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47. CHROMOSOMAL AND MOLECULAR EVOLUTION IN THE GENUS *BRACHYSCOME* (ASTEREAE)

KUNIAKI WATANABE[1], TETSUO DENDA[1], YOHEI SUZUKI[2], KEIKO KOSUGE[1], MOTOMI ITO[2], PHILIP S. SHORT[3] AND TETSUKAZU YAHARA[4]

[1] **Department of Biology, Faculty of Science, Kobe University, Tsurukabuto, 1-2-1, Kobe, 657, JAPAN**

[2] **Department of Biology, Faculty of Science, Chiba University, Chiba, 260, JAPAN**

[3] **National Herbarium of Victoria, Birdwood Avenue, South Yarra, Victoria, 3141, AUSTRALIA**

[4] **Department of Biology, Faculty of Science, Kyushu University, Fukuoka, 812, JAPAN**

Abstract

Intrageneric circumscription, interspecific relationships and chromosomal and molecular evolution of the Australian *Brachyscome* were examined using data from restriction site analysis of chloroplast DNA, karyotype analysis and DNA sequence analysis of the alcohol dehydrogenase gene (*adh*). Molecular data indicate that the genus *Brachyscome* is divided into four clusters at the base. Two of them are composed of the members of subgenus *Brachyscome* and the remains the members of subgenus *Metabrachyscome*. Further, Davis' superspecific groupings are not fully supported. The base chromosome number in *Brachyscome* is 9. The haploid chromosome numbers lower than 9 are the products of several independent dysploid reductions from $n = 9$, resulting from very unequal reciprocal translocations with loss of centromeric fragments. Decrease in total karyotype length in some *Metabrachyscome* species with $n = 9$ and some *Brachyscome* species with $n = 6$ to $n = 2$ correlates with the change of habit from perennial to annual in arid or seasonally dry conditions. In addition to the dysploid reduction in chromosome number, amphidiploidy also plays a considerable role in speciation in this genus. The interspecific relationships based on the amino acid sequences of the *adh* gene support those based on the karyotypes more than relationships inferred from fruit type and morphology. In contrast to the conservative nature of exon sequences of the *adh* gene, the intron length shows significant variation among species, due to inserted elements. Drastic genomic re-organization has occurred both at the molecular and chromosomal levels in the advanced species.

Introduction

Brachyscome Cass. is endemic to the Australasian region, and is predominantly Australian. This genus comprises about 80 species and occurs in a diversity of habitats from the high rainfall zones of the coasts and mountains to the arid regions of Central Australia. It has undergone substantial episodes of adaptive radiation and speciation in the widespread semi-deserts in Australia. Thus the study of this plant group might contribute to an understanding of the origin and evolution of Australian desert flora. This genus was divided into two subgenera by Davis (1948, 1949, 1955, 1959), *Brachyscome* and *Metabrachyscome* G.L.Davis, based on the presence or absence, respectively, of terminal appendages on the anthers. In addition, she grouped the species into six superspecies in the subgenus *Brachyscome* and five superspecies

in the subgenus *Metabrachyscome*. Davis gave heavy emphasis to fruit characters not only for species discrimination but also for the interpretation of phylogenetic relationships. This genus shows extraordinary chromosomal versatility, involving structural changes and variation in number from $n = 15$ to $n = 2$ as well as intraspecific polyploidy (Smith-White *et al.*, 1970; Watanabe & Short, 1992; Watanabe *et al.*, 1996), and thus offers excellent opportunities to examine chromosomal evolution from a phylogenetic perspective. Davis' phylogenetic tree (Davis, 1948, Fig. 123) is not consistent with the data based on the chromosome numbers, habits, reproductive modes and geographical distributions (Smith-White *et al.*, 1970; Watanabe & Short, 1992; Watanabe *et al.*, 1991, 1996, unpublished). Almost all the known species of subgenus *Brachyscome* with $n = 9$ occupy mesic regions in the south-east temperate zone and have a perennial habit, basal rosette growth form, erect flowering peduncles, self-incompatibility and conspicuous flowers. In contrast, lower chromosome numbers in subgenus *Brachyscome* are often associated with annual habit and special habitats such as flood plains, arid or seasonally dry areas and mallees subjected to frequent natural burning.

To obtain a more accurate estimate of phylogenetic relationship and the evolutionary trend of several characters, we carried out cytological and molecular research.

Molecular phylogenetic tree based on restriction site mutations in chloroplast DNA

We have analysed the restriction site mutations in chloroplast DNA of 31 taxa, representing two subgenera and nine superspecies in this genus (Suzuki *et al.*, unpublished). The genera *Lagenifera* Cass. (= *Lagenophora*) and *Solenogyne* Cass. have often been united and are putatively related to *Brachyscome* (Davis, 1950a, b). Hence, the Australian *Solenogyne dominii* L.G.Adams was included in the present analysis as the outgroup. Thirteen restriction endonucleases were used for digesting and fifteen clones from a lettuce chloroplast DNA clone bank (Jansen & Palmer, 1987) were used as probes for southern hybridization. Parsimony analyses of the data matrix (Table 1) were performed using branch-and-bound searches of PAUP version 3.1 (Swofford, 1993). As the result, a total of 66 site mutations was identified, 31 of which were phylogenetically informative. We detected only one insertion/deletion, c. 400 bp deletion was present in 12 taxa at the position of lettuce chloroplast DNA probe 1 (Table 1, Character No. 1). Cladistic analyses yielded 220 equally most-parsimonious trees with length 37 steps and consistency index 0.865. The strict consensus tree (Fig. 1) indicates that the genus *Brachyscome* is divided into four clusters at the base. Two of them are composed of the members of subgenus *Brachyscome* and the remains the members of subgenus *Metabrachyscome*. We cannot detect any synapomorphic mutations in chloroplast DNA to cluster *B. dichromosomatica* C.R.Carter representing the *B. lineariloba* complex with other members of the subgenus *Brachyscome*, although they have a terminal appendage on the anthers. One of the two *Metabrachyscome* clusters was composed of only the members of the superspecies *trachycarpa*. Except for this cluster, the superspecific circumscription of Davis is not fully supported. The base chromosome number for the Australian Astereae Cass. is regarded to be 9 based on its commonness (Smith-White *et al.*, 1970; Watanabe *et al.*, 1996). The tree supports a view that the base chromosome number in *Brachyscome* is also 9, and haploid chromosome numbers lower than 9 are regarded as the products of several independent dysploid reductions in several different lineages.

Mitotic metaphase karyotype of species

Conventional karyotype analyses were made on 54 taxa of *Brachyscome* and selected representatives of other Australian Astereae. Drawings are based on the mean of ten measurements. The reliability of the comparisons of the chromosome length measured in the respective species was partly confirmed in the hybrid cells of 35 successful interspecific or

TABLE 1. Matrix of restriction site data used in analysis of *Brachyscome*.

