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The Course of the First Meiotic Prophase in *Beta procumbens* and in the F_1 between *B. vulgaris* and *B. procumbens*

WALTER NAGL

Max-Planck-Institut für Pflanzengenetik, Ladenburg a. Neckar, Rosenhof

Summary. The succession of the stages of the first meiotic prophase in *Beta procumbens* has been ascertained by measurements of the nuclear volume and by other criteria. By this method it has been possible to relate the appearance of pairing gaps to the beginning separation of the chromosomes after pachytene. A zygotene-like stage of early diplotene has been found. Asynchronous condensation of the bivalents and reduction of the chiasma frequency have been observed in diplotene and diakinesis. — The lengths and arm ratios of the pachytene chromosomes of *Beta procumbens* were determined.

Stages similar to those in *B. procumbens* have been recognized in the F_1 hybrid between *Beta vulgaris* and *Beta procumbens*. The pachytene chromosomes appeared to be paired to a great extent. However, only some of the cells showed bivalents in diakinesis, in the majority desynapsis occurred during diplotene or early diakinesis. In metaphase I, bivalents, univalents, cases of secondary association due to stickiness and cases of fragmentation have been observed.

Introduction

The behavior of the chromosomes during the first meiotic prophase is of general interest for the elucidation of several genetic problems. Nevertheless, no conformity exists in the interpretation of the single stages (compare MOENS, 1964). Therefore the stages were filed, in the present paper, using as guides the nuclear volume of pollen mother cells, the degree of polyploidy of the tapetal cells, and several other criteria. Special reference is given to the pairing behavior of the homologues. — The species *Beta procumbens* was chosen as material, because this and the other species of the section *Patellares* are considered as potential sources of certain valuable characters not known to occur in the sugar beet: high resistance to leaf spot (*Cercospora beticola*), to sugar beet nematode (*Heterodera schachtii*), and to curley top virus (compare OLDEMEYER, 1954; KNAPP, 1958). For an attempt to transfer desirable genes from *Beta procumbens* to sugar beet, interspecific hybrids were bred (compare KNAPP, 1958; SAVITSKY, 1960). Observations on the structure and pairing behavior of the chromosomes of such a hybrid during the first meiotic prophase will be communicated in the second part of the present paper.

Material and Methods

Buds of plants grown in the green house of the institute were measured under a preparation microscope, and fixed partly with ethanol/acetic acid (3:1, 4 °C), partly with glutaraldehyde (5%, pH 7.2, 4 °C) for 3 hours. After fixation, the lengths of the anthers were measured, the anthers halved and stained with aceto-carmine (2%, with a trace of iron alum of 4% added immediately before use) at room temperature for 12 hours. The material fixed with ethanol/acetic acid was squashed, carefully heated, squashed again weakly, sealed and examined by phase contrast; permanent slides were made with the dry-ice technique. The pollen mother cells of anthers fixed with glutaraldehyde were used to establish the nuclear volumes (for method see NAGL, 1965), because volume is known to increase during mitotic and meiotic prophase (lit. at NAGL, 1969). The degree of polyploidy of the cells of the tapetal layer — known to rise during meiotic prophase of the pollen mother cells (compare MECHELKE, 1952, for *Antirrhinum*) — was established by counting all the chromosomes of a cell in normal or arrested mitosis, and — according to the method of REITBERGER (1956) — by counting the nucleolus-associated chromocenters in interphase nuclei. The significance of mean differences of the nuclear volumes was tested with the aid of the tables given by FISHER and YATES (1953).

It was more difficult to obtain good preparations of the meiotic nuclei in the hybrid than in *B. procumbens*. In the hybrid (an engrafted plant, cultivation no. 942 G5), the best results were obtained if the plant was maintained in a phyto-chamber at 10 °C for two or three weeks.

Results

1. *Beta procumbens*

An accurate description and understanding of the pairing behavior of the pachytene chromosomes is only possible, if the successive stages of the first meiotic prophase are well determined. In the present study, the stages were therefore filed by measurements of the bud size, of the anther length, of the polyploidy degree of the tapetal cells, and particularly of the volume of the meiotic nuclei (Table 1). Moreover, the relative site of a pollen mother cell and the thickness of the mucilaginous wall were guides in the establishment of the stage succession.

