Acta Bot. Neerl. 33(3), August 1984, p. 257–266.

STRUCTURAL CHANGES IN THE APEX OF THE FEMALE STROBILUS AND THE INITIATION OF THE FEMALE REPRODUCTIVE ORGAN (OVULE) IN EPHEDRA DISTACHYA L. AND E. EQUISETINA BGE

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SUMMARY

Histological changes in the apex of the female strobilus and the initiation of the reproductive organ (ovule) were investigated in two species of *Ephedra*. In contrast to the vegetative shoot apex, periclinal cell divisions take place frequently in the dermal layer of the apex of the female strobilus and of the ovule. These divisions in the former begin at the summit before the initiation of the ovule, or they begin at both the summit and peripheral region simultaneously with the ovular initiation. Two ovule primordia originate from the axils of the uppermost bracts by mitotic activity in the subdermal region, their outer part becoming two-layered as the result of regular periclinal divisions in the dermal cells. As compared with the ovule primordia, the residual shoot apex between the ovule primordia shows less periclinal cell divisions just before and after it becomes discernible. The occurrence of these divisions in the apex of the female strobilus and in the ovule primordia is compared with that in the male ones.

1. INTRODUCTION

In contrast with the classical concept that the sporangium-forming meristems of gymnosperms consist of two separate cell layers and that the sporangial initials are "hypodermal" in position (SCHNARF 1933; CHAMBERLAIN 1935), some deviations have been revealed in both micro- and macrosporangium forming meristems, in which the dermal cells divide periclinally and the sporangia originate from the resultant cells (ALLEN 1946; ERSPAMER 1952; FAGERLIND 1961).

Since these two patterns of structure and growth apparently resemble those in the differentiated (tunica-corpus organized) and undifferentiated vegetative shoot apices, respectively, ALLEN (1946), FOSTER & GIFFORD (1959) and FAGERLIND (1961) pointed out their correlations.

FAGERLIND (1961) and BRUNKENER (1973) thoroughly investigated this subject in the male and female sporangium-forming meristems of many taxa. Their studies confirmed the correlations suggested, but clearly showed certain differences

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between the developmental phases, and also showed intermediate types which are characterized by occasional or rare periclinal cell divisions in the dermal layer. BRUNKENER (1977) summarized the previous works from this viewpoint. He concluded that "in most gymnosperms studied the spore-producing meristems have attained the more advanced stage while the shoot apical meristems of most genera still are quite undifferentiated or show intermediate conditions". But, as BRUNKENER (1977) himself stated, precise pertaining information on the conditions in gymnosperms as a whole is still lacking especially as to the reproductive phase. A more comprehensive ontogenetical survey, advocated by FOSTER & GIFFORD (1959, 1974), is necessary to interpret the occurrence of different structural and growth patterns.

Ephedra is a representative of a group which possesses a uniseriate tunica in the vegetative shoot apex (GIFFORD 1943; SEELIGER 1954), but may exhibit more or less frequent periclinal cell divisions in the dermal layer of the male and female reproductive meristems during their development (STRASBURGER 1872; SINGH & MAHESHWARI 1962; FAGERLIND 1971; and others). Therefore, this group, together with the other Gnetophyta, is considered to be an exception to Brunkener's general conclusion. STRASBURGER (1872) and FAGERLIND (1971) studied the apices of male and also, but fragmentarily, female strobili in *Ephedra*, and stated their similarity in anatomy to the vegetative apex, but Fagerlind reported periclinal cell divisions in the dermal layer of the apices of the strobili of both sexes in the genus. The purpose of this paper is to describe accurately the histological changes in the apex of the female strobilus and the initiation of the reproductive organ (ovule) in *Ephedra*, with special reference to the periclinal cell divisions in the regions concerned.

2. MATERIALS AND METHODS

Material of *Ephedra distachya* L. and *E. equisetina* Bge. was collected at Tsukuba Medical Plant Research Station, National Institute of Hygienic Sciences in Japan (the voucher specimens are preserved in the Makino Herbarium, MAK 189060, 189061). Female strobili were fixed in formalin-acetic acid-alcohol (FAA) in early May. For microscopical studies some of the material was dehydrated in a tertiary butyl alcohol series, embedded in paraplast, cut into 10 μm sections and stained with Heidenhain's haematoxylin, safranin and fast green. In addition, *E. distachya* material was studied by means of scanning electron microscopy (SEM) after having been dehydrated in an ethyl alcohol series, critical-point dried and coated with gold.

