Microsporogenesis and microgametogenesis of *Excentrodendron hsienmu* (Malvaceae *s.l.*) and their systematic implications

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Flowers, microsporogenesis and microgametogenesis of *Excentrodendron hsienmu* in opening-functional flowers and non-opening flowers were studied to investigate the evolutionary relationships of *Excentrodendron*. *E. hsienmu* is a dioecious species that blossoms every 3–4 years, although large numbers of flower buds develop every year. The anther is tetrasporangiate, the tapetum is of the secretory type, the microspore tetrads are mainly tetrahedral, and the pollen grains are two-celled when shed. Four to six microsporocytes are seen on the transverse section of the anthers, and cytokinesis is simultaneous. The development of the anther wall conforms to the basic type and the anther wall is five or six cells thick, with a fibrous endothecium. The difference between the opening-functional and the non-opening flowers is mainly in the thickness of the anther wall. Early megasporogenesis in staminate flowers up to megaspore mother cell or megaspore tetrads has been observed. *Excentrodendron* shares with Dombeyeae only plesiomorphic features, but differs in anther wall development type and thickness. Most features of *Excentrodendron* are shared with *Pterospermum*, including such synapomorphic features as basic type of anther wall development, five- to six-cell-thick anther wall, biseriate tapetum at some places, and degeneration of microsporocytes, suggesting placement near *Pterospermum*.   © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, **150**, 447–457.

ADDITIONAL KEYWORDS: dioecy – Dombeyeae – flowering cycle – Malvales – *Pterospermum*.

INTRODUCTION

Recently, the order Malvales has been the subject of intensive phylogenetic studies, particularly on its core families (Judd & Manchester, 1997; Alverson *et al.*, 1998, 1999; Baum, Alverson & Nyffeller, 1998a; Baum, Small & Wendel, 1998b; Bayer, Chase & Fay, 1998; Bayer *et al.*, 1999; Whitlock & Baum, 1999; Nyffeler & Baum, 2000; Das, Mukherjee & Das, 2001; Whitlock, Bayer & Baum, 2001; Pfeil *et al.*, 2002; Andreasen & Baldwin, 2003; Baum *et al.*, 2004; Small, 2004; Tate *et al.*, 2005). The main conclusions of these studies include the exclusion of the family Elaeocarpaceae from the newly circumscribed Malvales, establish-

ment of a new family Muntingiaceae, further confirmation of a close relationship among the core families of Malvales, and establishment of the expanded Malvaceae. Despite these advances, our understanding of evolutionary relationships within some groups of the Malvaceae *s.l.* is still poor (Alverson *et al.*, 1999).

The genus *Excentrodendron* H. T. Chang & R. H. Miau (Malvaceae *s.l.*, formerly Tiliaceae) was separated from *Burretiodendron* Rehder solely on macromorphology (Chang & Miau, 1978). However, the segregation has not been accepted in most systematic and taxonomic treatments (Zhuge, 1990; Wu, 1991; Brummitt, 1992; APG, 2003; Bayer & Kubitzki, 2003). Tang & Gao's (1993) work on the pollen morphology of the *Burretiodendron* (*s.l.*), however, revealed two very distinct types of pollen, which agree with the circumscription of Chang & Miau's two genera, *Excentroden-*

