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The Cytology of *Brachycome lineariloba*

4. The 10-chromosome Quasi-diploid

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Abstract. Meiotic behaviour in PMC's and in ESMC's, the development of pollen and embryo sacs, and the chromosome constitution of endosperm in this species (species E) define its unique genetic system. It is not a normal diploid, and is perhaps best defined as a quasidiploid. — The somatic complement consists of a diploid set of four chromosomes plus a haploid set of two nonhomologous chromosomes. The latter are inherited solely *via* the pollen. — In meiosis in both PMC'c and ESMC's the two univalents divide at the first division and lag at second anaphase. Pollen grains which do not receive them are non-functional. The embryo sacs are of the normal Asteroid type, and each is derived from a chalazal megaspore. They do not transmit the univalent chromosomes. Following fusion of the polar nuclei and double fertilisation, the endosperm has a constitution of 14 chromosomes. — The system parallels the maternal inheritance of univalents in *Rosa canina* and in *Leucopogon juniperinus*, except that the univalents do not constitute a full haploid set, and except that their transmission is paternal. (Summary see p. 454.)

Introduction

The taxonomic species *Brachycome lineariloba* (DC) Druce comprises five different "chromosome number" species of arid and semi-arid ephemeral annuals. These include species with diploid numbers of 4, 8, 12, and 16, which do not constitute a polyploid series (Smith-White and Carter, 1970). In order of their discovery these have been designated species A, D, B, and C respectively. There is also a species (species E) which has a somatic complement of 10 chromosomes. On the basis of observations of somatic mitosis only, Smith-White and Carter (1970) assumed that this represented a diploid complement of five pairs. Subsequent work has shown that this simple assumption was wrong. For reasons which will become apparent, we have designated it a "quasi-diploid".

Species E is morphologically distinguishable from both species A and species D, but differs from species B and C only in quantitative characters such as whole plant size (Smith-White and Carter, 1970). In the field morphological discrimination between the species B, C, and E appears to be impossible.

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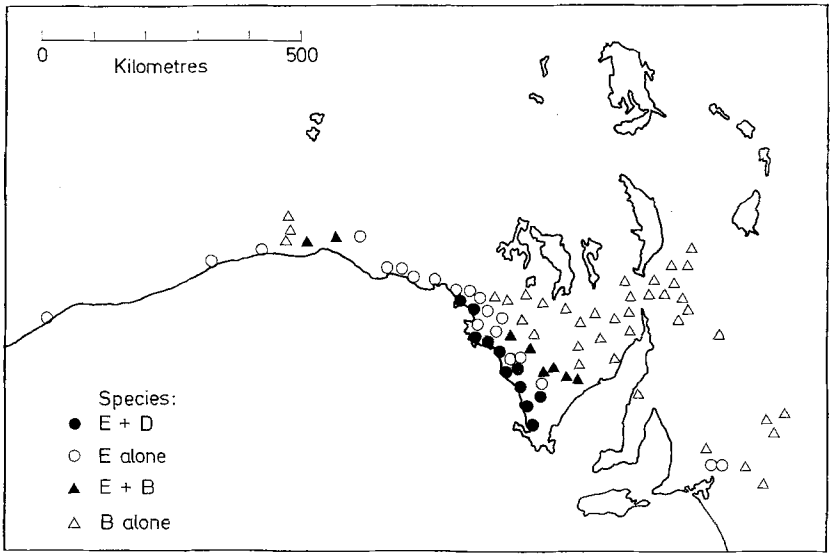


Fig. 1. The distribution of the quasi-diploid (species E) in relation to species D ($2n = 8$) and species B ($2n = 12$) in southern Australia

Species E is found over a range extending more than 1500 kilometers east to west in southern Australia (Fig. 1), and may occur alone or in association with either species B or species D. It has not been found simultaneously in association with both B and D, and is not found with either species A or species C.

Materials and Methods

For the examination of somatic mitosis and pollen mother cell meiosis, fixations were made in the field during August and September of 1970, 1971 and 1972. A modified Bradley fixative (4:3:1, chloroform, ethyl alcohol, propionic acid) was found most satisfactory, and was used exclusively during the later years. Newcomer's fixative, which was used earlier, gave comparable results. Material in either fixative stored well at about −5°C. Staining has been in 1% synthetic orcein in 45% propionic acid usually after 2 minutes cold hydrolysis in acidified (1% HCl) propionic orcein.

Examination of the pollen grain divisions was done by staining fresh pollen in propionic orcein. Material at this stage, fixed and stored in the modified Bradley fixative failed to stain either with propionic orcein or with aceto-carmine. The best preparations, particularly of the second pollen grain mitosis (the generative nucleus division) were obtained following overnight pretreatment with saturated para-dichlorbenzene at 5°C. Although the chromosomes could be seen fairly clearly inside the intact pollen grains at the first PG mitosis, heavy squashing and disruption of the pollen walls was necessary to study the second mitosis.

Examination of embryo sac mother cell meiosis and of embryo sac development has also been made by a squash technique, on material fixed in modified Bradley. Each ovule was dissected out from the fruit case and placed in a drop of acidified propionic-orcein. After 2 minutes a cover slip was applied, and then by very careful tapping the integuments were parted and the nucellus, with its contents, extruded. After observation to determine the stage and position of the contents, further tapping interspersed with observation, was made to coax out the nucellar contents. The kind of results which can be obtained by this method are illustrated in Figs. 20—28.