	Taxon	Character number 1111111111222222222333 12345678901234567890123456789012
1	*Brachyscome dentata*	11000000000010001000000001000000
2	*B. curvicarpa* A	11000000001010001000000001000000
3	*B. curvicarpa* B	11000000001010001000000001000000
4	*B. tetrapterocarpa*	11000000001010001000000001000000
5	*B. microcarpa*	10000000000010001000000001100000
6	*B. melanocarpa*	10000000000010001000000001100000
7	*B. multifida* A	10000000000010001000000001100000
8	*B. multifida* B	10000000000010001000000001000000
9	*B. scapigera*	10000000000010001000000001000000
10	*B. graminea*	1?000000000010001000000001000000
11	*B. debilis*	1?000000000010001000000001000000
12	*B. muelleri*	10000000000010000000000001000000
13	*B. radicans*	00000001100010000000000001000000
14	*B. decipiens*	00000001100010000000000001000000
15	*B. readeri*	00000001100010000000000001010000
16	*B. nodosa*	00000001100017??000000??01000000
17	*B. diversifolia*	00000000000010000000101011001000
18	*B. goniocarpa*	00000000000010000000101011001000
19	*B. eriogona*	00000000000010000000000101001001
20	*B. smithwhitei*	00000000000010000000000101001001
21	*B. cardiocarpa*	0?000000000010000?00000001000000
22	*B. dichromosomatica*	00000000000000000000000000?000000
23	*B. latisquamea*	0?101000000000???01???00000010010
24	*B. cheilocarpa*	0?101000000?00110?10000001010010
25	*B. ciliocarpa*	0?101000000?00110010000000010010
26	*B. halophila*	0?100000010000110?0000?000010010
27	*B. iberidifolia*	0?101000010000???00010000000010
28	*B. trachycarpa*	0?010110000101000?1?1010000000100
29	*B. ciliaris* A	0?010110000101000?1?1010000000100
30	*B. ciliaris* B	0?010110000101000?1?1010000000100
31	*B. ciliaris* C	0?010000000100000000?10000000000
32	*Solenogyne dominii*	00000000000000000000000000000000

For each character, 0 = plesiomorphy (same state as *Solenogyne dominii*); 1 = apomorphy; ? = missing data. Character 1 is c. 400 bp deletion.

cytotypic cross combinations (Watanabe *et al.*, 1975, 1976, 1991, unpublished; Watanabe & Smith-White, 1987). The parental morphology and size of metaphase chromosomes are maintained except that some of the NORs are frequently suppressed in some F_1-hybrids (Watanabe *et al.*, 1991). The condensation pattern of the chromosomes from the two parental sources is quite distinct at late mitotic prophase and prometaphase when F_1-hybrids involve both the long chromosome species, such as *B. eriogona* (J.M.Black) G.L.Davis and *B. lineariloba* (DC.) Druce, and the medium-sized chromosome species (Watanabe *et al.*, 1976;

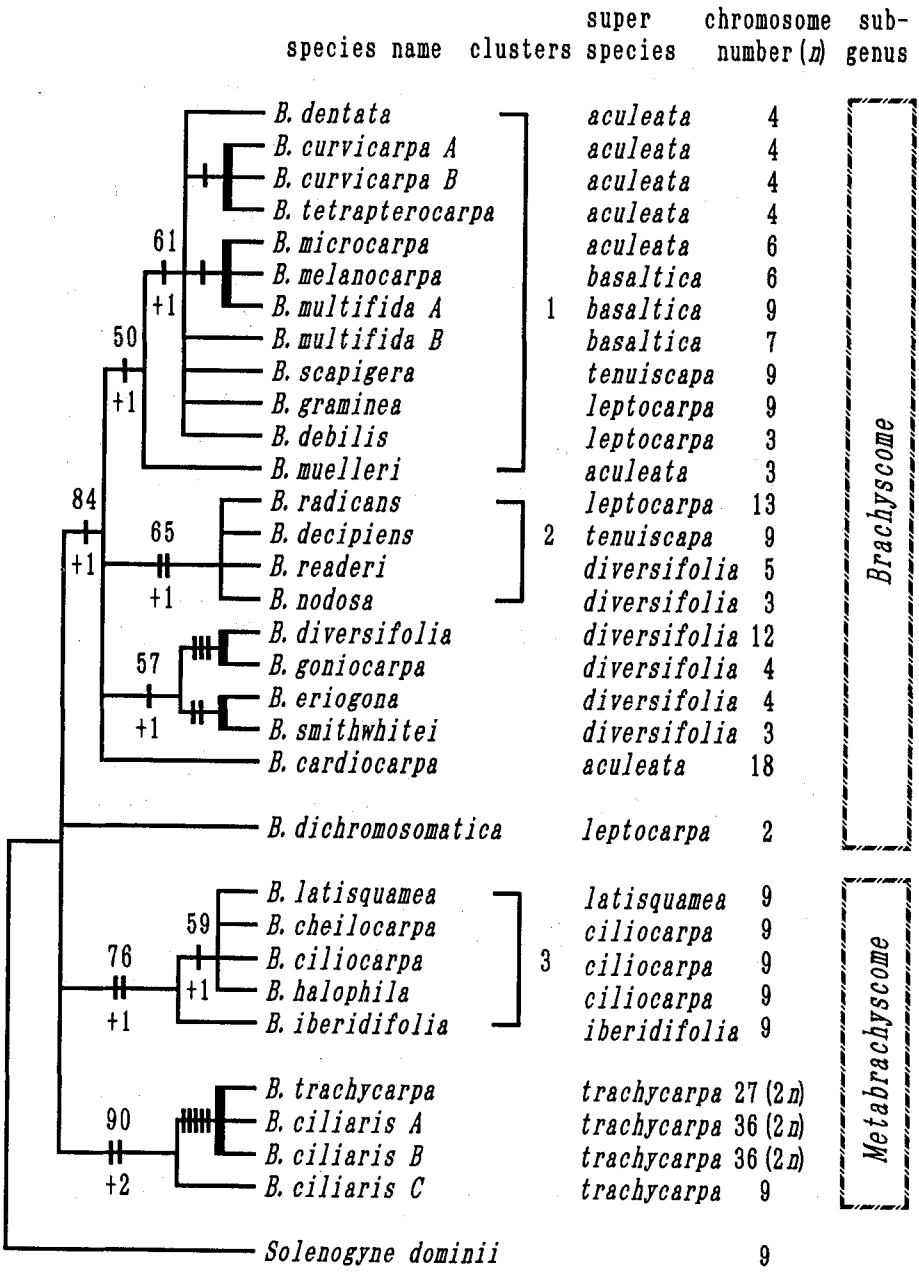
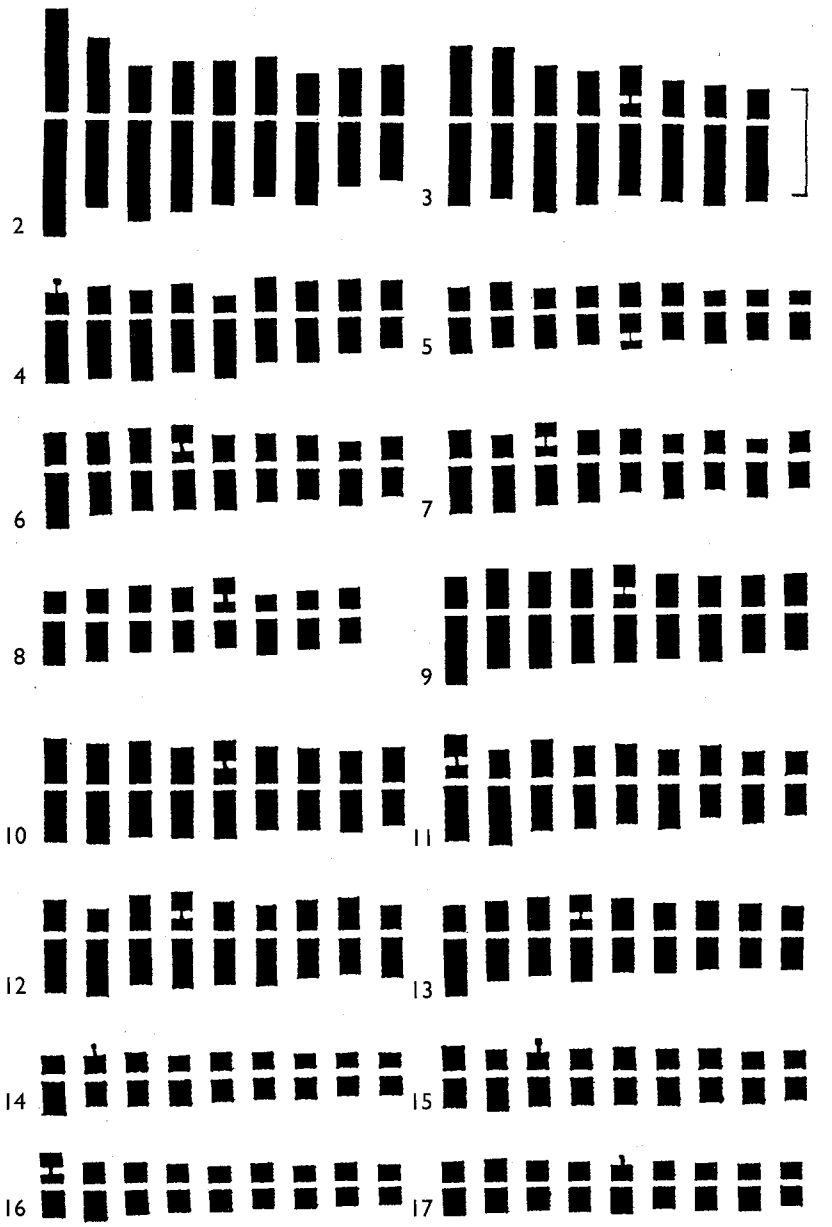