The very early stages, leptotene and synizesis ("synizetic knot"), do not exhibit enough details for the identification of single chromosomes. However, the glutaraldehyde-fixed cells give evidence of paired

Table 1. *Beta procumbens, succession of early meiotic stages in pollen mother cells, with reference to the bud size, the anther length, the degree of polyploidy of tapetal cells, and the volume of meiotic nuclei (*$P < 0.0002$)*

Stage of Meiosis	Diameter of Buds	Length of Anthers	Polyploidy (Tapetum)	Pollen Mother Cell Nuclei			
				N	Volume (μ^3)	*F*	*t*
Interphase	ca. 1.0 mm	ca. 0.3 mm	2x	56	535±22		
						24.19	1752*
Synizesis	ca. 1.0 mm	ca. 0.4 mm	4x	38	847±108		
						1.89	11.47*
Pachytene	ca. 1.0 mm	ca. 0.4 mm	4x	74	1074±79		
						8.93	6.65*
Late Pachytene	ca. 1.0 mm	ca. 0.5 mm	4x–8x	53	1298±235		
						4.08	21.51*
Early Diplot.	ca. 1.1 mm	ca. 0.5 mm	8x	45	2083±117		
						—	—
Diplotene	ca. 1.3 mm	ca. 0.6 mm	8x	—	—		
						—	—
Diakinesis	ca. 1.3 mm	ca. 0.6 mm	8x–16x	—	—		

regions and parallel sections of homologues during synizesis. No zygotene was observed.

During the early and middle pachytene, the nine bivalents do not show failures in the pairing, or structural differences between the homologues (Fig. 1 a). An analysis and characterization of the pachytene chromosomes was possible in several pollen mother cells. Difficulties occurred only in the localization and identification of the small chromomeres and of the centromeres of the five short chromosomes. The latter were assumed to lie within or very near to the only heterochromatic centromere of each of these short bivalents, since — as far as investigated in the tapetum — proximal heterochromatin could be identified in all mitotic chromosomes. The average lengths of the bivalents of 28 cells of a single anther, and some arm ratios, are noted in Table 2. The chromomere pattern and the decrease of the length of the nucleolus-organizing bivalent was studied in more detail and figured in Fig. 2.

Pairing gaps as well as small structural differences between the homologues appear first at late pachytene, or after pachytene (Figs. 2d–g). There are some morphological criteria which indicate clearly that these chromosomes are in the process of separation: The nuclei showing bivalents with gaps or bivalents like rope-ladders occur between pachytenic and diplotenic cells, they have a volume which is significantly higher than that of pachytene nuclei, there are also higher degrees of polyploidy in the tapetal layer, and the bivalents are shorter than during the completely paired stage. The chromo-

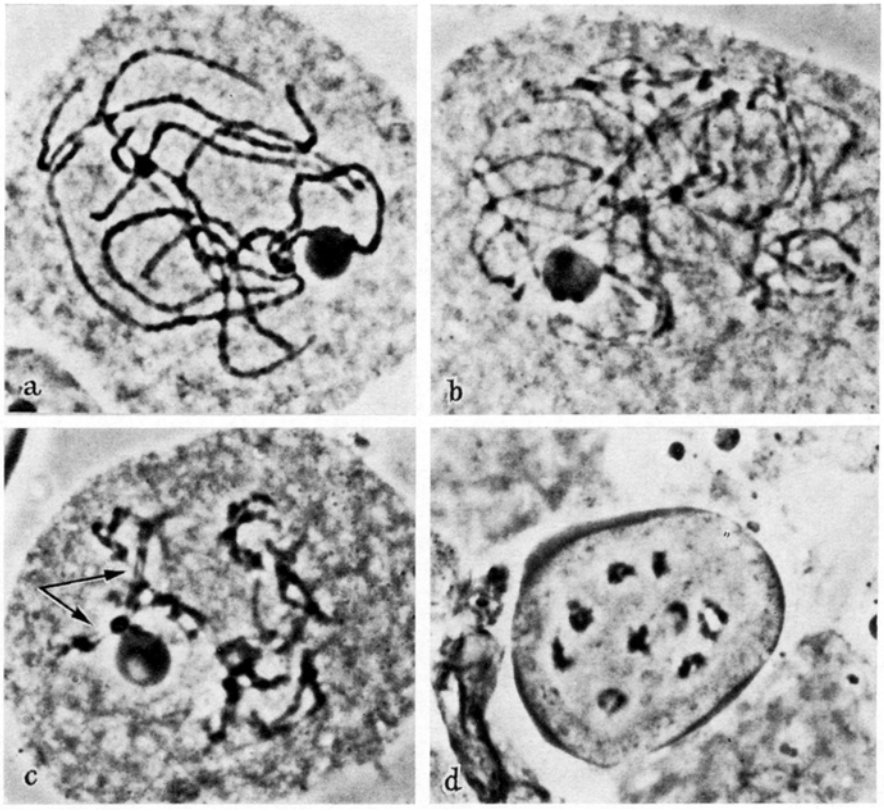

Fig. 1. Characteristic stages of the first meiotic prophase in pollen mother cells of *Beta procumbens*:
a) pachytene;
b) early diplotene;
c) late diplotene (arrows indicate bivalent delayed in contraction);
d) diakinesis (all 9 bivalents are visible).
Carmine-phase contrast; a–c) squashed, d) unsquashed; ×1500