3. DESCRIPTIVE NOTES

E. distachya and *E. equisetina* are small dioecious shrubs with decussately arranged, scaly leaves. The female strobilus usually bears four pairs of bracts; they are marked here acropetally as *br.* 1, *br.* 1′, *br.* 2, *br.* 2′, and so on. Two reproductive organs are enclosed within the uppermost pair of bracts (*br.* 4,

br. 4'), each of which is referred to as an ovule for convenience, and differentiates into two nucellar envelopes and a nucellus (for the conflicting opinions concerning the reproductive organ, see MARTENS 1971). The term "shoot apex" used in this paper refers to the portion above the uppermost bracts at the stage before the outline of the two ovule primordia becomes conspicuous. The same term is also applied to a minor outgrowth, i.e., residual apex, present between the two ovule primordia after their bulging has become distinct.

Recognition of developmental stages: Observations were made of developmental stages for convenience'sake divided into four phases (*fig. 1*), viz., Stage I: before the initiation of the ovule primordia; Stage II: when the ovule primordia just become visible; Stage III: when the ovule primordia, though still swelling, are not clearly outlined yet; and finally Stage IV: when the ovule primordia and the residual apex are clearly visible.

Frequency of periclinal cell divisions in the dermal layer: In order to get some insight into the developmental pattern of the shoot apex and ovule primordia, the frequency of recently and periclinally divided cells was examined in the dermal layer by using the following index:

$$\text{Frequency} = \frac{\text{Number of mother cells recently divided periclinally}}{\text{Number of cells observed}} \times 100.$$ The results are summarized in *table 1.*

Of three median longisections through the midribs of the uppermost bracts, the two lateral ones were used for the calculations. As indicated in *fig. 3* with circles, nine cells were observed at the summit and the flanks of the shoot apex (Stage I–III) or in the corresponding portions, i.e., residual apex and ovular apices, (Stage IV) in a section.

4. OBSERVATIONS

The developmental pattern of the apex of the strobilus is almost the same in

Table 1. Frequency of periclinally divided cells in the dermal layer. The total number of cells examined in brackets.

	E. distachya		*E. equisetina*	
	s	f	s	f
Stage I	17% (30)	2% (60)	0% (18)	0% (36)
Stage II	33% (42)	1% (84)	17% (18)	19% (36)
Stage III	25% (24)	44% (48)	13% (30)	73% (60)
Stage IV	33% (72)	83% (144)	25% (24)	90% (48)

s, f: The summit and the flanks of the shoot apex (Stage I–III) and the corresponding portions (Stage IV).