FIG. 1. Strict consensus tree for the 220 equally most parsimonious trees of *Brachyscome*. Bold line indicates the cluster of species which have same mutations. Numbers above the branches indicate the bootstrap values in 100 replicates and those below branches are the decay index values. Superspecies name and haploid chromosome numbers are given following each species name. *B. multifida* A = var. *dilatata*, B = var. *multifida*.



FIGS. 2–17. Mitotic metaphase karyotype idiograms of *Brachyscome* and allied genera. 2. *Olearia pimelioides* (2*n* = 18), 3. *Erodiophyllum elderi* (2*n* = 16), 4. *Minuria leptophylla* (2*n* = 18), 5. *Vittadinia cuneata* (2*n* = 18), 6. *Solenogyne dominii* (2*n* = 18), 7. *Lagenifera stipitata* (2*n* = 18), 8. *Calotis cuneifolia* (2*n* = 16), 9. *Brachyscome decipiens* (2*n* = 18), 10. *B. latisqamea* (2*n* = 18), 11. *B. aculeata* (2*n* = 18), 12. *B. lyrifolia* (2*n* = 18), 13. *B. obovata* (2*n* = 18), 14. *B. trachycarpa* (2*n* = 18), 15. *B. halophila* (2*n* = 18), 16. *B. tatei* (2*n* = 18), 17. *B. iberidifolia* (2*n* = 18). Scale bar = 5 μm.

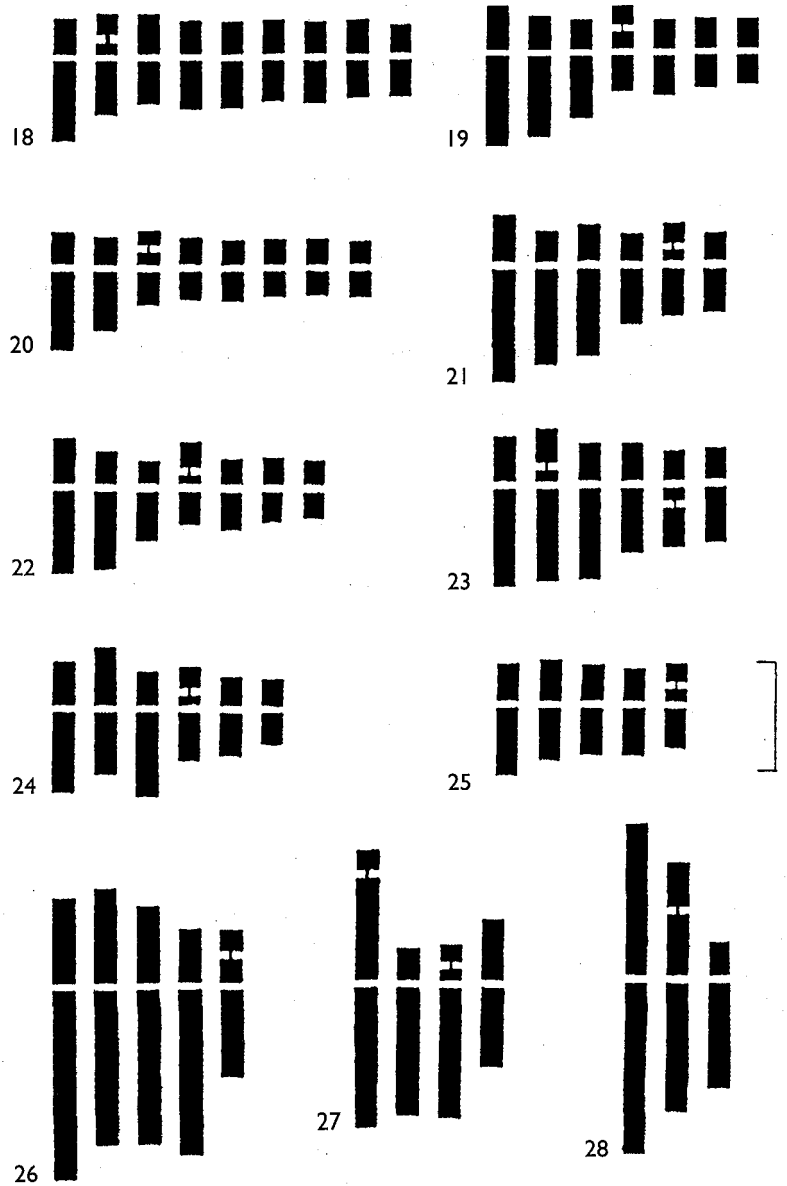
Watanabe & Smith-White, 1987). Otherwise, the hybrid chromosomes show almost synchronous condensation behaviour similar to that of the corresponding chromosomes in the parental material (Watanabe & Smith-White, 1987; Watanabe *et al.*, 1991). Among the Australian Astereae examined (*Erodiophyllum* F.Muell., *Olearia* Moench., *Calotis* R.Br., *Minuria* DC., *Vittadinia* A.Rich., *Solenogyne* and *Lagenifera*), the karyotypes of *Lagenifera* and *Solenogyne* closely resemble in chromosome size and morphology those of some taxa of *Brachyscome* (Figs. 2–17). Based on the karyotype comparison in allied genera and the extensive survey of chromosome number in Australian Astereae (Watanabe *et al.*, 1996), we can safely conclude that the ancestral base number in *Brachyscome* is 9.