A hypotonic treatment was found necessary for the examination of endosperm. Young fruits were placed in distilled water and the nucellus dissected out. The water was then removed and immediately replaced with propionic orcein. The slide was then placed in a Petri-dish moist chamber for ten minutes before applying the cover slip. This interval allowed the endosperm to become firmer. At its most mitotically active state the endosperm was found to be in a free nuclear state, and dividing nuclei were very easily disrupted. Before dissection, fruits were given para-dichlorbenzene treatment overnight at 5° C to cause chromosome contraction and some spindle disruption.

Observational Data

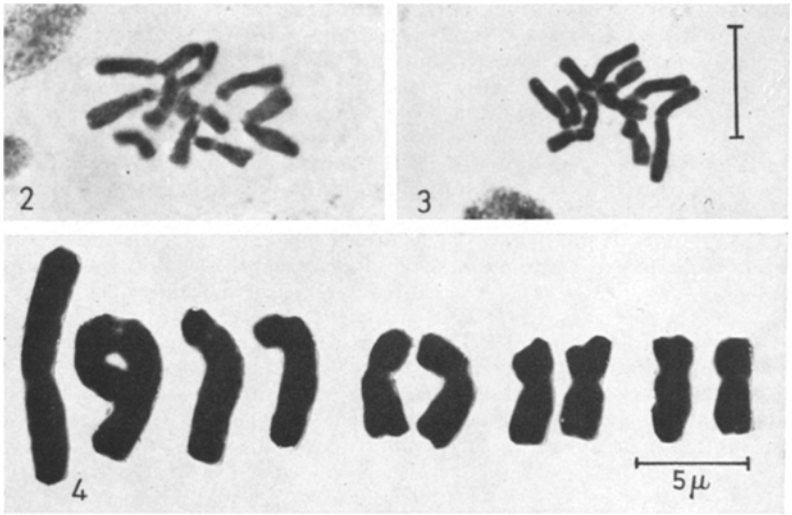
The Somatic Complement

The somatic complement of 10 chromosomes in species E is illustrated in Figs. 2–4. Two pairs of chromosomes are very distinctive. The largest pair (Chromosome I) comprises two nearly metacentric chromosomes which are about 12 microns long. The members of the second pair (Chromosome II) are sub-acrocentric and ca. 7 microns long. With care, a third pair can be distinguished in most cells at metaphase, as being sub-metacentric and almost 6 microns in length. Of the remaining four chromosomes, one is distinct in being more sub-acrocentric, and in showing an appearance of a secondary constriction. This is designated chromosome V. The other three are all less than 5 microns long and of similar morphology, but must constitute one pair (Chromosome IV) and one unpaired chromosome (Chromosome VI), and they have been arbitrarily arranged in this way in Fig. 4.

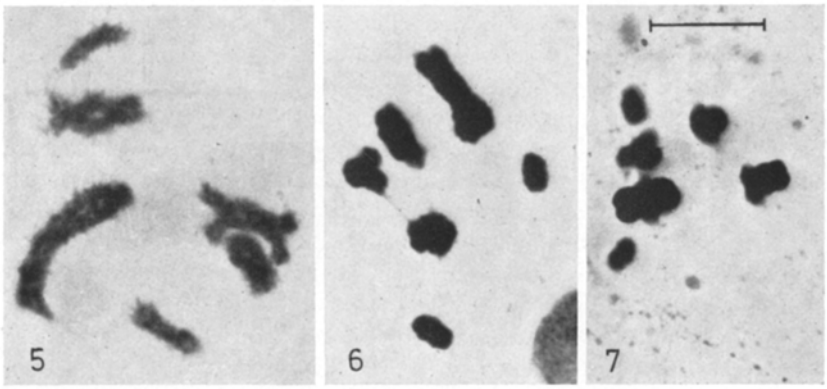
In gross morphology, the chromosome constitution of species E appears to be uniform throughout the species-range.

Meiosis in the Pollen Mother Cells

The unusual nature of *B. lineariloba* species E is evident throughout meiosis. At diplotene, diakinesis and first metaphase (Figs. 5–7) four bivalents are regularly formed, and, equally regularly, there are two unpaired univalent chromosomes. The larger bivalents I and II are easily distinguished; bivalent I usually has several chiasmata in both arms, and bivalent II may have several in the long arms but frequently lacks any in the short arms. The other two bivalents can be distinguished