Table 2. *Beta procumbens, average lengths and arm ratios of the pachytene chromosomes in pollen mother cells (the chromosomes were not individually identified, but ranged in every cell due to their actual lengths only; the identifiable SAT-bivalent, p.e., was the third-, fourth-, or fifth-longest)*

N	Length (μ)	*N*	Arm ratio
28	61±4.4	5	1.7—2.2
28	53±3.5	5	1.9—2.6
28	48±4.0	4	1.1—3.2
28	47±3.8	3	2.0—2.1
28	35±2.1	3	1.1—2.3
28	30±1.9	5	2.8—3.3
28	20±1.4	3	2.1—2.8
28	19±1.2	3	3.0—4.6
28	18±1.0	3	2.0—2.1

meres become more distinct during the process of chromosome separation. The pattern of the small chromomeres is not totally constant, but a similarity of their number and distribution cannot be ignored; the large, evidently heterochromatic chromomeres, show a constant pattern (Fig. 2). However, the two homologues of many bivalents do not possess the same structure in all regions within many cells (Figs. 2d, e): Either some chromomeres of one homologue seem to be displaced, or to have joined with the neighboring ones. Since these phenomena do not occur in a regular pattern, they may be interpreted as squashing artefacts, or as the expression of a differential coiling speed, but not as structural heterozygosity.

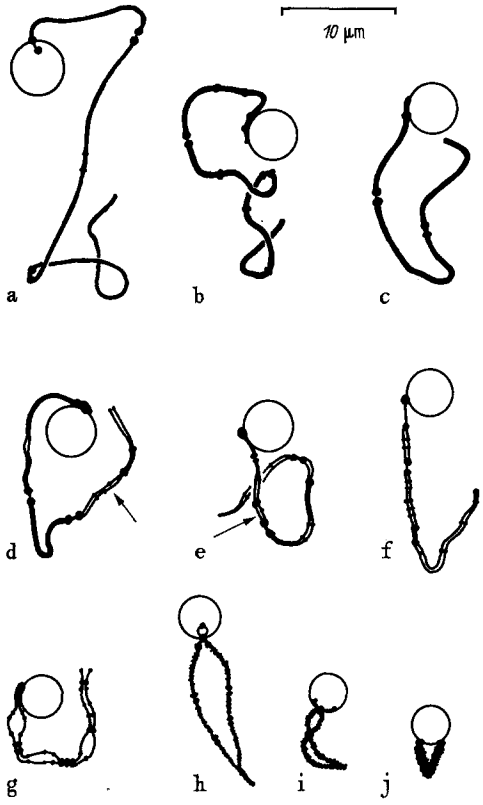

Fig. 2. The nucleolus-organizing bivalent of *Beta procumbens* during the first meiotic prophase (its length in μ is given in brackets):
a) early pachytene (60),
b—c) pachytene (47, 40),
d) late pachytene (34),
e—g) separation of homologues (30, 23, 20),
h—i) diplotene (15, 8),
j) early diakinesis (3.2).
Arrows in d) and e) indicate structural differences between the homologues

At the onset of diplotene the homologues become completely separated up to the points of contact or chiasmata (Fig. 1b). This stage, during which the single chromosomes are not individually distinguishable, would be easily misinterpreted as zygotene, if the criteria cited above had not demonstrated its post-pachytene nature. The diplotene coiling of the chromosomes which starts now occurs asynchronously. The bivalents appear therefore differentially contracted in most of the cells (Fig. 1c). The nucleolus-organizing bivalent was used as a marker: it is the fourth-longest during the middle pachytene, but the sixth-longest during the late diplotene, and the arm ratio of this bivalent was found to decrease from about 3.0 at early pachytene to about 1.5 at diplotene, indicating a more rapid coiling of the longer arm (compare Fig. 2). However, these observations are only an indication for an asynchronous behavior of the chromosomes, since no statistical tests were performed.