The *Brachyscome* species with $n = 9$ can be divided into two distinct groups by their chromosome length: medium-sized (Figs. 9–13) or short (Figs., 14–17). The total karyotype length of medium-sized chromosome species is nearly one and one half to twice the length of short chromosome species. Although the short chromosome species are confined to the subgenus *Metabrachyscome*, some of its other members, such as *B. latisquamea* F.Muell. (Fig. 10) and *B. lyrifolia* J.M.Black (Fig. 12), have medium-sized chromosomes. Since perennial or woody taxa of Australian Astereae have medium-sized chromosomes (Figs. 2, 3, 4, 6 and 7), the character state of "medium-sized chromosome" is considered to be plesiomorphic. The shortening of chromosomal and total karyotype lengths in some *Metabrachyscome* (Figs. 14, 15 and 17) correlates with the change of habit from perennial to annual as shown in Table 2. All species with $n = 9$ have rather unimodal and symmetrical karyotypes (Fig. 40). The annuals *Vittadinia cuneata* DC. (Fig. 5) and *Calotis cuneifolia* R.Br. (Fig. 8) and the exceptional perennial *Brachyscome tatei* J.M.Black (Fig. 16) have shorter chromosomal and total karyotype lengths.

TABLE 2. The relationships between haploid chromosome numbers (n), total karyotype lengths (TKL) and habits in *Brachyscome*.

Perennials or Shrub	n	TKL (μm)	Annuals	n	TKL (μm)
Cluster 1					
B. multifida var. *dilatata*	9	66.96	*B. muelleri*	3	64.62
B. multifida var. *multifida*	7	55.60	*B. tetrapterocarpa*	4	49.16
B. scapigera	9	54.98	*B. curvicarpa*	4	47.54
B. melanocarpa	6	54.02	*B. dentata*	4	36.96
B. microcarpa	6	50.72	*B. debilis*	3	31.94
B. graminea	9	47.02			
mean	7.7	54.88	mean	3.6	46.04
Cluster 2					
B. decipiens	9	68.84	*B. readeri*	5	52.34
			B. nodosa	3	25.02
			mean	4	38.68
Cluster 3					
B. latisquamea	9	69.86	*B. halophila*	9	40.52
			B. iberidifolia	9	34.68
			mean	9	37.60

Comparisons were made on the species karyotyped within each cluster in Fig. 1. There is a significant TKL difference at 5% between perennials and annuals by Mann-Whitney test.



FIGS. 18–28. Mitotic metaphase karyotype idiograms of *Brachyscome*. 18. *B. multifida* var. *dilatata* (2*n* = 18), 19. *B. multifida* var. *multifida* (2*n* = 14), 20. *B. basaltica* var. *basaltica* (2*n* = 16), 21. *B. basaltica* var. *gracilis* (2*n* = 12), 22. *B. nova-anglica* A (2*n* = 14), 23. *B. nova-anglica* B (2*n* = 12), 24. *B. microcarpa* (2*n* = 12), 25. *B. nova-anglica* C (2*n* = 10), 26. *B. campylocarpa* (2*n* = 10), 27. *B. eriogona* (2*n* = 8), 28. *B. muelleri* (2*n* = 6). Scale bar = 5 μm.

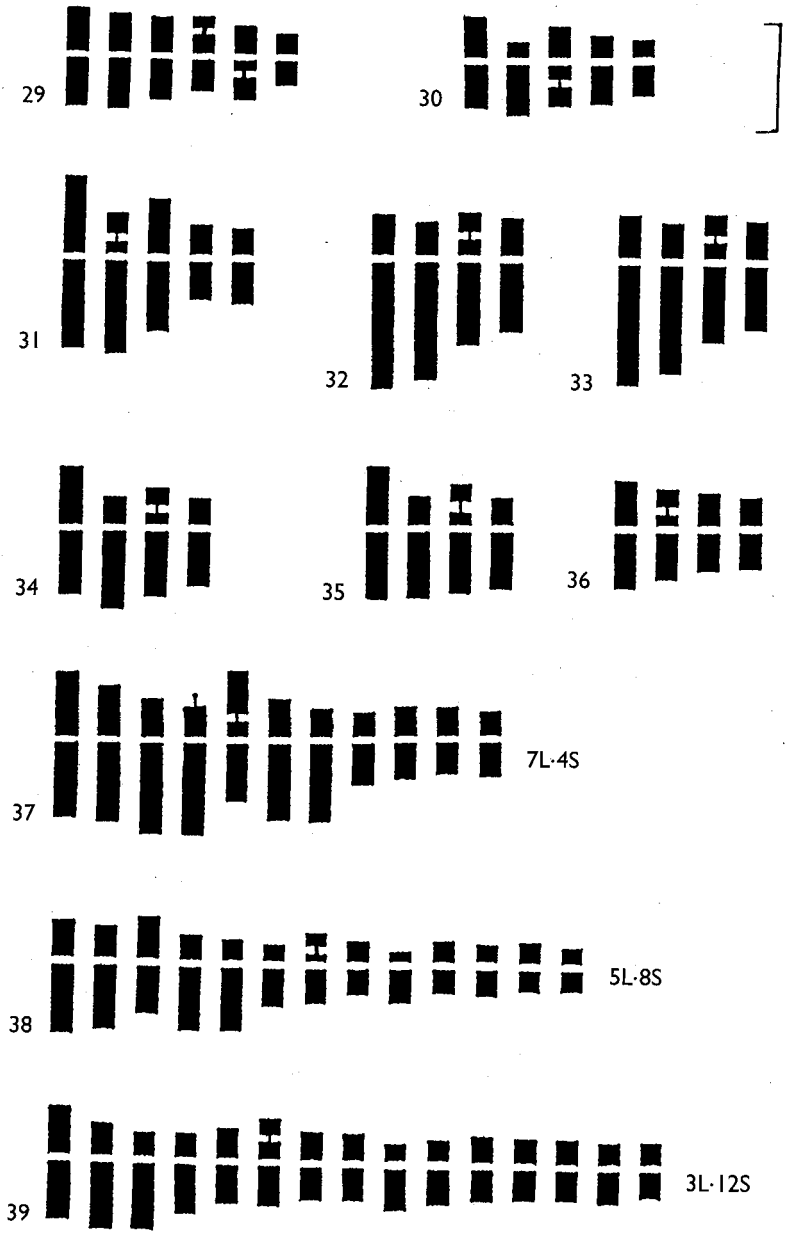
In contrast to these unimodal and symmetrical karyotypes of species with $n = 9$, the karyotypes in the species having haploid chromosome numbers lower than 9 are characterized as bimodal and asymmetrical (Fig. 40) with several distinctly longer chromosomes in the subgenus *Brachyscome* (Figs. 19–24). The chromosome numbers $n = 8$ to $n = 6$ seem best explained by reduction in number from 9, accomplished by very unequal reciprocal translocations with loss of centromeric fragments. Decrease in chromosome number appears to have proceeded stepwise with one or more species at each step. Because the number of longer translocation chromosomes increases as chromosome number decreases from $n = 8$ to $n = 6$, this means that the decrease in chromosome number could occur without losing large pieces of chromatin in these processes. Some examples of dysploid reduction in *B. multifida* DC. $n = 9$ (Fig., 18) to $n = 7$ (Fig., 19), in *B. basaltica* F.Muell. $n = 8$ (Fig. 20) to $n = 6$ (Fig. 21) and in *B. nova-anglica* G.L.Davis $n = 7$ (Fig. 22) to $n = 6$ (Fig. 23) and $n = 5$ (Fig. 25) are illustrated. These results also confirm that the dysploid reduction was achieved independently more than once and thus the lower chromosome numbers are suggested to have some selective advantage in their habitats. It is notable that the species with low chromosome numbers are largely confined to flood plains, arid or seasonally dry areas and mallees subjected to frequent natural burning. They are considered to be rapid colonizers, largely owing to their genetic conservatism. The natural disturbances such as fire, drought and flooding may temporarily denude a site of its biotic occupants and create ecological vacua serving as refuges for species with lessened chromosome numbers. Under the lessened biotic selective pressures of parasites, predators or competitors in such disturbed areas, these genetic systems that have restricted recombination (Grant, 1981; Levin, 1975; Glesener & Tilman, 1978) would be favoured and tend to spread rapidly since their well-adapted biotypes would have higher reproductive potential.