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dron and *Burretiodendron*, respectively, indicating that the segregation of *Excentrodendron* might be appropriate. On the other hand, the affinity of *Excentrodendron* is controversial. It has been referred to various taxa by different authors as a part of *Burretiodendron* or a closely related genus: *Colona* (Kostermans, 1961), *Craigia* (Chang & Miau, 1978), and *Schoutenia* and *Sicrea* (Zhuge, 1990), all members of the traditionally circumscribed 'Tiliaceae' but belonging to different subfamilies of Malvaceae *s.l.* However, recent molecular phylogenetic studies of the Malvales (Alverson *et al.*, 1999; Bayer *et al.*, 1999) suggest its placement in the Malvaceae *s.l.* subfamily Dombeyoideae, together with *Pterospermum*, Dombeyeae and *Eriolaena.* Our nrDNA sequence study has revealed a very close relationship between *Burretiodendron* and *Excentrodendron* and their close relationship with *Pterospermum* (Li, Tang & Shoup, 2004). However, *Excentrodendron* and *Burretiodendron* differ considerably in floral morphology from the above-mentioned genera, especially *Dombeya*, *Eriolaena* and *Pterospermum.* These disparities might have resulted from limited knowledge of both *Excentrodendron* and *Burretiodendron*, because only their gross morphology (Rehder, 1936; Chun & How, 1956; Kostermans, 1961; Chang & Miau, 1978; Zhuge, 1990), pollen morphology (Long, He & Hsue, 1985; Zhuge, 1990; Tang & Gao, 1993) and embryo sac development (of *Burretiodendron kydiifolium* Hsu & R. Zhuge) have been investigated (Tang, 1998). Our recent investigation of pollen morphology and reinvestigation of gross morphology revealed some incorrect or incomplete observations (Tang & Gao, 1993; Y. Tang, unpubl. data) from the lack of critical material and mistaken observations. In addition, although pollen of *Excentrodendron* is very different from that of *Burretiodendron*, their floral morphology is very similar. Such a combination has rarely been observed in other genera of Malvales. The difference in pollen morphology and the similarity in floral morphology raise an interesting question as to how floral morphology and pollen have evolved in these taxa.

To improve our understanding of *Excentrodendron* and to learn the affinity of *Burretiodendron* and *Excentrodendron* within the Malvaceae *s.l.*, we studied the gross morphology, anatomy, embryology, and molecular systematics of these two genera. Here the results of our study on the development of microspores and male gametophytes of *E. hsienmu* (W. Y. Chun & F. C. How) H. T. Chang & R. H. Miau during three consecutive years (2002–04) are presented.

MATERIAL AND METHODS

Excentrodendron hsienmu is a dominant or codominant tree species of montane rain forests in south-western China, and adjacent Vietnam and Laos (Tang, Mao & Gao, 2005a). It is exclusively restricted to limestone mountains and hills, and, thus, is a characteristic species of limestone flora.

Field observations and collection of the material were carried out during three consecutive years (2002–04) at the site of one population in Jinping County, Yunnan Province in south-western China. Buds of staminate flowers at different stages were collected from late February to mid-March 2002, but sectioning of the material indicated that, except for very few preparations, almost all of the material was at the stage of microsporocytes (microspore mother cells), although most developmental stages up to pollen grains were identified in the preparations. Field investigations later revealed that flowers did not open that year. Material was again collected in 2003, but was not sectioned because it was later found that once again flowers did not open. Collection of the material continued during 17–25 March 2004 when flowers blossomed. Material collected in 2002 and 2004 was studied systematically to compare differences in microsporogenesis and male gametophyte development.

Flower buds were fixed in modified FAA (89 : 6 : 5) or FPA on a daily basis. The morphology of the flowers was observed continuously during the flowering season from 2002 to 2004. The fixed material was treated with conventional methods of dehydration, transparency, and embedded in paraffin. Tissues were sectioned, both transversely and longitudinally, at 10 µm thickness with a Leica Rotary Sectioner using standard techniques. Sections were stained with a saffranin–Fast Green combination or with Heidenhain's iron alum-haematoxylin, and mounted with Canadian gum. The sections were observed with a Zeiss microscope and photomicrographs were taken with photographic facilities associated with the microscope.

RESULTS

THE FLOWERS

Flowers of *E. hsienmu* did not open every year, although large numbers of flower buds developed. Field observations from 2002 to 2004 revealed that the flower buds did not open in 2002 and 2003 but blossomed in 2004. Taking into account the age of the seedlings and saplings found under large female trees, the flowers seem to blossom *c.* every 3–4 years. Usually only very few flowers open in other years. The study of flower numbers indicated that the abundance of flower buds is not significantly different between blossoming and nonblossoming years.

Two types of flower or inflorescence were found on different individual trees. One type was a compound

cyme of three to 12 flowers and the other mostly a solitary flower or rarely a cyme of two flowers. It was found later through sectioning of the flower buds and field examination of opened flowers that the flowers in compound cymes are staminate, whereas solitary flowers or flowers in the small cymes are pistillate. Therefore, *E. hsienmu* is dioecious. Both staminate and pistillate flowers are borne in axils of leaves.