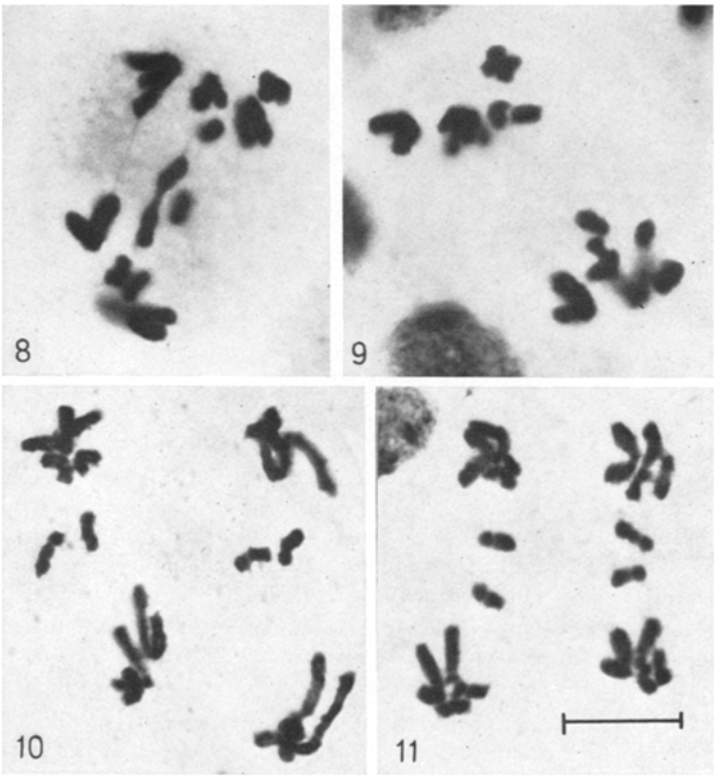
Figs. 2—4.[1] The somatic chromosomes of *B. lineariloba* E. Figs. 2 and 3. Metaphase. Fig. 4. An idiogram. The two chromosomes at right are unpaired and one of these (chromosome V) has a secondary constriction



Figs. 5—7. Meiosis in PMC's of *B. lineariloba* E. Fig. 5. Late diplotene. Fig.6. Diakinesis. Fig. 7. First metaphase. There is a constant configuration of four bivalents and two univalents

as a larger (III) and a smaller (IV), and both may have two or three chiasmata. The two univalents have never been seen to associate either with one another or with the other chromosomes of the set.

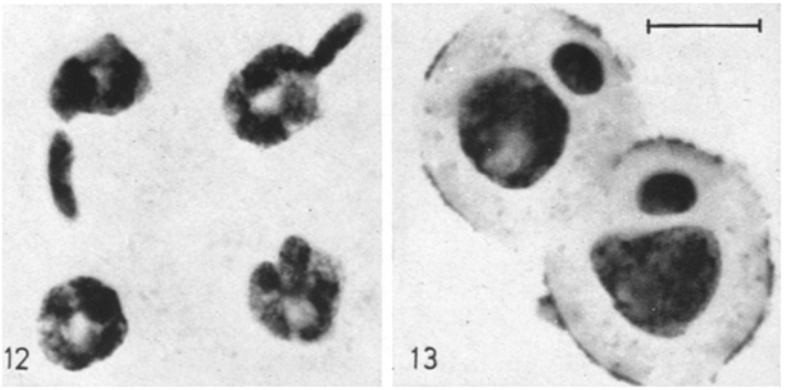
1 The bars on Figures 2–29 correspond to a length of 10 microns.



Figs. 8—11. Meiosis in PMC's of *B. lineariloba* E. Figs. 8 and 9. First anaphase. The univalents divide. Figs. 10 and 11. Second anaphase. The univalents lag in each spindle. One of these carries the secondary constriction, and can be identified as chromosome V

At first anaphase (Figs. 8 and 9) the bivalents disjoin normally. The univalents divide late on the spindle, and one chromatid of each univalent passes to each pole. Thus at late first anaphase and at second metaphase each pole or plate contains four double-chromatid chromosomes and two single-chromatid chromosomes. This behaviour appears to be completely consistent; the univalents have not been seen to pass undivided to either pole, to misdivide, or to become excluded in the cytoplasm.

In the second division, the single-chromatid chromosomes do not divide again, and at second anaphase both lag in each spindle (Fig. 10 and 11). Again, the behaviour is highly regular and the two second anaphase spindles in each mother cell are symmetrical, each having identical laggards. At this stage a difference in the morphology of the two



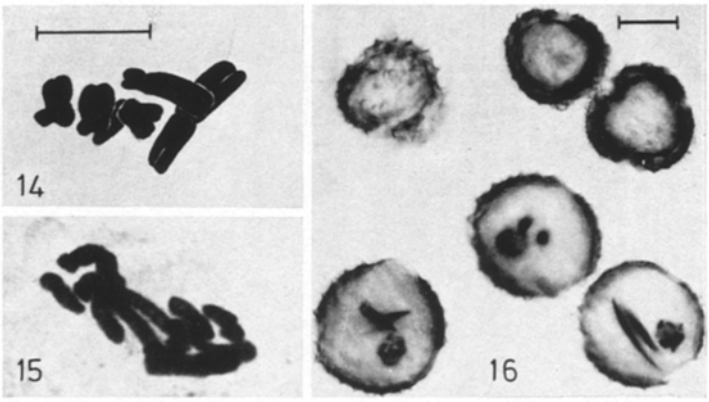
Figs. 12 and 13. Meiosis in PMC's of *B. lineariloba* E. Fig. 12. Second telophase. Two accessory nuclei are formed; one in each second division. Fig. 13. Two microspores containing accessory nuclei

laggard chromosomes is most obvious. One is slightly larger than the other, and the larger one often shows a distinct secondary constriction. It is sometimes possible to observe this distinction in diploid somatic mitoses (Fig. 4).