As a result of successive chromosomal changes, karyotypes in the species with $n = 8$ to $n = 3$ became more asymmetrical as evolution progressed, shifting from metacentric to subtelocentric (Fig. 40A) and undergoing increasing differentiation in chromosome length between the translocated and nontraslocated chromosomes (Fig. 40B). But, symmetrical karyotypes again evolved in advanced species such as *B. nova-anglica* C with $n = 5$ (Fig. 25)

TABLE 3. Relation to karyotype asymmetry and variance in chromosome size within genome to chromosome number in *Brachyscome*.

Taxa with higher chromosome numbers	*n*		A	V	Taxa with lower chromosome numbers	*n*		A	V
B. multifida	9	I	0.178,	0.013	*B. multifida*	7	I	0.220,	0.033
var. *dilatata*		II	3.72,	0.44	var. *multifida*		II	3.97,	1.84
B. basaltica	8	I	0.172,	0.025	*B. basaltica*	6	I	0.352,	0.014
var. *basaltica*		II	2.97,	1.11	var. *gracilis*		II	5.04,	2.69
B. nova-anglica A	7	I	0.221,	0.025	*B. nova-anglica* B	6	I	0.306,	0.005
		II	3.74,	1.80			II	5.14 ,	1.57
B. nova-anglica B	6	I	0.306,	0.005	*B. nova-anglica* C	5	I	0.162,	0.008
		II	5.14,	1.57			II	3.99,	0.36

n: haploid chromosome numbers, A: average, V: variance, I: average of arm difference ratio (see Fig. 40A), II: average of chromosome length (see Fig.40B). Comparisons were made within the species complex. There is no significant difference at 5% between higher and lower chromosome numbers by Mann-Whitney test.



FIGS. 29–39. Mitotic metaphase karyotype idiograms of *Brachyscome*. 29. *B. ptycocarpa* ($2n = 12$), 30. *B. whitei* ($2n = 10$), 31. *B. angustifolia* ($2n = 10$), 32. *B. tetrapterocarpa* ($2n = 8$), 33. *B. curvicarpa* ($2n = 8$), 34. *B. dentata* ($2n = 8$), 35. *B. papillosa* ($2n = 8$), 36. *B. chrysoglossa* ($2n = 8$), 37. *B. nivalis* ($2n = 22$), 38. *B. radicans* ($2n = 26$), 39. *B. stolonifera* ($2n = 30$). Scale bar = 5 μm.

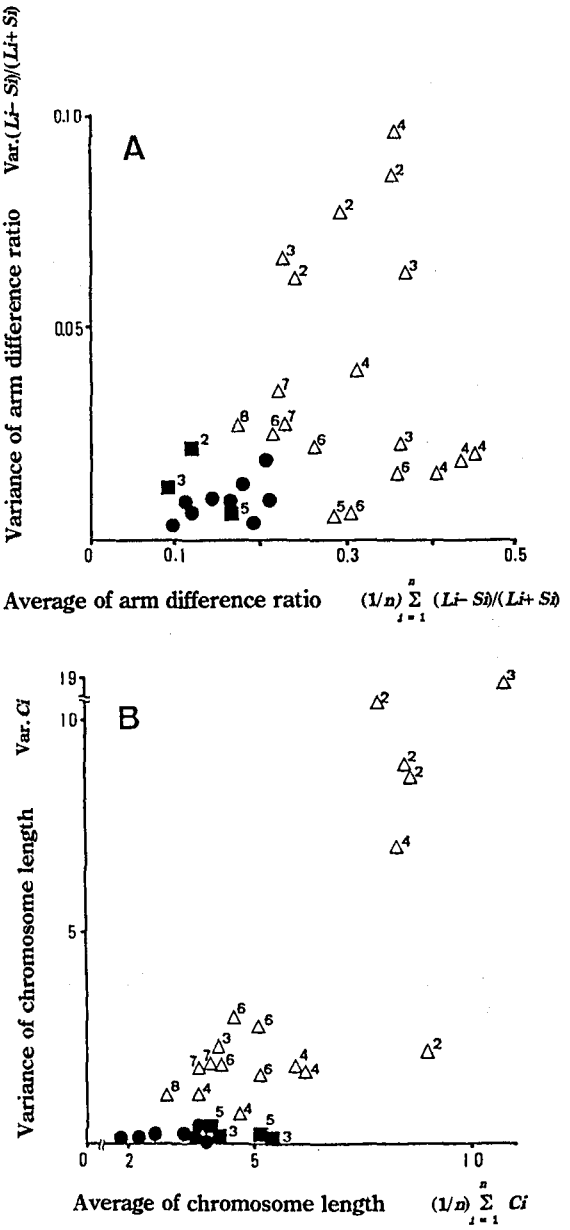


FIG. 40. Karyotype asymmetry and variance in chromosome length within genome in *Brachyscome* species. A. Vertical axis shows the variance of arm difference ratio. Horizontal axis shows the average of arm difference ratio. B. Vertical axis shows the variance of chromosome length. Horizontal axis shows the average of chromosome length. *Li*: long arm length, *Si*: short arm length, *Ci*: chromosome length, *n*: haploid chromosome number within respective species. Note two extremes of the species with $n = 9$ shown black circles and the species with lower chromosome numbers shown black squares have symmetrical karyotypes. The other species shown by triangles have rather asymmetrical karyotypes. Number on the symbol means haploid chromosome number.