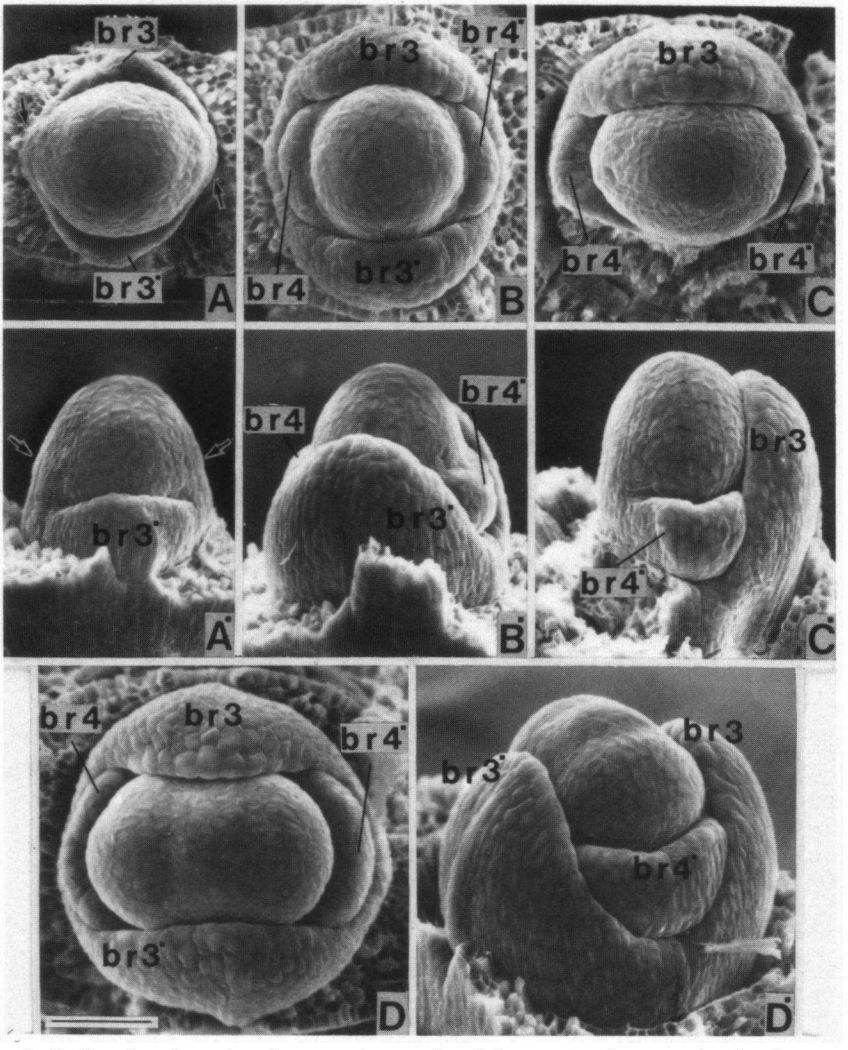

Fig. 1. *E. distachya.* Scanning electron micrographs of shoot apices in successive developmental stages (A–D:apical views; A′-D′lateral views of the same apices, respectively). These developmental stages correspond to those of the following figures. Arrows indicate the initiation of the uppermost pair of bracts (*br.* 4, 4′) arranged decussately in respect of the pair below them (*br* 3, 3′). Scale indicator: 100 μm.

Ephedra distachya and in *E. equisetina,* so that, unless one of the two exhibits a special peculiarity, the following description applies to both species.

Stage I: Before the ovule primordia are initiated (figs. 1A–4A)

At this developmental stage, the uppermost pair of bracts is being initiated (see arrows in *fig. 1A, A′*), and the shoot apex above them is conical in outline (*figs. 2A–4A*).

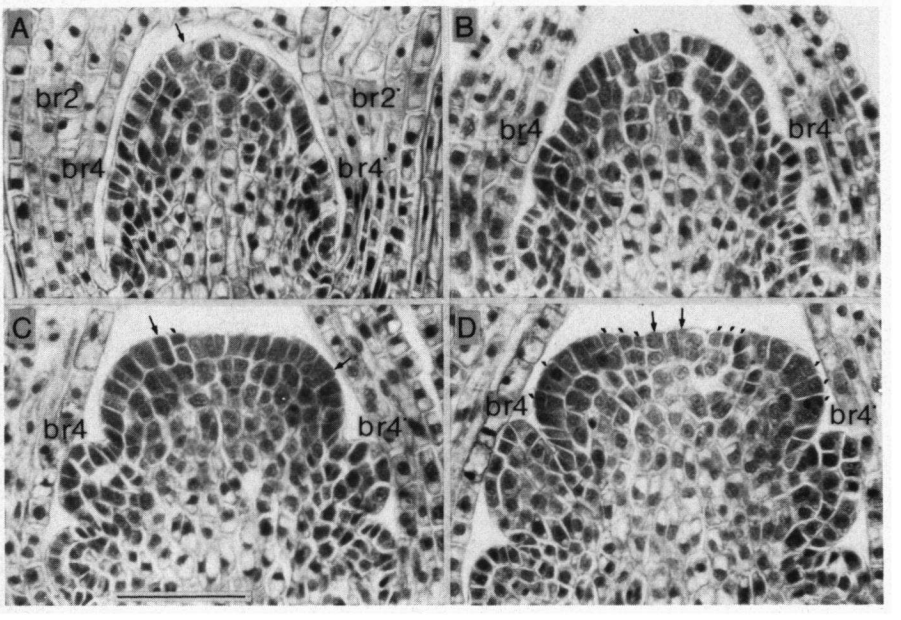

Fig. 2. *E. distachya.* Median longisections of shoot apices and ovule primordia (A–D). Note the periclinal cell divisions in the dermal layer which begin at the summit and before the initiation of the ovule primordia (A). Later on, active periclinal cell divisions take place in the ovule primordia (C, D). Arrows and dots indicate periclinal cell divisions. *Br.* 2, 2′ and *br.* 4, 4′ (shown in *fig. 1*) are pairs of bracts. Scale indicator: 100 μm.

