Micro- and Nucleolar-organizing B-Chromosomes in *Calycadenia* (Asteraceae)

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Recent reviewers of B-chromosomes in plants and animals (Battaglia 1964, Jones 1975) concur that examples of nucleolar organization by B-chromosomes are extremely rare, perhaps limited to one plant and one insect. Biosystematic studies in the genus *Calycadenia* (Carr 1975a, b, 1977) have led to the discovery of at least three recognizable forms of nucleolar-organizing B-chromosomes, one type in each of five different taxa embracing three species of the genus. Another kind of B-chromosome, unusual because of its extremely small size (less than $1\ \mu$), was also found in a species of *Calycadenia*. The characterization of the several types of B-chromosomes found in *Calycadenia* is the purpose of this paper.

Calycadenia is an annual, predominantly self-incompatible genus in the tarweed subtribe of the Heliantheae (Asteraceae). It is comprised of about a dozen species confined mostly to arid regions bordering the Central Valley of California. At least two of the species consist of several chromosome races differentiated chiefly by translocations and in one instance also by aneuploid reduction (Carr 1975a, 1977). An additional cytological peculiarity of *Calycadenia* is the fact that most, if not all species in the genus have two pairs of nucleolar-organizing chromosomes despite the fact that they are diploids with low chromosome numbers ($n=4,5,6,7$ and 9 ; Carr 1975a).

Materials and methods

Observation of meiosis in microsporocytes provided the information on occurrence, behavior and morphology of B-chromosomes presented herein. Some of the floral bud material examined in this study was collected directly from natural populations (Table 1). Fruits were also collected directly from natural populations (Table 1), from which plants were grown under greenhouse conditions for use in a hybridization program (see Carr 1975a for details). These parental and subsequent hybrid greenhouse-grown plants provided the balance of the material used in this study. Fixation, slide preparation and estimation of pollen fertility were accomplished as described elsewhere (Carr 1975a).

Table 1. Populations of *Calycadenia* in which B-chromosomes have been observed†

<i>C. ciliosa</i> Greene race Dry Creek
663 Tehama Co., 6.0 mi W of Vestal Rd. on Hwy. 36, n=6*
<i>C. multiglandulosa</i> DC. ssp. <i>cephalotes</i> (DC.) Keck
566 Napa Co., 1.5 mi SW of Angwin on Howell Mt. Rd., n=6*
577 Sonoma Co., Jct. of Hacienda Lane and Occidental Rd., n=6*
639 Marin Co., E city limits of Forest Knolls, n=6*
<i>C. multiglandulosa</i> ssp. <i>robusta</i> Keck
627 Santa Clara Co., 3.3 mi NE of Skyline Blvd. on Page Mill Rd., n=6*,††
630 Santa Clara Co., 1.2 mi SW of San Martin, n=6*
<i>C. oppositifolia</i> (Greene) Greene
789 Butte Co., Upper Bidwell Park, near Chico, n=7**
<i>C. pauciflora</i> Gray race <i>Pauciflora</i>
606 Colusa Co., 0.5 mi NW of Fout Spr. Boys Ranch, n=5*
645 Tehama Co., 8.0 mi SW of Paskenta on rd. to Mud Flat Campground, n=5*
646 Tehama Co., 7.1 mi NW of Paskenta on Toomes Camp Rd., n=5*
652 Colusa Co., 3.6 mi SW of Leesville on Bartlett Spr. Rd., n=5*
671 Lake Co., 2.2 mi SW of Rieff Rd. on Morgan Valley Rd., n=5*
<i>C. pauciflora</i> race <i>Ramulosa</i>
680 Lake Co., SW Lakeport, n=6*
<i>C. pauciflora</i> race <i>Tehama</i>
738 Tehama Co., 0.1 mi S of Hwy. 36 on Tedoc Rd., n=6*
743 Tehama Co., 1.4 mi W of Mountain Rd. on Colyer Spr. Rd., n=6*

† All populations are located in the state of California (U.S.A.) and all collection numbers are those of the senior author.

* Nucleolar-organizing B's also present.

†† Micro-B's also present.

** Marco-B's also present.

Results

Nucleolar-organizing B-chromosomes

Nucleolar-organizing B-chromosomes were found in a total of 14 populations (Table 1) comprising six taxa of three species of *Calycadenia*. At least three different types could be distinguished on the basis of size. The morphology and behavior of nucleolar-organizing B-chromosomes are described systematically below.