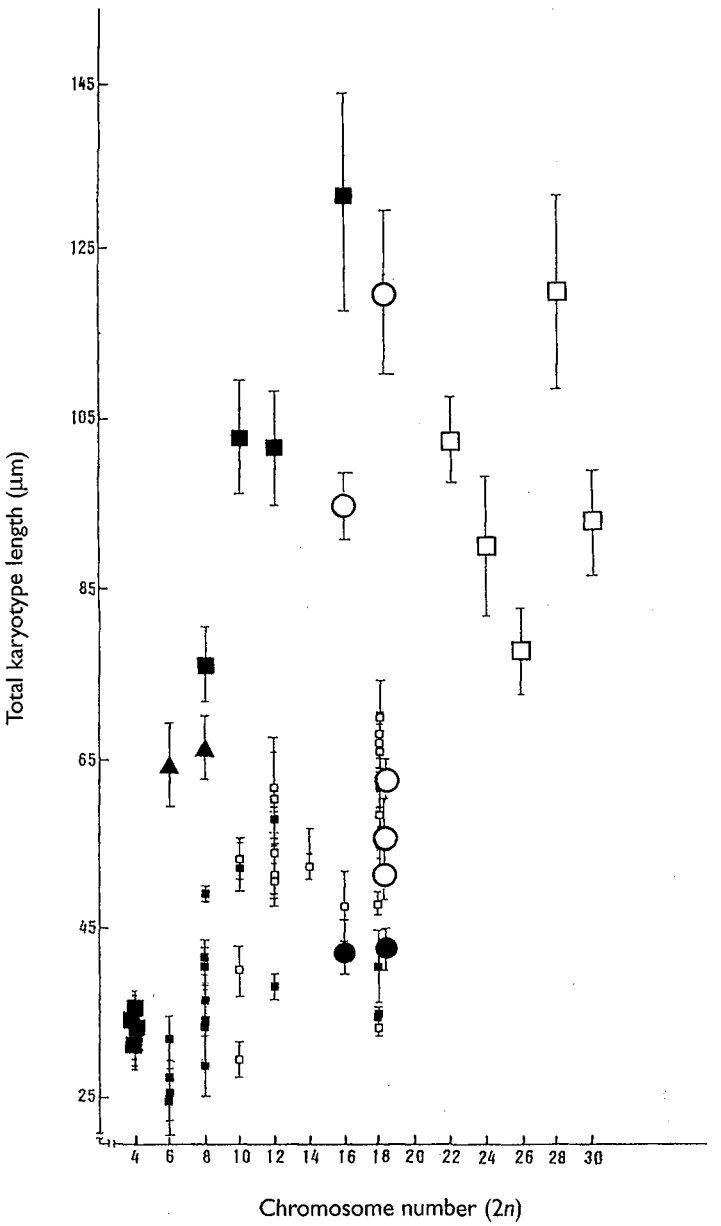


FIG. 41. The relationships of total karyotype length (μm), chromosome numbers (2*n*) and habits. White large circles are other Australian Astereae excluding *Brachyscome*. The annuals *Calotis cuneifolia* (2*n* = 16) and *Vittadinia cuneata* (2*n* = 18) shown black large circles have shorter total karyotype length. Black large boxes are the annuals *Brachyscome lineariloba* complex. Black large triangles are the annuals *B. muelleri* (2*n* = 6) and *B. eriogona* (2*n* = 8). Large clear boxes are the perennial *Brachyscome* species with 2*n* = 22 to 2*n* = 30. Small boxes are *Brachyscome* species with 2*n* = 6 to 2*n* = 18. Black boxes mean they are annuals. Note that annuals, except the *B. lineariloba* complex, *B. muelleri* and *B. eriogona*, have shorter total karyotype length.

and *B. ptychocarpa* F.Muell. with $n = 6$ (Fig. 29). There can be no doubt that *Brachyscome* chromosomes have often undergone successive changes of this kind moving at one time towards asymmetry and at another towards symmetry (Table 3).

The substantial reduction in total karyotype length in the *Brachyscome* species with $n = 6$ to $n = 2$, in addition to some *Metabrachyscome* species with $n = 9$, also correlates with the change of habit from perennial to annual (Table 2). There appears to be an evolutionary trend towards decrease in the total karyotype length that is correlated with the trend from perennial to annual. Total karyotype length provides a good estimate of DNA content and genome size (Levin & Funderberg, 1979), and annuals tend to have smaller genomes than perennials in general (Bennett, 1972). As exceptions, the annuals *B. muelleri* Sond. ($2n = 6$), *B. eriogona* ($2n = 8$) and the *B. lineariloba* complex B ($2n = 12$), C ($2n = 16$), D (= *B. breviscapis* C.R.Carter, $2n = 8$) and E ($2n = 10$) have extraordinarily longer chromosome and karyotype length (Fig. 41). Although the reasons why annuals such as *B. muelleri* and *B. eriogona* have such longer chromosome and karyotype length have not been explained at present, the increase in total karyotype length in the *B. lineariloba* complex is due to successive amphidiploidy. The apparent increase in drought resistance or water stress tolerance and of vigour and fecundity is certainly associated with an increase in chromosome number in the *B. lineariloba* complex (Watanabe & Smith-White, 1987). These longer chromosomes show the distinct differential condensation pattern compared to the shorter chromosomes of other species with low chromosome number at prometaphase in the hybrid cells (Watanabe *et al.*, 1976).

The *B. lineariloba* complex includes *B. dichromosomatica* ($2n = 4$), *B. breviscapis* (2n = 8) and *B. lineariloba* E ($2n = 10$), B ($2n = 12$) and C ($2n = 16$). *Brachyscome breviscapis* ($2n = 8$) may be of amphidiploid origin between a species with two long early condensing chromosome pairs and another unknown *B. dichromosomatica*-like species with two medium-sized late condensing pairs. The additional medium-sized and late condensing chromosomes in *B. lineariloba* E ($2n = 10$), B ($2n = 12$) and C ($2n = 16$) are also considered to be derived from the *B. dichromosomatica*-like parent (Watanabe & Smith-White, 1987). The evidence that these chromosomal races and species have a recent amphidiploid origin involving *B. dichromosomatica* as a mother is supported by the possession of exactly the same restriction fragment patterns of chloroplast DNA in preliminary analysis. *Brachyscome dichromosomatica* can easily cross with other members of the subgenus *Brachyscome* and produce viable F_1-hybrids (Watanabe *et al.*, 1976, 1991).

The chromosome condensation behaviour of *B. dichromosomatica* is similar to those of shorter chromosomes in the *B. lineariloba* complex (Watanabe & Smith-White, 1987). The candidate of father species with longer chromosomes in the *B. lineariloba* complex may be the *B. eriogona*- or *B. muelleri*-like species. The view that these chromosomal races of the *B. lineariloba* complex have a recent amphiploid origin (Watanabe & Smith-White, 1987) is also supported by the drastic increases of the total karyotype length, in contrast to the small difference between $n = 4$ and $n = 8$ in other species (Fig. 41).