MICROSPOROGENESIS

The anther is two-locular and tetrasporangiate (Fig. 1). The archesporium differentiates beneath the epidermal cells in each of the four lobes of the very young anther (Fig. 2) and consists of a row of hypodermal cells. The archesporial cells undertake a periclinal division to form the primary parietal cell to the outside and the primary sporogenous cell to the inside (Fig. 3). The secondary sporogenous cells function as microsporocytes (microspore mother cells) by further enlarging, and are polygonal, elliptical, or irregular in shape, containing dense cytoplasm with large nuclei. The microsporocytes are usually arranged more or less regularly in two rows on the longitudinal sections (Fig. 6), and four to six microsporocytes are seen on transverse sections (Figs 5, 7). Just before meiotic divisions, microsporocytes become less polygonal, elliptical, or irregular, and are deeply stained. The degeneration of microsporocytes was observed in some preparations (Figs 8, 16). At the end of meiosis I, two nuclei are formed but no cell wall is formed (Fig. 9), followed by meiosis II. Therefore, cytokinesis is simultaneous, giving rise to tetrahedral tetrads (Figs 10, 17); but other types of tetrad are also observed: some bilateral (Fig. 11), a few tetragonal, and occasionally T-shaped or linear (Figs 12, 13). While one nucleolus is common in the newly formed microspore of tetrads, two, three, and four nucleoli are also observed (Figs 17, 18).

MICROGAMETOGENESIS

The tetrads released from the callose wall enlarge, developing into uninucleate microspores. The nucleus of the early uninucleate microspore is located at the centre of the microspore, which contains dense cytoplasm. A large vacuole is formed later at the centre of the microspore. As a result, the nucleus is then located at one end of the microspore (Fig. 14). The nucleus of the gametophyte undertakes one mitotic division to form a large generative cell and a small lenticular generative cell. The generative cell is distant from the wall whereas the generative cell is close to the wall. The mature pollen grains are spherical or subprolate, and are two-celled at the stage of release. Three to four pollen pores can often be observed on

pollen grains and the pollen grains are five- or six-porate. In the course of enlargement of the pollen grains, the tapetal cells are gradually absorbed, and completely disintegrated when the pollen grains mature.

THE ANTHER WALL AND TAPETUM

The epidermis of the anther primodium develops directly into the epidermis of the anther wall. The epidermal cells divide only anticlinally to increase the number of cells. When the anther reaches maturity, division of the epidermal cells ceases. The epidermal cells thin and flatten, but they are persistent in mature anthers. Substances were observed developing in epidermal cells. As a result, the epidermal cells are deeply stained and are distinctly different from the endothecium at the stages of microsporocytes and newly released microspores. The substances increase with anther development and are different between endothecium and epidermal cells but are absorbed in mature anthers. The epidermis is continuous throughout its development.

The primary parietal cells divide periclinally to form two layers of cells (Figs 4, 5). The outer layer functions directly as the endothecium and the inner layer divides once more to form the middle layer and the tapetum. Thickening of the endothecium is observed at the stage of the microspore, with the nucleus at one end. The endothecium becomes fibrous in mature anthers (Fig. 15). The cells of the middle layer further divide to form a two-cell-thick middle layer. In some places the tapetal cells can also further divide to form a two-layer tapetum. Therefore, the tapetum is uniseriate in most parts, but biseriate in some. As a result, the mature anther wall consists of five to six layers: epidermis, endothecium, two middle layers, and one or two tapetum layers (Figs 5, 7). The middle layers start to degenerate at the late stage of microsporocytes or just before division of the functional microspore mother cell due to the increase in size of cells of the endothecium and tapetum. The development of the anther wall conforms to the basic type (Davis, 1966).