Calycadenia multiglandulosa ssp. *robusta*: In two populations of this taxon individuals with 0-3 nucleolar-organizing B-chromosomes about 3 μ in length were detected (Table 1; Fig. 1). The nucleolar-organizing function of this B-chromosome when present in single dose is very strong and it can be seen associated with the nucleolus in nearly 100% of the microsporocytes, often to the exclusion of either of the two A-chromosome nucleolar-organizing pairs. When two or more B-chromosomes are present the ratio of A- to B-chromosomes associated with the nucleolus increases. The B-chromosomes in this taxon may pair with each other when more than one is present (cf. B's in ssp. *cephalotes*, Fig. 4), but they appear incapable of true pairing with any of the A-chromosomes. During the first meiotic division univalent B-chromosomes generally precede the A-chromosomes to the poles in random fashion without dividing equationally. Equational division of B-

chromosomes in this and the next two taxa occurs almost always at the second meiotic division, but rarely may occur at the first anaphase. At the second meiotic metaphase the B-chromosome appears submetacentric and obviously smaller than the A-chromosomes (Fig. 2, right inset).

Figs. 1-5. Meiotic behavior of nucleolar-organizing B-chromosomes in *Calycadenia multiglandulosa*. 1, ssp. *robusta*, one B_I organizing the nucleolus (arrow) + 6_{II} at diakinesis. 2, ssp. *robusta* × ssp. *cephalotes*, composite showing "cephalotes" B (left inset) and "robusta" B (right inset) with A karyotype, all at metaphase two. 3, ssp. *cephalotes*, two B_{II} + one B_I organizing the nucleolus + 6_{II} at diakinesis. 4, ssp. *cephalotes*, two B_{II} + one B_I (arrow) + 6_{II} at metaphase one. 5, ssp. *robusta* × ssp. *cephalotes*, 6_{II} + "cephalotes" B (C) and "robusta" B (R) paired at metaphase one. (Bars = 10 μ).

Calycadenia multiglandulosa ssp. *cephalotes*: In three populations of this taxon individuals with 0-5 nucleolar-organizing B-chromosomes about 2 μ in length were detected (Table 1; Fig. 3). It is possible to compare the morphology of this B-chromosome directly with that of the one described above in the hybrid between *C. multiglandulosa* ssp. *robusta* and ssp. *cephalotes* (Fig. 2, left and right insets; cf. Carr 1977, Fig. 3). The B-chromosome in ssp. *cephalotes* is smaller than the one in ssp. *robusta* and is apparently nearly acrocentric. The frequency of nucleolar organization by the B-chromosome from ssp. *cephalotes* is about equivalent to that of ssp. *robusta* and the meiotic behavior is also the same. Although these B-chromosomes tend to be sticky they apparently do not truly pair with the A-chromosomes. They appear to pair normally with one another (Figs. 3, 4) and the "cephalotes" type also occasionally pairs with the "robusta" type in the hybrid between the subspecies (Fig. 5).

Calycadenia pauciflora race *Ramulosa*: In one population of this taxon

individuals with 0–3 nucleolar-organizing B-chromosomes about $2.5\ \mu$ in length were detected (Table 1; Fig. 6). The nucleolar-organizing function of this B-chromosome is hard to detect, especially in individuals with more than one B-chromosome present. In some individuals with a single B-chromosome, the B-organizer is associated with the nucleolus in about one out of four microsporocytes. It can also occasionally be seen to organize a second smaller nucleolus, independent of the one organized by the A-pairs (Fig. 6). Although occasional associations between nucleolar-organizing pairs can appear rather strong, they are probably due to stickiness rather than true pairing. "Stickiness" in the sense used herein

Figs. 6–11. Meiotic behavior of nucleolar-organizing B-chromosomes in *Calycadenia pauciflora*. Figs. 6–8. Race *Ramulosa*. 6, one B_I organizing small nucleolus (arrow) + 6_{II} at diakinesis. 7, late separation of B-chromatids (arrow) at telophase two. 8, two B-chromatids (arrows) incorporated in one pole at telephase two. 9–11, race *Pauciflora*. 9, B_{II} organizing nucleolus + 5_{II} at diakinesis. 10, lagging B-chromatids after equational division at telophase one. 11, two B-chromatids (arrows) excluded from the poles at telophase two. (Bars = $10\ \mu$).

refers to apparent achiasmate associations between chromosome segments that are chiefly heterochromatic, especially nucleolar-organizing regions or arms. Associations of this sort appear to result from the physical tackiness of the chromosomal material rather than from structural homology. Even the rather infrequent associations between B-chromosomes in this taxon usually appear to be the result of stickiness. The meiotic behavior of the B-chromosomes in this taxon is the same as in the preceding cases except that during equational division at the second meiotic metaphase the chromatids of the B-chromosome separate late, often lagging on the metaphase plate (Fig. 7). The late separation of the B-chromatids may result in their exclusion from the A-chromosome nuclei or alternatively sometimes results in the inclusion of both B-chromatids in the same microcyte nucleus (Fig. 8).

