      GO TO 204
   12 K=IPAR(NUM,3) $L=NUM $KA=IPAR(NUMB,3) $LA=NUMB
C DETERMINE N (FIRST DATA LINE OF SMALLEST SET)
  204 LL=L-1
      N=0
      IF(LL.EQ.0)13,14
   14 DO 15 JA=1,LL
      N=N+IPAR(JA,1)
   15 CONTINUE
      N=N+1
      GO TO 205
   13 N=1
C DETERMINE NA (FIRST DATA LINE OF LARGEST SET)
  205 NA=0
      LLA=LA-1
      IF(LLA.EQ.0)16,17
   17 DO 200 IA=1,LLA
      NA=NA+IPAR(IA,1)
  200 CONTINUE
      NA=NA+1
      GO TO 206
   16 NA=1
  206 WRITE(3,32) (HA(L,IS),IS=1,20),(HA(LA,IT),IT=1,20)
   32 FORMAT(//,40X,*SET A*,3X,20A4,/,45X,*VS*,/,40X,*SET B*,3X,20A4,//
     C,120(1H-))
      IF(I.GT.1) GO TO 100
      DO 52 IZ=1,2
      IF(IZ.EQ.1)53,54
   53 LX=L $NX=N
      GO TO 207
   54 LX=LA $NX=NA
  207 WRITE(3,33)(HA(LX,IS),IS=1,20)
   33 FORMAT(1X,20A4,//,1X,*ARM LENGTHS*,/)
      IT=NX+IPAR(LX,1)-1
      DO 34 IS=NX,IT
      WRITE(3,35)(DATA(IS,IU),IU=1,19,2),(DATA(IS,IV),IV=2,20,2)
   35 FORMAT(1X,10F8.2,/,1X,10F8.2,//)
   34 CONTINUE
      WRITE(3,36)
   36 FORMAT(1X,*STANDARD ERRORS*,/)
      DO 50 IS=NX,IT
      WRITE(3,51)(STERR(IS,IU),IU=1,19,2),(STERR(IS,IV),IV=2,20,2)
   51 FORMAT(1X,10F8.3,/,1X,10F8.3,//)
   50 CONTINUE
      WRITE(3,211)
  211 FORMAT(120(1H-))
   52 CONTINUE
  100 WRITE(3,217)
  217 FORMAT(1X,///,1X,*IDENTICAL CHROMOSOMES (AT 5% PROB. LEVEL)*,//)
      WRITE(3,218)
  218 FORMAT(3X,*SET A*,3X,*SET B*,5X,*ALL MATCHES IN B*,/,50(1H-))
C  COMPARE SET A CHROMOSOMES WITH SET B
      DO 60 IW=1,21
      DO 60 IWW=1,60
      IPAIR(IWW,IW)=0
   60 CONTINUE
      KD=N+(IPAR(L,1)-1)
```

```
      DO 70 MC=N,KD
      DO 20 M=1,10
      TA=DATA(MC,2*M-1) $TAA=DATA(MC,2*M)
      IX=(MC-KD+IPAR(L,1)-1)*10+M
      IPAIR(IX,1)=IX
      MN=2 $MM=0
      KE=NA+(IPAR(LA,1)-1)
      DO 21 MB=NA,KE
      DO 71 MA=1,10
      MM=MM+1
      IF(DATA(MC,2*M-1).EQ.0.0)GO TO 70
      IF(DATA(MB,2*MA-1).EQ.0.0)GO TO 20
      TB=DATA(MB,2*MA-1)
      IF(TA.GT.TB)24,25
   24 TC=TA-TB
      GO TO 208
   25 TC=TB-TA
  208 TD=STERR(MC,2*M-1)*STERR(MC,2*M-1)
      TE=STERR(MB,2*MA-1)*STERR(MB,2*MA-1)
      T=TC/SQRT(TD+TE)
      NF=IPAR(L,2)+IPAR(LA,2)-2
      IF(T.LT.TTEST(NF))26,38
   26 TB=DATA(MB,2*MA)
      IF(TAA.GT.TB)27,28
   27 TC=TAA-TB
      GO TO 209
   28 TC=TB-TAA
  209 TD=STERR(MC,2*M)*STERR(MC,2*M)
      TE=STERR(MB,2*MA)*STERR(MB,2*MA)
      T=TC/SQRT(TD+TE)
      IF(T.LT.TTEST(NF))29,38
   29 IF(MN.GT.18)MN=18
      IPAIR(IX,MN)=MM
      MN=MN+1
   38 IF(MM.EQ.IPAR(LA,3))30,71
   30 IF(MN.EQ.2)GO TO 71
   71 CONTINUE
   21 CONTINUE
   20 CONTINUE
   70 CONTINUE
C CATALOG UNIQUE CHROMOSOME MATCHES
      DO 72 MC=1,K