The tapetal cells are uninucleate and not enlarged at the stage of microsporocytes (Fig. 5). They are more or less square or polygonal, with large nuclei and stain weakly. Shortly before meiotic division of the microsporocytes, the tapetal cells enlarge and elongate radially, with some becoming binucleate by nuclei division. The tapetum degenerates while the uninucleate microspore develops into mature pollen. At the stage of the microspore, with the nucleus at one end, the tapetum is still clearly visible. The residual tapetum is sometimes observed in the mature anther. The tapetum is therefore of the secretory type.
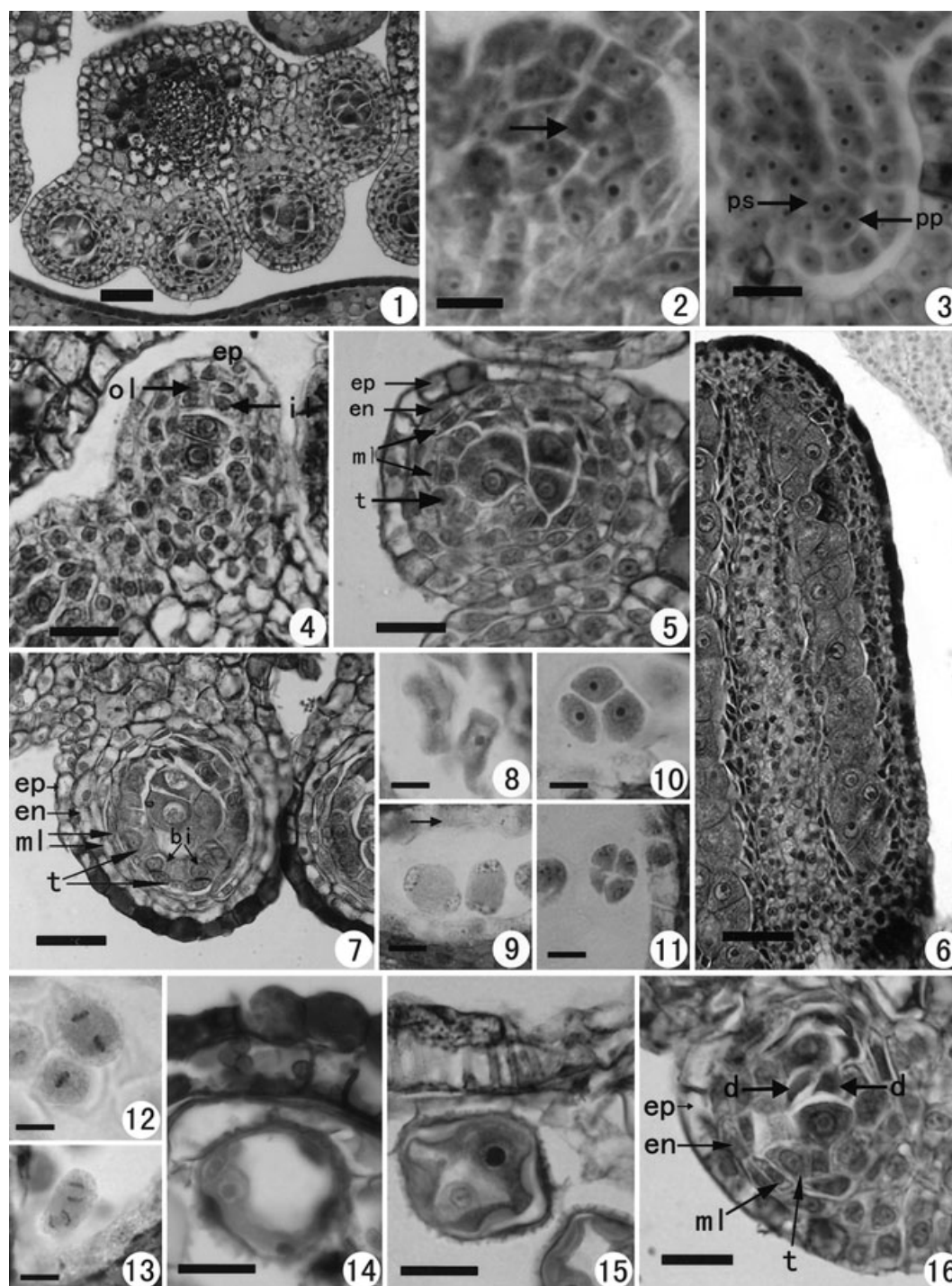
Figures 1–16. Microsporogenesis of *Excentrodendron hsienmu*. Fig. 1. Tetrasporangiate anther. Fig. 2. Archesporial cell of microspore (arrow). Fig. 3. Division of archesporial cell to primary sporogenous cell and primary parietal cell. Fig. 4. Division of primary parietal cell to secondary parietal cells. Fig. 5. Division of secondary parietal cells to endothecium, middle layer and tapetum. Fig. 6. Two rows of microsporocytes arranged on longitudinal section. Fig. 7. Five- to six-cell-thick anther wall. Fig. 8. Degenerating microsporocytes. Fig. 9. Meiosis I. Figs 10–13. Microspore tetrads. Fig. 10. Tetrahedral tetrad. Fig. 11. Bilateral tetrad. Fig. 