There are other examples of amphidiploidy as a speciation process in this genus. Among the taxa having haploid chromosome numbers more than 10, *B. diversifolia* Fisch. & Meyer ($2n = 24$), *B. tenuiscapa* Hook.f. var. *tenuiscapa* ($2n = 28$) and *B. cardiocarpa* ($2n = 36$) are clearly polyploid derivatives because other, lower, chromosome numbers were found in these taxa. However, the chromosome number $n = 11$ for *B. nivalis* (Fig. 37), $n = 13$ for *B. radicans* (Fig. 38) and $n = 15$ for *B. stolonifera* (Fig. 39), are the only known chromosome numbers for their respective species. The species with $n = 9$ have unimodal and symmetrical karyotypes as shown in Figs. 9–17 and the species with haploid chromosome numbers lower than 9 have bimodal and asymmetrical karyotypes as shown in Figs. 19–24 and 30. Thus the high chromosome numbers and bimodal and asymmetrical karyotypes in the species with *n*

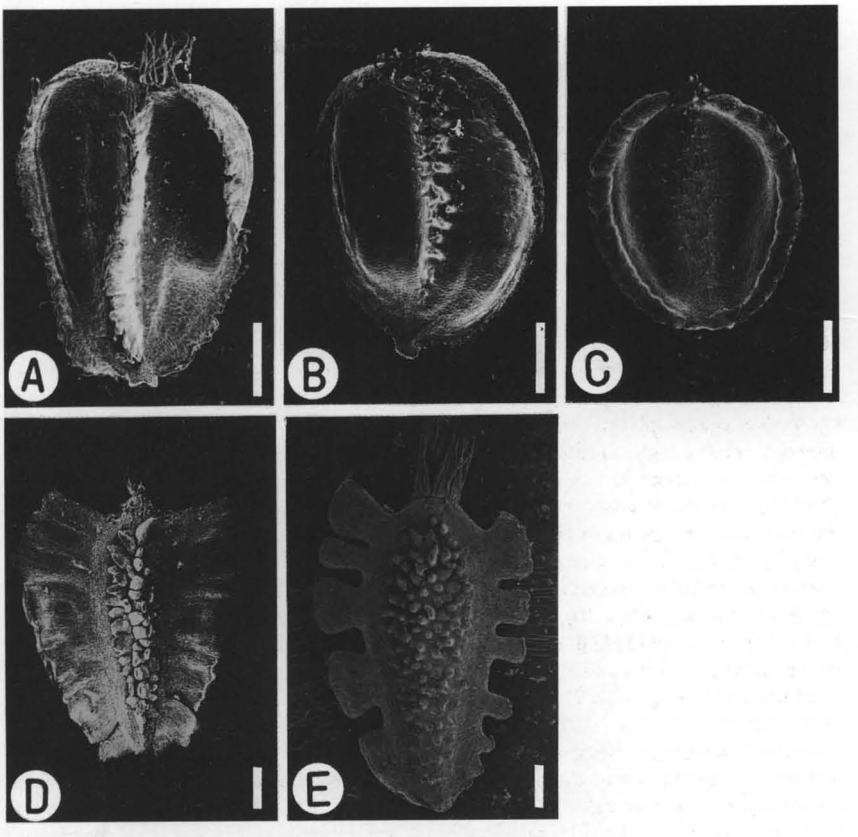


FIG. 42. The fruit morphology of the *Brachyscome dentata* complex. A: *B. tetrapterocarpa*, B: *B. curvicarpa*, C: *B. chrysoglossa*, D: *B. papillosa*, E: *B. dentata*. Scale bar = 500 μm.

= 11 to $n = 15$ support the view that they are of amphidiploid origin from the species having haploid chromosome numbers lower than 9. They are perennial, highly restricted in distribution and occur in specialized habitats.

The interspecific relationships of the *B. dentata* complex based on the *adh* gene sequences

Since the work by Davis (1948, 1949, 1955), a number of species complexes have been recognized within her superspecies (Smith-White *et al.*, 1970; Stace, 1981; Watanabe & Short, 1992). These include the *B. dentata* complex, which comprises the five species *B. tetrapterocarpa* G.L.Davis, *B. curvicarpa* G.L.Davis, *B. chrysoglossa* F.Muell., *B. papillosa* G.L.Davis and *B. dentata* Gaud. They have the same chromosome number, $n = 4$, and similar winged fruits (Fig. 42). *Brachyscome chrysoglossa* has fruits and vegetative morphology resembling that of *B. curvicarpa*, except that the fruits are light brown and the ray florets are yellow in contrast to the dark brown or almost black fruits and white rays of the latter species.

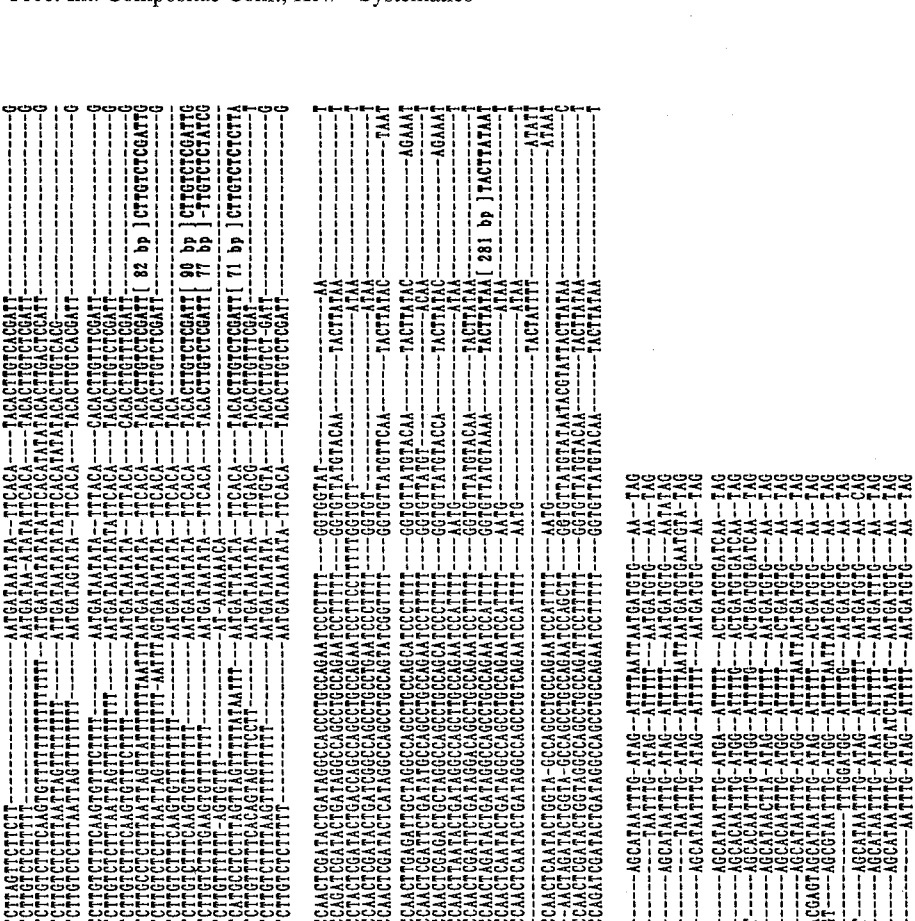


FIG. 43. Aligned *adh* intron 3 sequences from 11 *Brachyscome* species. Gaps are indicated as dashes. The target sequences are in bold. The long inserted segments are given only by the number of base pairs.