As in the dermal layer of the vegetative shoot apex, a clear uniseriate layer is present in the apex of the strobilus (*figs. 2A–4A*), but in *E. distachya* periclinal cell divisions may have taken place at the summit of this layer (see arrow in *fig. 2A*; 17% in *table 1*). In the subdermal region, the cells are highly meristematic, and numerous dividing cells can be observed. No distinct cell arrangement pattern is present in this region (*figs. 2A–4A*).

Stage II: When the ovule primordia are just being initiated (figs. 2A–4B)
Owing to the initiation of the ovule primordia, the shoot apex slightly increases in width in the directions of the uppermost pair of bracts (FAGERLIND 1971), but the general outline of the shoot apex remains hemispherical (*fig. 1B, B′*).

Periclinally divided and dividing cells are of frequent occurrence in the dermal layer (*figs. 2B* and *3B*). In *E. distachya* these divisions are almost restricted to the summit area as in the previous developmental stage (see arrow in *fig. 4B*; 33% in *table 1*; FAGERLIND 1971), while in *E. equisetina* they also occur in the peripheral region (19% in *table 1*, for example). The dermal cells which have not divided periclinally become radially elongated in *E. distachya*, but they do not extend so much in *E. equisetina*. Cells of the subdermal region are actively dividing in both species, and periclinal cell divisions are prominent at the flanks (nearest to the uppermost bracts) where the swelling of the ovule primordia

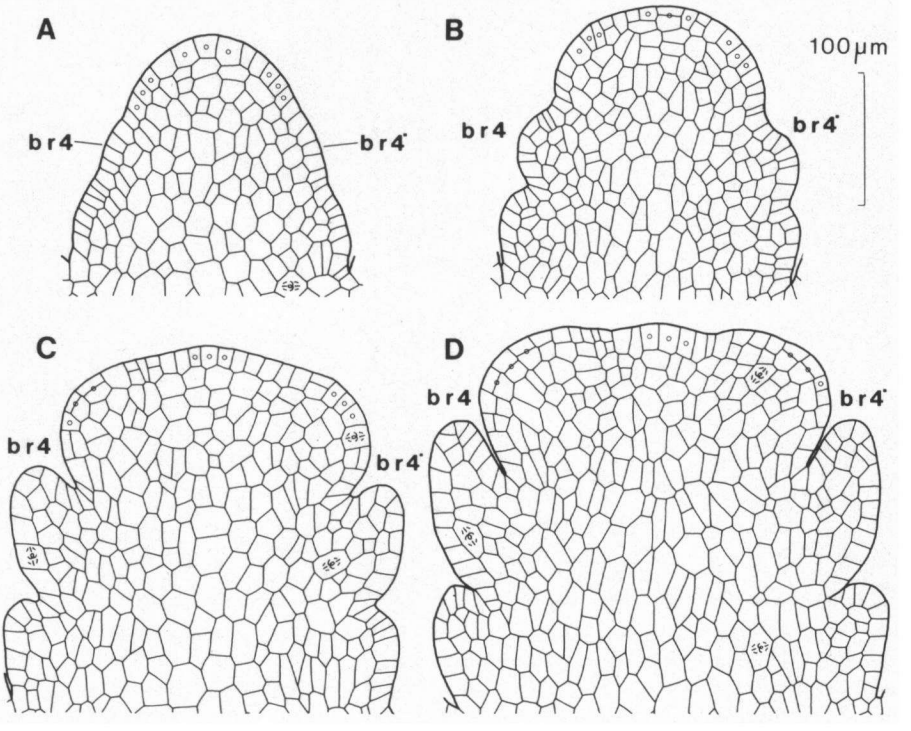

Fig. 3. *E. equisetina*. Diagrammatic representation of median longisections of shoot apices and ovule primordia (A–D). Note the uniseriate dermal layer in the shoot apex (A) and a similar layer at the summit of the shoot apex (C) and in the residual shoot apex (D). Cells marked by circles were used for the calculation of the relative frequency of periclinal cell divisions (see text for further explanation). *Br.* 4, 4′: the uppermost bracts.

is remarkable (*figs. 2B* and *3B*, see also PANKOW 1962; FAGERLIND 1971).