12. T-shaped tetrad. Fig. 13. Linear tetrad. Figs 14, 15. Anther wall. Fig. 16. Degenerating microsporocyte. Scale bars: Figs 1, 6 = 50 μm; Figs 2–5, 7–16 = 20 μm. Abbreviations: bi, binucleate tapetal cells; d, degenerating microsporocytes; en, endothecium; ep, epidermis; il, inner secondary parietal layer; ml, middle layer; ol, outer secondary parietal layer; pp, primary parietal cell; ps, primary sporogeneous cell; t, tapetum.
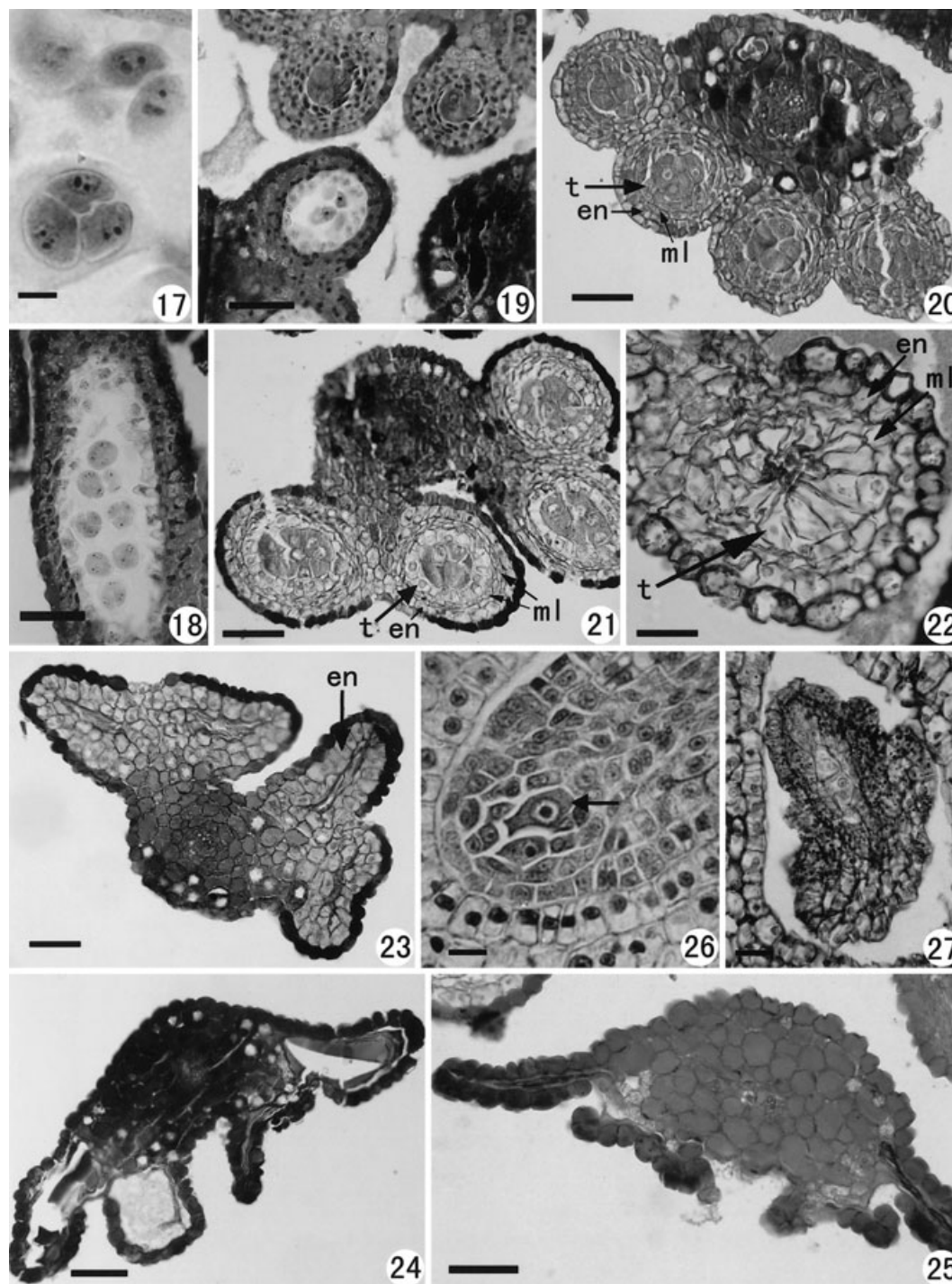
Figures 17–27. Figs 17, 18. Two to four nucleolus in tetrads. Scale bars = 50 μm. Fig. 19. Disparity development of microsporogenesis in different anthers. Scale bar = 50 μm. Figs 20– 25. Development and degeneration of microspores in pistillate flowers. Fig. 20. Microsporocyte with four-cell-thick anther wall. Fig. 21. Degenerating microsporocytes. Fig. 22. Residual microsporocytes in the centre, elongated tapetal cells and middle layer cells. Figs 23–25. Different stages of degenerating anthers. Scale bars: Figs 20, 21, 23–25 = 50 μm; Fig. 22 = 20 μm. Abbreviations: en, endothecium; ml, middle layer; t, tapetum. Figs 26, 27. Early development and degeneration of megaspores in staminate flowers. Fig. 26. Degenerating megaspore mother cell. Fig. 27. Degenerating megaspore tetrad, with two integuments demarcated. Scale bars = 20 μm.