Calycadenia pauciflora race Pauciflora: In 5 populations of this taxon individuals with 0-4 large nucleolar-organizing B-chromosomes about $5\ \mu$ in length were detected (Table 1; Fig. 9). The strength of nucleolar organization of this B-chromosome when present as a univalent often exceeds that of both A-nucleolar-organizing pairs. As in the previous case there are infrequent associations between the B-chromosome of this taxon and the nucleolar-organizing arms of the A-chromosomes but these are probably due to stickiness of nucleolar organizers rather than true chromosomal homology. When two B-chromosomes are present they are nearly always paired with each other (cf. Fig. 9). Equational division of univalent B-chromosomes from race Pauciflora occurs most frequently at the second meiotic anaphase following their random movement to the poles of the dyad. However, about 20% of the time equational division occurs during the first meiotic division but lags behind that of the A-chromosomes (Fig. 10). As a result the B-chromatids are often excluded from the microcytes, or at least from their A-chromosome nuclei (Fig. 11).

These B-chromosomes appear to have a significant effect on the fertility of the plants possessing them. Nine controlled interpopulational hybrid individuals of race Pauciflora with one B-nucleolar-organizing chromosome apiece averaged 86% pollen stainability whereas two hybrids with two B-chromosomes apiece averaged 64% pollen stainability and one hybrid with three B's had 24% stainable pollen. Thirty-five such hybrids lacking B-chromosomes had a mean pollen stainability of 93%.

Calycadenia pauciflora race Tehama: In two populations of this taxon individuals with 0-2 large B-chromosomes similar to those seen in race Pauciflora were detected. A small sample of microsporocytes suggests the nucleolar-organizing function of these B-chromosomes (Fig. 12), but more material is needed to further document this function and to make observations on their meiotic behavior.

Calycadenia ciliosa race Dry Creek: The nucleolar-organizing function of a large B-chromosome from a population of this taxon is strongly suggested by observations of a small number of microsporocytes (Fig. 13). More material is needed for further substantiation and to characterize the meiotic behavior of this B-chromosome.

Figs. 12-18. 12, 13: Meiotic behavior of nucleolar-organizing B-chromosomes in *Calycadenia*. 12, *C. pauciflora* race Tehama, B_{II} organizing one of three nucleoli (arrow) + 6_{II} at diakinesis. 13, *C. ciliosa* race Dry Creek, B_{II} organizing nucleolus (arrow) + 6_{II} at diakinesis. (Bars = $10\ \mu$). 14-18: Micro- and macro-B-chromosomes in *Calycadenia*. Figs. 14-17. Meiotic behavior of micro-B-chromosomes in *Calycadenia multiglandulosa* ssp. *robusta*. 14, two clusters of micro-B's (arrows) at early prophase. 15, micro-B's (arrows) + 6_{II} at diplotene-diakinesis. 16, micro-B (arrow) + 6_{II} at metaphase one, note terminal chromomeres. 17, micro-B's (arrows) excluded from nucleus of microcyte. 18, *C. oppositifolia*, one $B_I + 7_{II}$ at metaphase one. (Bars = $10\ \mu$).

Micro-B-chromosomes

Microsporocytes in some individuals of one population of *Calycadenia multiglandulosa* ssp. *robusta* were found to contain very small structures less than $1\ \mu$ in length that are interpreted here as micro-B-chromosomes (cf. Carter and Smith-White 1972). These micro-B-chromosomes appear to be unstable in the micro-

sporocytes of a single plant with numbers ranging from perhaps 0 to about 10. Their small size and irregular clumping (Figs. 14–16) make accurate determinations difficult. In one cell at least seven micro-B's were clustered tightly but irregularly together. The condensation and staining of these structures varied little from early prophase of meiosis to the formation of microspores (Figs. 14–17). In all cells at all stages where critical observations were possible the micro-B's appeared to be double stranded, each strand possessing a light-staining proximal region with a single darker staining chromomere at each end (Fig. 16). No form of division of micro-B-chromosomes could be substantiated at any stage of meiosis of the A-complement. In some cells the micro-B-structures appeared to be of approximately the same width as a pachytene A-pair of chromosomes.

Macro-B-chromosome

A B-chromosome of dimensions only slightly smaller than chromosomes of the A-complement has been detected in one population of *Calycadenia oppositifolia* (Fig. 18). This is the only known occurrence of B-chromosomes in a 7-paired species of *Calycadenia*. The sample of microsporocytes available did not contain stages necessary to detect the potential nucleolar-organizing function of this B-chromosome, nor to characterize its meiotic behavior.