Stage III: The ovule primordia are still developing without clear outline (figs. 1C–4C)

Although the shoot apex increases in size as a whole, its growth is strongest in the directions of the uppermost pair of bracts (*fig. 1C, C'*; STRASBURGER 1872, 1879), so that in longisections the shoot apex appears to be ovate in outline (*figs. 2C* and *3C*). At this developmental stage periclinal cell divisions in the dermal layer seem to decrease a little in number at the summit (25% and 13% in *table 1*); therefore, in many cases there is a uniseriate dermal layer at the summit and none of the cells in this layer divide periclinally (*figs. 2C* and *3C*). However, in the peripheral region many dermal cells are dividing, or have divided periclinally (see arrows in *figs. 2C* and *4C*; *fig. 3C*). These divisions are most numerous at the flanks (44%, 73% in *table 1*). In *E. distachya* the dermal cells which had not divided periclinally have become radially extended as in the previous stage. Mitotic activity occurs in the greater part of the subdermal

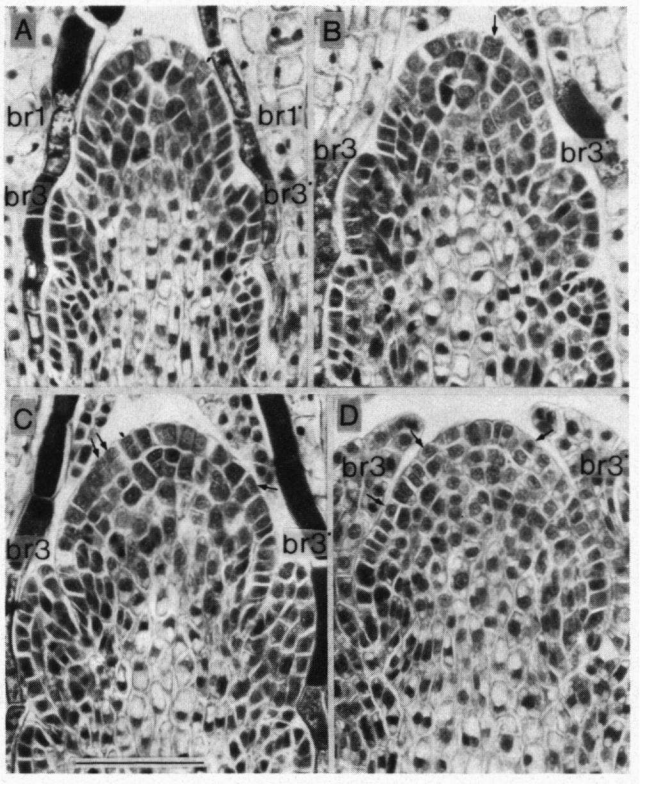

Fig. 4. *E. distachya.* Transmedian longisections (perpendicular to the median ones shown in *figs. 2* and *3*) of shoot apices (A–D). Arrows and dots indicate periclinal cell divisions. *Br.* 1, 1′ and *br.* 3, 3′ are the lower pairs of bracts. Scale indicator: 100 μm.

region, the cells dividing periclinally, especially at the flanks, and anticlinally (*figs. 2C–4C*; PANKOW 1962). Near the summit some dermal cells have already become vacuolated.

Stage IV: When the ovule primordia and the residual shoot apex are clearly visible (figs. 1D–4D)

The two ovule primordia are clearly discernible as lateral outgrowths, and at the apical part of the furrow above them the residual apex of the strobilus is visible as a small bulge (*figs. 1D, D′, 2D* and *3D*; STRASBURGER 1872, 1879; PANKOW 1962).

At this developmental stage, in the ovule primordia almost all dermal cells have formed derivatives by periclinal divisions; in other words, the outer part of the ovule primordia has become two-layered as the result of periclinal divisions in the original dermal cells (*figs. 2D* and *3D*; 83% and 90% in *table 1*; STRASBURGER 1872, 1879; PANKOW 1962), but these divisions are not so conspic-

uous towards the bases of the uppermost bracts. The cells constituting these regions usually have become elongated, especially so in *E. distachya*. Within the ovule primordia the cells below the two-layered outer part are mitotically very active (*figs. 2D* and *3D*; PANKOW 1962).