At the end of the second division the laggards are left out of the four telophasic nuclei, and reconstitute two small accessory nuclei, one in each spindle. Each contains two chromosomes (Figs. 12 and 13). Cytokinesis then usually results in the formation of four microspores, two of which contain, and two lack an accessory nucleus. Very often, the accessory nuclei show a delayed entry into the telophasic or interphasic condition. The accessory nuclei are only infrequently involved in the formation of microcytes; the frequency of tetrads with microcytes was found to vary from below 2% in some anthers to about 5% in others. Thus, after liberation of the microspores, substantial counts have shown that very nearly 50% of the microspores contain accessory nuclei, and such counts vouch for the regularity of the whole sequence of anomalous meiotic behaviour.

Pollen Grain Development

In the *Compositae* mature pollen is trinucleate containing a vegetative nucleus and two spindle-shaped gamete nuclei (Davis, 1966; Tanaka and Terasaka, 1972). This is also the case in the quasi-diploid *B. lineariloba* species E. (Fig. 16), but since there are, initially, two kinds of microspores, two kinds of mature pollen grains (PG) are to be expected. In fact, only 45–50% of the mature pollen (at maximum) contains cytoplasm and nuclei. Half of the pollen is degenerate.



Figs. 14—16. The second PG mitosis (and mature pollen) in *B. lineariloba* E. Fig. 14. Camera lucida drawing of metaphase. Fig. 15. Anaphase. Fig. 16. Good and bad pollen. The small pollen grains are devoid of protoplasmic contents

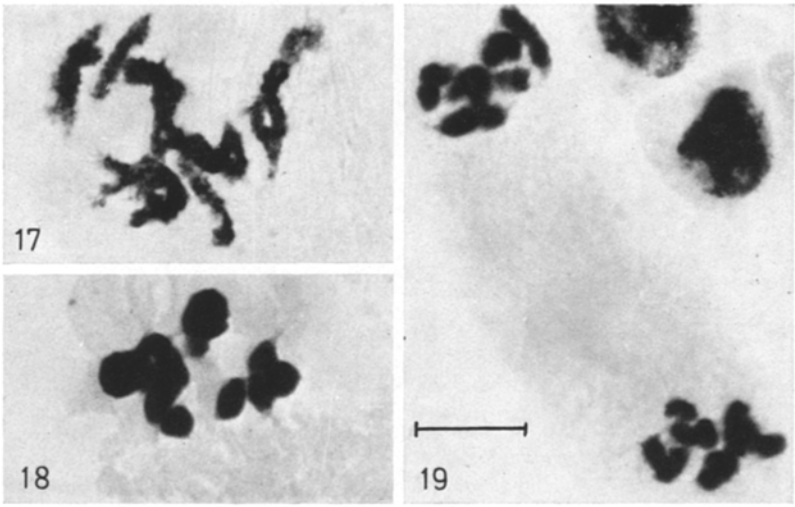
The fate of the accessory nuclei in those microspores which contain them, prior to the first PG mitosis has been difficult to establish. However, 14 counts at metaphase of the first mitosis have been made (D.W.K.), six of these showing four chromosomes, and eight showing six chromosomes. Therefore, the accessory nuclei, when present, must fuse with the normal nuclei; very probably this fusion happens with the breakdown of the nuclear membrane at the onset of the first mitosis.

Subsequent to the first PG division, the generative nucleus becomes spindle-shaped, and it retains this form into the second PG division, the gamete mitosis, thus foreshadowing the shape of the gamete nuclei. During prophase, metaphase and anaphase of the gamete mitosis the chromosomes are arranged as a tightly grouped elongated aggregate (Figs. 14 and 15), and the division is a difficult one to illustrate. However, 21 counts have been made at this stage, and in all the chromosome number has been six. One cell is illustrated in Fig. 14. Chromosomes I and II can be clearly recognised in this cell.

Thus the 50% of degenerate pollen must be derived from microspores lacking the accessory nuclei. Their degeneration occurs after the first PG division, but before the second. The basic set of four chromosomes are apparently genetically deficient for the purpose of pollen maturation.

The Ovule and the Embryo Sac Mother Cell

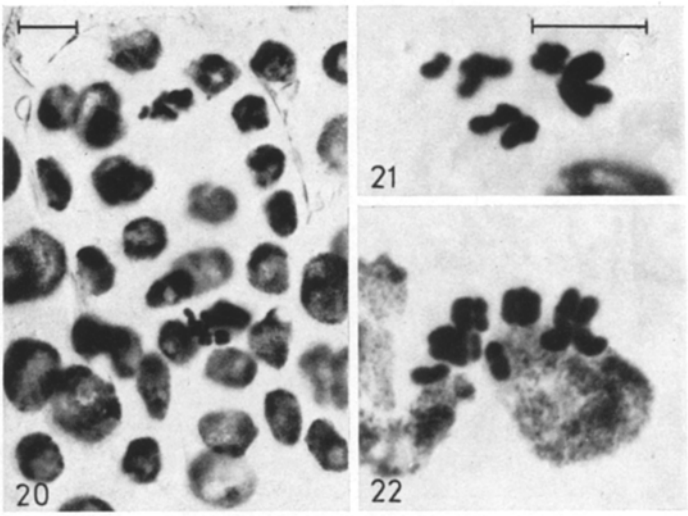
The young ovule conforms to the Composite type (Davis, 1966). It is anatropous, unitegmic, and tenuinucellar. A single-layered nucellus surrounds a single archesporial cell which functions directly as the embryo sac mother cell (ESMC).