The number of chiasmata is reduced between the middle and the late diplotene from 28 to 21 per chromosome complement. In the stage of diakinesis, numbers of 12, 13 or 14 are recognizable (Fig. 1d). — The further course of meiosis in *Beta procumbens* occurs in the normal, well-known way.

2. *Beta vulgaris* × *Beta procumbens*

The relationships between the bud size, the anther length, the polyploidy degree in the tapetal layer, and the nuclear volume in the pollen mother cells are disturbed in the hybrid. Moreover, the quality of the preparations was not as good as in the studied parent, evidently due to physiological changes in the cells of the hybrid. Measurements of the nuclear volumes were, therefore, not possible. However, a course of the first meiotic prophase in the hybrid similar to that in *B. procumbens* may be assumed, due to the similar structures observed in the nuclei.

The nuclei in the stages leptotene and synizesis exhibit the same structure as those of *Beta procumbens*. During the pachytene (Fig. 3a), the chromosomes seem to be almost completely paired. The rough surface of the bivalents and their relative shortness may be the expression of many small unidentified pairing gaps and loops, or of physiological disturbances. The centromere region is often extremely stretched, at least in the greater part of the pollen mother cells.

Chromosome separation occurs in early diplotene before onset of the condensation (Figure 3b), leading to a similar loosening of the bivalents as it was described for *B. procumbens*. The coiling asynchrony of the bivalents is more extreme in the hybrid than in the investigated parent. The diakinetic cells exhibit different patterns of desynapsis: In some cells, one to eight bivalents are visible (rods, crosses, rings: Figs. 3c to e), in other cells only univalents were observed. Frequently two — possibly the two corresponding — univalents are lying close together (Fig. 3f). This is not the case with the greater part of the univalents in prometaphase I (Figure 3g).

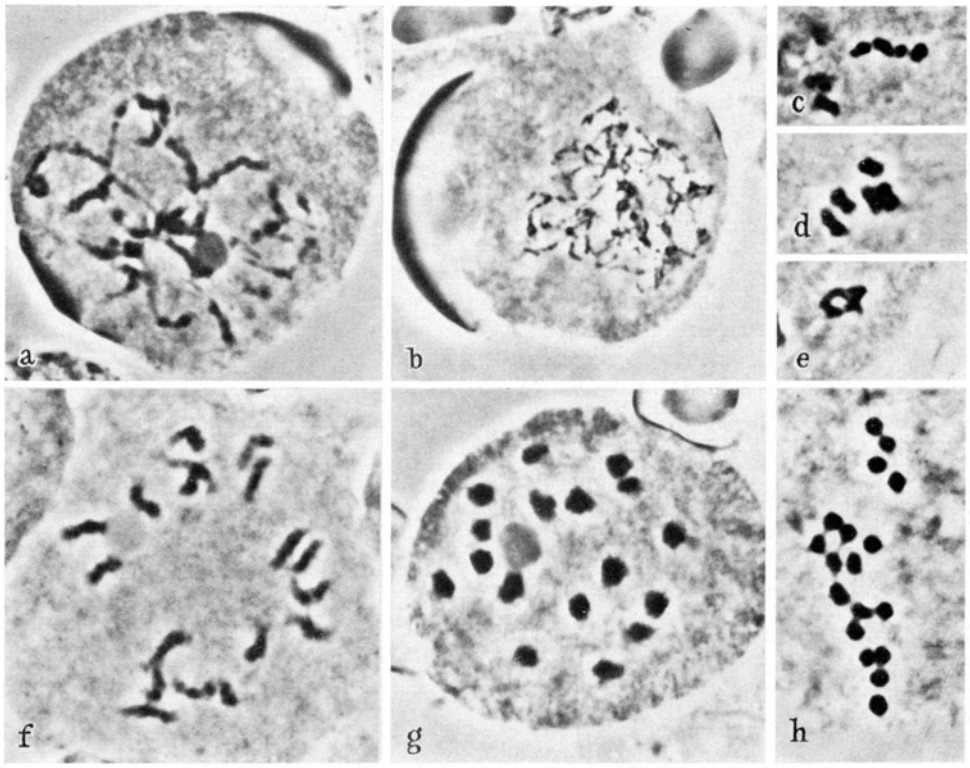

Fig. 3. First meiotic prophase in the hybrid *Beta vulgaris* × *B. procumbens* (F_1):
a) pachytene,
b) diplotene,
c—e) bivalents at diakinesis,
f) diakinesis showing univalents (the close site of the corresponding chromosomes indicates desynapsis),
g) pro-metaphase I (several chromosomes are lying close together),
h) meta-anaphase I (note secondary associations between the chromosomes).
Carmine-phase contrast, × 1500

Different irregularities were found in metaphase I and anaphase I. Evidently no cell behaves in a totally normal way, although bivalents are identifiable in some of them. Since also secondary associations (due to stickiness) occur between two or more chromosomes (Fig. 3h) or even between all chromosomes, the real bivalents are not recognizable with certainty. As was already shown by SAVITSKY (1960), chromosome fragmentation and anaphase bridges are the results of these disturbances, leading to microspore formations as pentads, hexads, and other polyads.