Degeneration of anthers in pistillate flowers

Apart from microsporogenesis in staminate flowers, the development of anthers and early microsporogenesis were also observed in pistillate flowers. Microsporogenesis up to microsporocytes is usually observed, but further development beyond microsporocytes aborted. At the stage of megaspore mother cells, tightly arranged microsporocytes are common (Fig. 20). At the late stage of microsporocytes, tapetal cells elongate radially, but the endothecium and epidermis are similar to the anthers of staminate flowers (Fig. 21). Two-cell-thick middle layers are thin. With further development of megasporogenesis, microsporocytes begin to degenerate and become disorganized, with tapetal cells further expanding to occupy the space of the microsporocytes. However, the two middle layers enlarge (Fig. 22). The microsporocytes, tapetum and middle layers degenerate finally, with empty sporangium (Figs 23, 24). Finally only epidermal cells remain (Fig. 25). Therefore, the anthers become sterile in mature pistillate flowers. Although the size of the sterile stamens is much smaller than that of the fertile stamens of the staminate flowers, they are apparent in pistillate flowers.

Early development of the ovary and ovule in staminate flowers

During the development of microspores and pollen, the development of ovary primordia and early ovules is also observed in the staminate flowers. Ovules are not demarcated when microsporocytes are formed. The megasporial archesporium is observed later when the microspore tetrads are formed. The megaspore archesporial cell sometimes develops into a megaspore mother cell or sometimes into tetrads (Figs 26, 27) when pollen grains mature. Two integuments are demarcated. However, further development of the ovule did not proceed and no other developmental stages after megaspore mother cell or tetrads were observed. As a result, only a rudimentary, obscure ovary is present in staminate flowers.