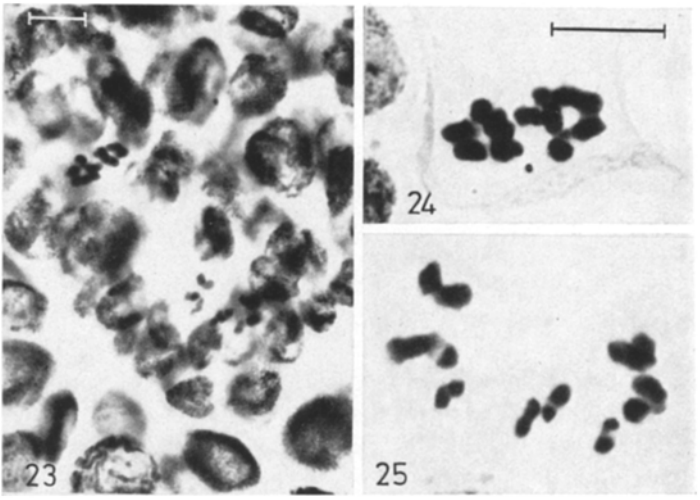
Figs. 17—19. Meiosis in the ESMC of *B. lineariloba* E. Fig. 17. Diplotene. Fig. 18. First metaphase. Fig. 19. First anaphase. The two univalents divide at the first division

In the ESMC, meiosis conforms with that described for the PMC's in general characteristics, but there are some important differences. At diplotene (Fig. 17) and first metaphase (Fig. 18) four bivalents and two univalents were found in all nine cells seen in these stages (C.R.C.). At first anaphase (Figs. 19 and 20–22) the bivalent chromosomes separate and the univalents divide (at least usually) so that each anaphase pole contains four double chromatid chromosomes and two single-chromatid chromosomes. At these stages the distinct chromosomes I and II can always be recognised. Occasional departure from univalent division is suggested by data presented later on the distribution of accessory nuclei in megaspore tetrads. Occasionally, but more frequently than in PMC meiosis, the univalents must pass undivided to one pole. Cytokinesis and cell wall formation follows immediately after first telophase. This is a situation which does not occur in the PMC's, but is again conformable with available anatomical descriptions of development in the *Compositae* (Davis, 1966).

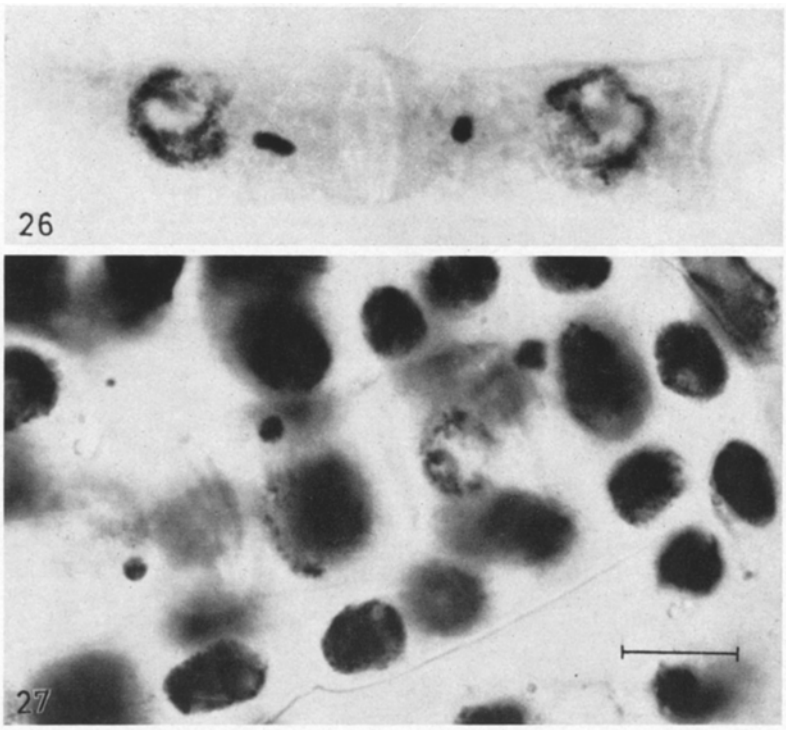
Second metaphase, second anaphase and second telophase stages are illustrated in Figs. 23–25 and 26. At second anaphase the two single-chromatid chromosomes do not divide, and lag in the centre of the spindle, just as they do in second anaphase in PMC meiosis. Cytokinesis follows immediately, to give a linear tetrad of megaspores, of which the chalazal one is usually the largest (Fig. 27).