      DO 73 MA=2,18
      IF(IPAIR(MC,2).EQ.0)GO TO 72
      IF(IPAIR(MC,MA).EQ.0)GO TO 101
      ME=IPAIR(MC,MA)
      IF(IPAIR(ME,19).GT.0)GO TO 73
      IPAIR(ME,19)=ME $IPAIR(ME,21)=MC $IPAIR(MC,20)=ME
      GO TO 72
   73 CONTINUE
C CHECK FOR EXTRA MATCHES = FIRST ORDER
  101 DO 102 MD=2,18
      IF(IPAIR(MC,MD).EQ.0)GO TO 72
      ME=IPAIR(MC,MD) $MF=IPAIR(ME,21)
      DO 103 MG=2,18
      IF(IPAIR(MF,MG).EQ.0)GO TO 102
      MH=IPAIR(MF,MG)
      IF(MH.EQ.ME)GO TO 103
      IF(IPAIR(MH,19).GT.0)GO TO 104
      IPAIR(ME,19)=0 $IPAIR(ME,21)=0 $IPAIR(MF,20)=0
      IPAIR(MH,19)=MH $IPAIR(MH,21)=MF $IPAIR(MF,20)=MH
      IPAIR(ME,19)=ME $IPAIR(ME,21)=MC $IPAIR(MC,20)=ME
      GO TO 72
  104 DO 105 MI=2,18
      MK=IPAIR(MH,21)
      IF(IPAIR(MK,MI).EQ.0)GO TO 103
      MJ=IPAIR(MK,MI)
      IF(MJ.EQ.MH)GO TO 105
      IF(IPAIR(MJ,19).GT.0)GO TO 105
      IPAIR(MH,19)=0 $IPAIR(MH,21)=0 $IPAIR(MK,20)=0
      IPAIR(MJ,19)=MJ $IPAIR(MJ,21)=MK $IPAIR(MK,20)=MJ
      IPAIR(ME,19)=0 $IPAIR(ME,21)=0 $IPAIR(MF,20)=0
      IPAIR(MH,19)=MH $IPAIR(MH,21)=MF $IPAIR(MF,20)=MH
      IPAIR(ME,19)=ME $IPAIR(ME,21)=MC $IPAIR(MC,20)=ME
      GO TO 72
  105 CONTINUE
  103 CONTINUE
```

```
  102 CONTINUE
   72 CONTINUE
C WRITE CHROMOSOME MATCHES
      DO 107 MC=1,K
      ME=1
      DO 108 MD=2,18
      IF(IPAIR(MC,MD).EQ.0)GO TO 108
      ME=ME+1
  108 CONTINUE
      IF(IPAIR(MC,2).NE.0)GO TO 117
      WRITE(3,118)IPAIR(MC,1)
  118 FORMAT(4X,I3)
      GO TO 107
  117 IF(IPAIR(MC,20).NE.0)GO TO 115
      WRITE(3,116)IPAIR(MC,1),(IPAIR(MC,MF),MF=2,ME)
  116 FORMAT(4X,I3,13X,18I4)
      GO TO 107
  115 WRITE(3,109)IPAIR(MC,1),IPAIR(MC,20),(IPAIR(MC,MF),MF=2,ME)
  109 FORMAT(4X,I3,5X,I3,5X,18I4)
  107 CONTINUE
      NN=0
      DO 110 MG=1,K
      IF(IPAIR(MG,20).EQ.0)GO TO 110
      NN=NN+1
  110 CONTINUE
      AN=NN $BN=K $CN=KA
      DN=(AN/BN)*100
      EN=(AN/CN)*100
      WRITE(3,111)DN
  111 FORMAT(//,10X,F6.2,*% OF SET A MATCHES WITH SET B*,/)
      IF(K.EQ.KA)GO TO 114
      WRITE(3,112) EN
  112 FORMAT(10X,F6.2,*% OF SET B MATCHES WITH SET A*,/)
  114 WRITE(3,113)
  113 FORMAT(//,120(1H-))
   10 CONTINUE
 3000 CONTINUE
      END
12706 4303 3182 2776 2571 2447 2365 2306 2262 2228 2201 2179 2160 2145 2131 2120
 2110 2101 2093 2086 2080 2074 2069 2064 2060 2056 2052 2048 2045 2042 22042 2042
```

Lawrence, M. E. (1980). Senecio L. (Asteraceae) in Australia: chromosome numbers and the occurrence of polyploidy. *Australian Journal of Botany, 28*(2), 151-165.

NOTE:
This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:
http://dx.doi.org/10.1071/BT9800151