It may be noted that the frequency of all these irregularities was much higher in the plant from the green-house than in the same plant after cooling at 10 °C in the phyto-chamber for two weeks.

Discussion

It may be emphasized that the stages of late pachytene and early diplotene might be misinterpreted as zygotene, without intimate knowledge of the stage succession. This was first shown by MOENS (1964) in the tomato, and later on confirmed in other species. In the terms of MOENS (1964), the stages "late pachytene" and "early diplotene" described in this paper for *Beta*, may be called "schizotene" and "diffuse stage", respectively. However, these terms were avoided due to their ambiguous use in the literature. The intimate knowledge of the stage sequence is also important in preventing a misinterpretation of the chromosome separation in terms of defective pairing at pachytene.

Structural differences between the homologues of *Beta procumbens* appearing during the process of separation may be caused by the squashing technique (compare OEHLKERS and EBERLE, 1957; v. WANGENHEIM, 1957), or due to an asynchronous condensation of the homologues. The latter conception may be confirmed by the findings of asynchronous contraction of the single bivalents in *B. procumbens*, but also in other *Beta* species (WALIA, 1968). Real structural heterozygosity is unlikely, because the chromosomes show the structural differences more frequently in the stages of chromosome separation and of diplotene than during the middle pachytene.

The F_1 hybrid *Beta vulgaris* × *B. procumbens* has paired chromosomes in the pachytene, as was also demonstrated in the hybrid *B. webbiana* × *B. vulgaris* (WALIA, 1969). Contrary to the latter, the "*pro-*

cumbens-hybrid" possesses several bivalents in most of the diakinetic cells. The present cytological findings, however, do not explain, whether or not pachytene pairing and chiasma formation is due to partial homology — as was supposed in respect of similar events in haploid plants (ERNST, 1940; LEVAN, 1945) and in other interspecific hybrids (lit. at RIEGER, 1963; JOHN and LEWIS, 1965). In this connection it may be noted that even the formation of synaptinemal complexes was found in interspecific hybrids (MENZEL and PRICE, 1966) as well as in the course of an achiasmatic meiosis (GASSNER, 1969). Although bivalents do occur in the pachytene and diakinesis of the F_1 *B. vulgaris* × *B. procumbens*, desynapsis, fragmentation, stickiness and secondary association of the chromosomes during meta-anaphase I — as well as the disturbed second division — lead to the formation of polyads and a high degree of sterility (compare SAVITSKY, 1960).

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Zusammenfassung

Die Stadienabfolge während der ersten meiotischen Prophase bei *Beta procumbens* wurde auf Grund verschiedener Kriterien, vor allem durch Messungen des Kernvolumens, bestimmt. Dadurch konnte das Auftreten von Paarungslücken auf die post-pachytänische Trennung der Chromosomen zurückgeführt werden. Während des frühen Diplotäns wurde ein Zygotän-ähnliches Stadium gefunden. Asynchrone Kondensation der Bivalente und Reduzierung der Chiasmenzahl wurden im Diplotän und in der Diakinese beobachtet. — Die Längen- und Armlängen-Verhältnisse der Pachytän-Chromosomen von *Beta procumbens* wurden bestimmt.

Die gleichen Stadien wie bei *Beta procumbens* traten auch in der F_1-Hybride zwischen *B. vulgaris* und *B. procumbens* auf. Die Pachytän-Chromosomen lagen offenbar weitgehend im gepaarten Zustand vor. Dennoch besaß in der Diakinese nur ein Teil der Zellen Bivalente, im größeren Teil der Zellen erfolgte während des Diplotäns oder der frühen Diakinese Desynapsis. In der Metaphase I wurden Bivalente, Univalente, Fälle von sekundärer Assoziierung (infolge stickiness) sowie Fälle von Chromosomen-Fragmentierung beobachtet.

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Dr. WALTER NAGL
Botanisches Institut der Universität Wien
Rennweg 14
A-1030 Wien (Austria)