Figs. 20—22. Meiosis in ESMC of *B. lineariloba* E. Fig. 20. The nucellar unit at first anaphase or second metaphase. Figs. 21 and 22. The two poles of the same ESMC, squashed out of the nucellar envelope. Two single chromatid chromosomes are present in the bottom plate. In the top plate, one of these is obscured



Figs. 23—25. Meiosis in ESMC of *B. lineariloba* E. Fig. 23. The nucellar unit. Figs. 24 and 25. The same ESMC has been squashed out of the nucellar envelope. The two spindles are asynchronous. In the bottom spindle, at second anaphase the laggard univalents are present

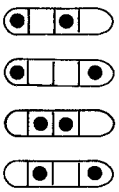
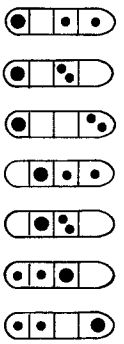
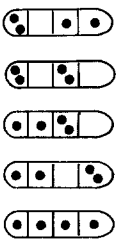
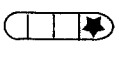
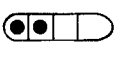
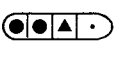


Figs. 26 and 27. Meiosis in ESMC of *B. lineariloba* E. Fig. 26. A second division spindle at telophase. A primary cell wall has been formed, and the two univalents lie on opposite sides of this wall. Fig. 27. A megaspore tetrad with accessories in three megaspores. These accessories remain condensed

All the observations of ESMC meiosis from diplotene to second anaphase thus conform with the behaviour described for PMC meiosis. However, analysis of the presence and distribution of accessory nuclei in the megaspore tetrads suggests that the control of univalent behaviour is less rigorous in the ESMC's than in the PMC's. These univalents may often pass to opposite poles at A II, to form single chromosome accessories (Fig. 26).

The distribution of accessory nuclei in 33 megaspore tetrads which have been analysed is illustrated diagrammatically in Table 1. The data are given a two-way classification. First, the tetrads are either conformable or non-conformable with first anaphase splitting of univalent chromosomes. Second, in each half-tetrad the two univalents may together form a single accessory nucleus, as they do in the pollen, or may each form separate accessory nuclei. This is referred to as "fusion", and there may then be three or four accessory nuclei in some tetrads.

Table 1. Distribution of accessory chromosomes in ESMC's in the quasi-diploid

	Fusion		Part-fusion		Non-fusion	
	m ch		m ch		m ch	
Split at A_I		2 3 1 1 7		2 1 1 1 1 2 1 9		3 1 2 1 6 13
Non-split at A_I		1		1		
Unexplained		1		1		

Presumptive constitution of accessory nuclei: ● one, ● two, ✱ four univalents, · possible fragment chromatin, ▲ abnormal accessory body, *m* micropylar end, *ch* chalazal end

Twenty-nine tetrads are conformable, two require first division non-splitting, and two must require other abnormalities. It is usually possible to distinguish larger and smaller accessory nuclei, and presumptively these are two-chromosome and one-chromosome accessories.

Among the 29 tetrads conforming with first division splitting of the univalents, seven showed fusion in both second division "half mother cells", nine showed fusion in one half mother cell only, and thirteen had no fusion in either half mother cell. Thus among the 58 half mother cells represented in the data, 23 show fusion and 35 non-fusion (ca. 0.40:0.60). The data are scarcely sufficient to test an assumption that the occurrence of fusion or non-fusion is independent in sister half mother cells ($\chi^2_{(2)} = 3.58$ $P = 0.18$).

It is possible, but unlikely that some of the cases where four accessory nuclei are present in a megaspore tetrad arise from the sequence: the

univalents do not split at first division, but the two univalents pass to opposite poles; they then divide in the second division. Although this behaviour seems unlikely, and no evidence supporting it has been seen, there is no way in which it can be rigorously excluded.

The four non-conformable or irrational tetrads seen require comment. Two suggest that there was failure of first division univalent splitting, with both univalents passing to the same anaphase pole. In one of these cases the two univalents, after splitting in the second division all passed to the one pole to give a giant four-chromosome accessory nucleus, and in the other the daughter single-chromatid chromosomes separated to opposite second-division poles to give two two-chromosome accessories. The two irrational tetrads are difficult to interpret. One may involve chromosome fragmentation. In the other, two of the accessory nuclei were of a size suggesting two-chromosome constitutions, one was smaller, and one was of a peculiar triangular shape not usually attained by nuclei.

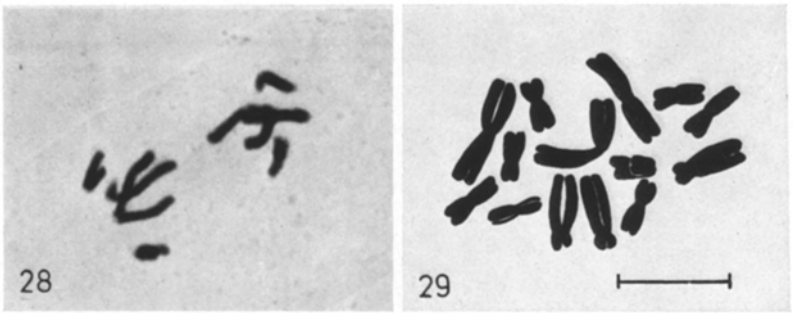
The accessory nuclei in megaspore tetrads are always much smaller and more dense than they are in young microspore tetrads. In fact, they fail to undergo telophasic and interphasic development, and it seems likely that this failure leads to their degeneration before the onset of the first megaspore (ES) mitosis.