Discussion

Nucleolar-organizing B's

In view of the near absence of reports of nucleolar-organizing B-chromosomes in the literature (cf. Battaglia 1964, Jones 1975) it is peculiar for them to be so common in *Calycadenia*. The occurrence in *Calycadenia* of two nucleolar-organizing pairs of A-chromosomes is also apparently rare or at least unusual in diploid species. Some species of *Calycadenia* are chromosomally very active to the extent that they are characterized by a series of populations differentiated from one another by reciprocal chromosome translocations (Carr 1975a). Furthermore, these translocations often involve nucleolar-organizing chromosomes. Thus, it is tempting to speculate an origin of nucleolar-organizing B's from products of abnormal meiosis in complex structural heterozygotes produced by hybridizing species or populations. The apparent lack of chromosomal differentiation within *Calycadenia multiglandulosa* (Carr 1977) would in this case appear to necessitate interspecific hybridization to produce the effects outlined above.

Alternatively, it is perhaps equally probable that the aneuploidy common in *Calycadenia* has been involved in the production of the nucleolar-organizing B's by supplying a centromeric fragment onto which a nucleolar-organizing region could be readily translocated. The presence of four nucleolar-organizing chromosomes in the basic set increases the possibility of this occurrence.

The nucleolar-organizing B-chromosomes in *Calycadenia* are obviously not all identical and it seems probable that they have had more than one origin. Those of *C. multiglandulosa* ssp. *robusta* and ssp. *cephalotes*, although slightly different in size and morphology are partially homologous as indicated by apparent chi-

asmate pairing in artificial hybrids between the subspecies. The two types quite probably had a common origin and subsequently became differentiated through gain or loss of chromosomal material. Several such modifications of B-chromosomes in *Aster ageratoides* have been described in detail by Matsuda (1970). *Calycadenia pauciflora* races Tehama and Pauciflora and *C. ciliosa* have nucleolar-organizing B's that are similar to each other in size and appearance, but differ in these features from those of *C. multiglandulosa*. Considering these facts and the overall genetic relationships of these groups (Carr 1977), a common origin for the B-chromosomes of the former three taxa that is independent from the origin of the B-chromosomes in *C. multiglandulosa* is suspected. For the same reasons it seems probable that the B-chromosomes in *C. pauciflora* race Ramulosa were independently derived.

Except for the nucleolar-organizing function, the meiotic behavior of the B-chromosomes reported on here is in line with that reported in many other species (cf. Battaglia 1964, Jones 1975). Likewise, the reduction of fertility in plants with increased numbers of nucleolar-organizing B's seen in *Calycadenia pauciflora* race Pauciflora is in agreement with many other reports on the effects of B-chromosomes in general.

Micro-B's

The diminutive size of the structures referred to herein as micro-B's make them difficult to characterize with certainty. Other workers have reported B-chromosomes of similar dimensions (eg. Carter and Smith-White 1972), but the intensity of staining, the morphology and the behavior in the instances known to us are more typical of larger B's than the micro-B's reported here. Although division of the micro-B's in microsporocytes of *Calycadenia* could not be substantiated, their small size and the difficulty of observation in the material available precludes any firm conclusion in this regard. The morphology of the micro-B's repeatedly seen in many cells is remarkably similar to that of the centromeric regions of the chromosomes of *Hyacinthus orientalis* and *Secale cereale* as illustrated by Lima-de-Faria (1956, Figs. 6 and 9, respectively). The possible occurrence of "naked" centromeres in natural plant populations would be of considerable interest to students of chromosome evolution, but more definitive evidence beyond the scope of this report is needed to make such a claim about the micro-B's in *Calycadenia*.

Macro-B's

The macro-B's of one population of *Calycadenia oppositifolia*, although only slightly smaller than A-chromosomes of that species, are no larger than some of the nucleolar-organizing B-chromosomes in other species of *Calycadenia*. In fact, they are about the same size as those found in *C. pauciflora* races Tehama and Pauciflora and *C. ciliosa* race Dry Creek. When more material from this population of *C. oppositifolia* is available it may demonstrate a nucleolar-organizing function for this B-chromosome as well. As $n=7$ is considered basic in *Calycadenia* (Carr 1975), the occurrence of B-chromosomes of any sort in a 7-paired species like *Calycadenia oppositifolia* presumably indicates an origin through some means

other than as a remnant of aneuploid reduction.

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Summary

Cytological studies of seven taxa in four species of the genus *Calycadenia* revealed the existence of several types of B-chromosomes. Of special interest is the occurrence of at least three forms of nucleolar-organizing B's in a total of six taxa in three species of the genus. Also of note is the occurrence in one species of an unusual structure less than 1 μ in length that is interpreted as a micro-B chromosome. Its morphology is reminiscent of that of a "naked" centromere. The B-chromosomes of *Calycadenia* are variable in size, morphology, and meiotic behavior. They have probably had multiple origins, perhaps as abnormal meiotic products in structural heterozygotes produced by hybridizing populations or species or as by-products of aneuploid reduction.

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