In the residual shoot apex, many of the dermal cells appear to have divided periclinally (arrows in *fig. 2D*; 33% and 25% in *table 1*), but occasionally they apparently do not divide in this way (*fig. 3D*; STRASBURGER 1872). In either case, both cells of the outer layer and those of the underlying region become vacuolated (*figs. 2D* and *4D*).

At the lateral parts of the furrow between the two ovule primordia, periclinal cell divisions have also taken place in some of the dermal cells (arrows in *fig. 4D*).

5. DISCUSSION

Observations of the successive ontogenetic stages show that periclinal cell divisions take place in the dermal layer of the apex of the female strobilus (FAGERLIND 1971). The time and place of initiation of these divisions differ, but slightly, in the two species investigated. In *E. distachya* they commence shortly before the initiation of the reproductive organs, or ovules, (Stage I), starting from the summit area of the shoot apex. In *E. equisetina* the first periclinal cell divisions occur at the stage when the ovule primordia are initiated (Stage II) and both at the summit and in the peripheral region. In both species the change in the dermal layer is followed by frequent periclinal cell divisions in the peripheral region (Stage III, IV) and by fewer periclinal cell divisions at the summit of the shoot apex (Stage III) or in the residual shoot apex (Stage IV). These findings agree with the morphological change in the apices of some male strobili as reported by FAGERLIND (1971) as far as the periclinal divisions at the summit are concerned. Probably they take place before the initiation of the male reproductive organ. Therefore, a most probable or definite relation might be present between the transition from the vegetative to reproductive growth and the periclinal cell divisions in the dermal layer of the summit area. Although STRASBURGER (1872) did not report these divisions in the apex of the male strobilus and FAGERLIND (1971) did not find them in the other species he investigated either, this contradiction seems to be explicable by the stronger development of the apex of the male strobilus as compared with the less extensive female one, which can be easily deduced from their gross morphology (cf. GIFFORD & CORSON 1971). In fact, periclinal cell divisions in the apices of some male strobili shown by FAGERLIND (1971) probably take place less frequently than those shown in this paper.

It is not so easy to define the exact location of the site of initiation of the ovule primordia in the shoot apex before they appear as distinct bulges (Stage II, III). However, the two areas closest to the uppermost bracts correspond to their places of origin (FAGERLIND 1971), because the structure and the develop-

mental pattern of the summit and of these places distinguish themselves from the surrounding areas.

BRUNKENER (1977) regarded the meristem of the microsporangium as differentiated in a tunica and a corpus, on account of the presence of a well-defined dermal layer during its development (SCHNARF 1933; FAGERLIND 1961, 1971; see also STRASBURGER 1872; LAND 1904; MAHESHWARI 1935; SINGH & MAHESHWARI 1962). However, FAGERLIND (1971) reported the incidence of periclinal cell divisions in the dermal layer of the male reproductive organ (which differentiates into a pair of bracteoles and an "antherophore" bearing several sporangia) just after (and before) this organ becomes a manifest outgrowth, and also in that of the young antherophore and its microsporangia (see also STRASBURGER 1872; FAGERLIND 1961; SINGH & MAHESHWARI 1962). Apparently these divisions take place very often during the whole ontogeny of the male reproductive organ, and Brunkener's typification (cf. FAGERLIND 1971) only seems to apply to the microsporangium formation proper. As shown in this paper, active periclinal cell divisions take place in the same way in the initiating (Stage II,III) and just initiated ovule primordia (Stage IV; STRASBURGER 1872, 1879; PANKOW 1962), and also during the development of the (future) nucellus (STRASBURGER 1872; MEHRA 1950; SEELIGER 1954; FAGERLIND 1961, 1971; PANKOW 1962; SINGH & MAHESHWARI 1962; LEHMANN-BAERTS 1967; TAKASO, in preparation). The frequent occurrence of such divisions in the male and female reproductive organs warrant the conclusion that their meristems are not so clearly differentiated as those of the vegetative shoot apex.

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to Dr. F. Bouman and Prof. A. D. J. Meeuse of the University of Amsterdam for their valuable advice and correcting the English. I am also indebted to Dr. H. Tobe of Chiba University and Prof. M. Ono of Tokyo Metropolitan University for their suggestions and encouragement.

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