Embryo Sac Development

Davis (1964) states that in seven species of *Eubrachycome*, including two species of the superspecies *leptocarpae* to which *B. lineariloba* belongs, embryo sac development conforms to the *Polygonum* type, that is, the embryo sac is monosporic and 8-nucleate, and is developed from the chalazal megaspore.

In *B. lineariloba* species E, the embryo sac must be monosporic, since the four megaspores are separated by cell walls at the end of meiosis. The chalazal megaspore is usually the larger, even immediately after meiosis, but further obvious embryo sac development does not occur for some time. In older ovules, the four megaspores are present, but accessory nuclei or accessory chromatin is no longer evident.

The first ES mitosis has been observed in four cases, three in prophase and one in anaphase (Fig. 28). In each case, it was the chalazal megaspore showing development, with the other megaspores in stages of degeneration, and in each case four chromosomes were clearly distinguishable, they were haploid, and lacking the univalent chromosomes. Six other late stage megaspore tetrads have been observed, and in all, the chalazal megaspore is developing as the embryo sac. Mature embryo sacs have been seen to contain an egg apparatus of two synergids, an



Figs. 28 and 29. Embryo sac development in *B. lineariloba* E. Fig. 28. First mitosis, anaphase. The univalent chromosomes V and VI are absent. Fig. 29. Endosperm mitotic metaphase. Camera lucida drawing showing basic triploidy but the presence of only one each of chromosomes V and VI

egg nucleus, two polar nuclei (or a fusion primary endosperm nucleus) and several antipodal nuclei or antipodal cells. It is thus confirmed that ES development conforms to the type described by Davis, and the ES develops from the chalazal megaspore, but it is still possible that occasional megaspore competition may occur. The accessory nuclei must degenerate whenever they are initially present.

The Endosperm

In the *Compositae*, the endosperm is ephemeral, and the mature seed is ex-endospermic. Degeneration of the endosperm commences within a few days of post-fertilisation development: according to Davis (1966) the endosperm is usually *ab initio* cellular, but the earliest divisions may be without wall formation. Our observations show that the free nuclei development lasts a good deal longer.

Under such circumstances, with an extremely ephemeral existence, the determination of endosperm chromosome number is difficult. Since the embryo sac is monosporic, and the endosperm originates from a double fusion of two polar nuclei with a male gamete nucleus, the chromosome number in endosperm of normal sexual composites is triploid (Maheshwari, 1950).

Four successful endosperm counts have been achieved for *B. lineariloba* species E. In all, the chromosome number has been determined as 14, and it has been possible to determine that the larger chromosomes (Chromosomes I and II) are each represented three times (Fig. 29). Endosperm constitution thus conforms with the inheritance of the univalents *via* the pollen, and with their elimination in embryo sacs.

Discussion

The Genetic System of the Species

It has been noted that *B. lineariloba* species E, with 10 somatic chromosomes, has a range of over 1500 km east to west in southern Australia, that it may occur alone (*i.e.* in the absence of other chromosome-species of the *B. lineariloba* complex) or together with either species D ($2n=8$) or B ($2n=12$), but not simultaneously with both. Also, where species E and either B or D occur together they exist patchily as a fine mosaic. The anomalous PMC meiosis of species E is constant over the whole of its range.

These observations show that species E is a self-maintaining entity. It is a species with a genetic system of its own. Three different hypotheses can be offered and examined on the nature of this genetic system.

(a) The species is an asexual apomict. This is the simplest and most obvious hypothesis for any species with an anomalous chromosome constitution. Apomixis could be by generative apospory, as reported for *Brachycome ciliaris* by Davis (1964) or by nucellar embryony.

(b) The species is sexual, with a segregational system. Two kinds of pollen, and two kinds of embryo sac operate, — one kind having four chromosomes of a basic set, the other the same four plus two additional "accessory nucleus" chromosomes. Selfing would then yield a segregation ratio:

$$0.25\ 4_{II}:0.50\ 4_{II}+2_{I}:0.25\ 6_{II}.$$

Uniformity could then be attained by zygotic elimination of the 4_{II} and 6_{II} embryos, and seed fertility would have a maximum of 50%.

(c) The species is sexual, with a complementary gametic system. Either 4-chromosome pollen grains and 6-chromosome embryo sacs only operate, and the univalents are maternally inherited, or 6-chromosome pollen grains and 4-chromosome embryo sacs only operate, and the univalents are paternally inherited.

Such a mechanism is reminiscent of the inheritance of *rigens* and *curvans* complexes in *Oenothera muricata* (Renner, 1921, 1925), of the maternal inheritance of univalents in *Rosa canina* (Hurst, 1931) and especially of univalent inheritance in the sexual triploid *Leucopogon juniperinus* (Smith-White, 1948, 1955, 1959). Complementary gametic inheritance has not been reported in the *Compositae*.

Even initially, the hypothesis of apomixis was not favoured. The flowerheads, although small, are not inconspicuous, and are visited by many small insects. A significant pollen role is suggested by the regularity of the meiotic process in pollen mother cells. The establishment of the features of pollen development and of embryo sac development rules out apomixis as a regular reproductive mechanism.