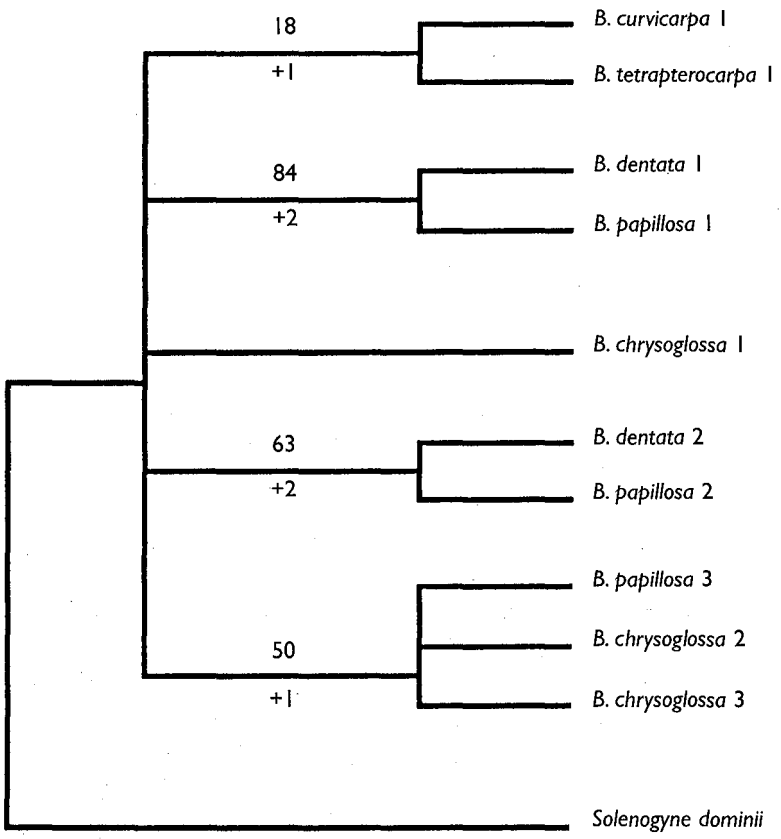


FIG. 44. Strict consensus tree for 17 equally most parsimonious trees for the *B. dentata* complex based on the 77 amino acid sequences deduced from the 233 nucleotide sequences of *adh* exon 4. Numbers above the branches indicate the bootstrap values in 100 replicates and those below branches are the decay index values.

Brachyscome tetrapterocarpa is indistinguishable from *B. curvicarpa*, except for the four distinct wings on the fruit. The fruit wing of *B. dentata* is irregularly dissected. *Brachyscome papillosa* has entire winged fruit but is similar vegetatively to *B. dentata*. The karyotype of *B. tetrapterocarpa* is similar to that of *B. curvicarpa* and the total karyotype lengths of both *B. tetrapterocarpa* and *B. curvicarpa* are distinctly longer than those of *B. dentata*, *B. papillosa* and *B. chrysoglossa* (Figs. 32–36). The karyotype of *B. dentata* is also similar to that of *B. papillosa* except that the long arm of Chromosome 2 is distinctly longer than that of Chromosome 1 in *B. dentata*, but these two long arms are almost the same length in *B. papillosa*. The karyotype of *B. chrysoglossa* is more similar to those of *B. papillosa* and *B. dentata*. All three species have the similar longest metacentric chromosome. In short, *B. chrysoglossa* is more similar to *B. papillosa* and *B. dentata* in karyotype but to *B. curvicarpa* and *B. tetrapterocarpa* in fruit and gross morphology. To clarify these interspecific relationships and differentiation at the molecular level, we have preliminarily compared the nucleotide sequences of a nuclear gene expected to show greater divergence than the chloroplast gene.

Primers for the *adh* gene were designed and PCR was performed. One to three PCR products per species were obtained from each of eleven species.

We determined sequences for three fourths of the 5' end of exon 4 and all of intron 3. The amplified segments of exon 4 are of uniform size with 233 bp; differences in fragment length are due to the differences in the length of intron 3. The sequences of intron 3 are aligned to reduce the number of insertions and deletion but increasing the similarity (Fig. 43). They had adequate homology to achieve alignment, except for the presence or absence of long insertions (from 77 bp to 282 bp). These insertions were flanked by direct repeats of 5 to 12 bp, suggesting that these are inserted elements (Denda *et al.*, 1995). Both *B. chrysoglossa* 2, 3, *B. papillosa* 2 and *B. tetrapterocarpa* 1, and *B. dentata* 1 and *B. papillosa* 1 have similar insertions at exactly the same sites.

Among the 11 species sequenced for intron 3, the inserted elements have been found only in those species with the advanced chromosome numbers such as $n = 4$ and 3 in spite of the fact that the species with $n = 9$ also have the same target sequences. It is interesting that the species with $n = 9$ are conservative both at the chromosomal and molecular levels in comparison with those of species with the advanced chromosome number. Some chromosomal rearrangements may be caused by the activation of inserted elements at the molecular level as suggested by McClintock (1984).

We preferred the generation of a more refined phylogenetic tree based on amino acid sequences deduced from the nucleotide sequences because most of the site mutations in exon 4 were synonymous. Figure 44 is the strict consensus tree for 17 equally most parsimonious trees for the *B. dentata* complex resulting from a branch-and-bound search in PAUP version 3.1. Australian *Solenogyne dominii* was included in this analysis as the outgroup. Except for *B. chrysoglossa* 1, segments of different taxa form four clusters: *B. tetrapterocarpa* 1 and *B. curvicarpa*; *B. dentata* 1 and *B. papillosa* 1; *B. dentata* 2 and *B. papillosa* 2; *B. chrysoglossa* 2,3 and *B. papillosa* 3. Thus *B. chrysoglossa* is more closely related with *B. papillosa-dentata* group than *B. curvicarpa-tetrapterocarpa* group. The former three species with the duplicated *adh* genes seem to have been derived recently from a common ancestor. This interspecific relationship based on the amino acid sequences of the *adh* gene supports those based on the karyotypes more than relationships inferred from fruit type and morphology. Thus, some fruit and morphological characters seem to have evolved in parallel.

Conclusions

The data presented here suggest the following conclusions:

1. The genus *Brachyscome* is divided into four clusters. Two of them are composed of the members of subgenus *Brachyscome* and the remains the members of subgenus *Metabrachyscome*.
2. Davis' superspecific groupings are not fully supported.
3. The base chromosome number in *Brachyscome* is 9.
4. The chromosome numbers $n = 8$ to $n = 2$ are the products of several independent dysploid reductions from 9, accomplished by translocation with loss of centromeric fragments.
5. Decrease in total karyotype length correlates with the change of habit from perennial to annual in arid or seasonally dry conditions.
6. Karyotype symmetry is highest in mesic, perennial species with $n = 9$. The degree of karyotype asymmetry increases as the chromosome number decreases. The karyotype symmetry increases again in some of advanced species.
7. The taxa having $n = 11$ to $n = 15$ are of amphidiploid origin. They are highly restricted in distribution and occur in specialized habitats.

8. Interspecific relationships within the *B. dentata* complex based on the amino acid sequences of the *adh* gene support those based on karyotypes more than relationships inferred from fruit type and morphology. Some fruit and morphological characters have evolved in parallel in this species complex.
9. Genomic reorganization has occurred both at the molecular and chromosomal levels in the advanced species.

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