The second hypothesis — that of a sexual segregational system seems unlikely since the species often occurs as pure stands; and it never occurs simultaneously with both the $2n=8$ and $2n=12$ species. The combined data on the chromosome constitutions of viable pollen grains ($n=6$; 21 observations); embryo sacs ($n=4$; 4 observations) and endosperms ($3n=14$; 4 observations) eliminate the segregational hypothesis, although it is still possible that illegitimate inheritance of the addition chromosome may sometimes occur.

Zygotic elimination of embryos with unfavourable chromosome constitutions is also excluded, since 90% or more of the mature seeds contain viable embryos.

The third hypothesis, that of complementary gametism, is supported by a number of observations. The observations may be summarised as follows:

a) At maximum, 50% of the pollen is viable. The pollen contains six chromosomes at the second pollen-grain mitosis. At the first pollen grain division, both 4-chromosome and 6-chromosome constitutions are developing. Thus the stage of death of 4-chromosome pollen is clearly defined.

b) Meiosis in the ESMC's is essentially similar to that in the PMC's. The behaviour of the divided univalent chromosomes at A II however, is more erratic.

c) The ESMC meiosis results in a linear row of separate haploid megaspores. The embryo sac is monosporic, and, at least usually, is developed from the chalazal megaspore.

d) The mature ES is the standard Asteroid (*Polygonum*) type, with two synergids, an egg nucleus, two polar nuclei, and three antipodal nuclei. The polar nuclei fuse to form the primary endosperm nucleus.

e) Developing embryo sacs have a four-chromosome constitution.

f) In the megaspores, accessory nuclei remain highly condensed, and fail to achieve telophasic and interphasic development. They are not visible in older megaspores, and apparently degenerate.

g) The chromosome number in the endosperm is 14. The expected chromosome number relationships between embryo and endosperm on the hypothesis of apomixis and of complementary gametism, with alternatively male and female transmission of the addition chromosomes, are shown in Table 2. In the case of apomixis, this relationship could be 10/10 if there is no fusion of polar nuclei, 10/20 if there is such fusion, or even 10/24 or 10/26 with pseudogamy.

The available evidence thus strongly supports the hypothesis of complementary gametism. Perhaps surprisingly, the addition univalents must be inherited *via* the pollen and not *via* the embryo sacs, and in this they differ from the known comparable cases of *Rosa canina* and *Leucopogon juniperinus*.

Table 2. Expected embryo-endosperm chromosome number relationships with apomixis and complementary gametism

	Hypothesis		
	Apomixis	Complementary gametism	
		Male transmission	Female transmission
Embryo	10	4+6=10	6+4=10
Endosperm	10+10=20[a]	(4+4)+6=14	(6+6)+4=16

[a] With pseudogamy this could be (10+10)+6 or (10+10)+4.

Table 3. Expected results from a reciprocal program of hybridisation on the basis of apomixis and complementary gametism

Cross seed × pollen	Hypothesis		
	Apomixis	Complementary gametism	
		Male transmission	Female transmission
D × E	4_{II}	$4_{II}+2_I$[a]	4_{II}
E × D	$4_{II}+2_I$	4_{II}[a]	$4_{II}+2_I$
E × B	$4_{II}+2_I$	$4_{II}+2_I$	6_{II} or $4_{II}+4_I$[a]
B × E	6_{II}	6_{II} or $4_{II}+4_I$	$4_{II}+2_I$[a]

[a] Discriminant against apomixis.

A final establishment of this unusual genetic system is possible by a totally different experimental method. Reciprocal crosses between the quasi-diploid species E, and the related species D ($2n=8$) and B ($2n=12$) should give the results indicated in Table 3.

In the case of apomixis, there would of course be strict maternal correlation. The results footnoted in the table are discriminant against apomixis and against one another. The alternative possibilities given in column 3 row 4 and column 4 row 3 depend on the possible homology or non-homology of the addition chromosomes of the quasi-diploid with the extra paired chromosomes of species B. This experimental programme has been hindered by difficulties of seed germination which have been recently overcome.

Summary

One species of the *Brachycome lineariloba* complex (species E) possesses 10 somatic chromosomes, but is not a true diploid. It is a sexual species with an extensive distribution in southern Australia.

In PMC meiosis, four bivalents and two non-homologous univalents are regularly formed. The univalents divide at the first division and lag in each spindle at the second. As a result, in each tetrad two microspores each contain an accessory nucleus of two chromosomes as well as a major nucleus of four chromosomes.

The accessory nuclei merge with the major nuclei at the first PG division. Only these pollen grains, possessing six chromosomes, survive to the second PG division.

In ESMC's, meiosis is essentially similar to that in the PMC, but the univalent chromosomes, which are excluded from the second anaphase groups, do not develop into accessory nuclei. The functional embryo sacs, which are developed from the chalazal megaspores, have a 4-chromosome constitution.

The sexual nature of the genetic system is confirmed by the chromosomal constitution of the endosperm (4+4 from ES, 6 from male gamete = total 14).

The genetic system of this species involves the unilateral paternal transmission of two univalents. Since these univalents are consistently present, they do not satisfy the criteria of B-chromosomes. The mode of inheritance parallels the maternal inheritance of univalents in *Rosa canina* and in *Leucopogon juniperinus*, except that the univalents do not constitute a full haploid set. The species is neither diploid nor triploid. It is best defined as a quasi-diploid.

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