Differences between material collected in 2002 (flower buds did not open) and 2004 (flowers blossomed normally)

The following differences were found between 2002 and 2004 material. (1) Most of the material collected in 2002 was at the stage of the microspore mother cell regardless of the size of the flower buds, which was not observed in the 2004 material. In 2004 material, different stages of microsporogenesis and male gametophytes corresponded with the enlargement of flower buds. (2) Development was abnormal in most material collected in 2002. Different stages of microsporogenesis were present among different anthers of the same flower or among different microspores within the same locule: microsporocytes of an anther were undergoing meiosis while only microsporocytes were present in other anthers, or the microsporocytes acquired their maximal enlargement and were just prior to meiosis in many anthers whereas only microsporocytes were present in one anther (Fig. 19). Such phenomena were not observed in the 2004 material. (3) The thickness of the anther wall was also different. The middle layer was mostly only one cell thick and the anther wall was usually four cells thick in the 2002 material, but the anther wall was five or six cells thick with two layers within the middle layer and a two-layered tapetum in some places in the 2004 material. In addition, the biseriate tapetum observed in the 2004 material was not observed in the 2002 material. (4) Fibrous thickening was observed on the endothecium in only a few preparations of the 2002 material. In contrast, thickening was commonly observed on the endothecium in 2004 material. (5) Pollen grains of the 2002 material were not as full as those of the 2004 material.

DISCUSSION

Floral biology and fruiting cycle

Flowers of *Excentrodendron (Burretiodendron) hsienmu* have been described as bisexual (Chang & Miau, 1978) or unisexual (Chun & How, 1956; Zhuge, 1990). Chang & Miau (1978) regarded bisexual flowers of *Excentrodendron* as a key character to segregate it from *Burretiodendron*. The present study indicates that flowers of *Excentrodendron* are unisexual and this species is dioecious, which is the same as *Burretiodendron*, reconfirming the observations and descriptions by Chun & How (1956) and Zhuge (1990), but not those of Chang & Miau (1978), as they incorrectly described *Excentrodendron* as bisexual.

Many tree species intermittently produce large seed crops within a population, termed mast fruiting. Although the mature trees of *E. hsienmu* develop large numbers of flower buds every year, they blossom and set fruit every 3–4 years. During the study period from 2002 to 2004, flowers did not bloom in 2002 and 2003, but flowered in 2004. Some differences were observed in microsporogenesis and male gametophyte development between 2002 and 2004 material. Although flowers did not blossom in 2002, most of the developmental stages in microsporogenesis and male gametophytes were observed. However, they were somewhat abnormal compared with those observed in 2004 material. Most of the material collected in 2002 was found to have only microspore mother cells regardless of the size of the flower buds. The conspic-

uous differences mainly included the thickness of the anther wall and characteristics of the tapetum. In addition, disparity in development was observed in only 2002 material. These differences might be related to resource availability for floral development and fruit set. The results also raise an interesting question about how staminate and pistillate plants synchronize blossoming. The degeneration of cells, including microsporocytes and pollen grains, was reported from some members of traditionally circumscribed Sterculiaceae (Rao, 1949, 1952a). The present study implies that the degeneration of cells might be related to the reproductive biology of flowers. Had not field investigations been continued for 3 years, the abnormal features might have been regarded as normal. A further study of mast fruiting of *E. hsienmu* would provide useful information for the protection and conservation of this species.

DIOECY IN *EXCENTRODENDRON*

Malvaceae *s.l.* is characterized by hermaphroditic flowers, and dioecy is found only in a few taxa, e.g. the tribe Sterculieae and a few other genera (Hutchinson, 1967; Bayer & Kubitzki, 2003). No studies seem to have covered as to whether bisexual or unisexual flowers are derived in Malvaceae *s.l.* The mature flowers of *E. hsienmu* are unisexual and the plants are dioecious. However, the present study has revealed early megasporogenesis and microsporogenesis in staminate and pistillate flowers, respectively. Staminate and pistillate flowers are not distinguishable at the early developmental stages because the development of anther sporangia is observed in both staminate and pistillate flowers. The development of megaspores in staminate flowers and microspores in pistillate flowers ceases before they mature. In consideration of the fact that a closely related species, *Burretiodendron kydiifolium*, has sterile stamens that are very similar to fertile stamens in size and shape (Y. Tang, unpubl. data) and that most species of the Malvaceae *s.l.* have hermaphroditic flowers, unisexuality and dioecy in *Excentrodendron* might be derived. A few species of *Dombeya*, to which *Burretiodendron* (*Excentrodendron* was part of this genus) is related (Bayer *et al.*, 1999), are also dioecious (Humeau & Thompson, 2001). Dioecy in the Malvaceae *s.l.* is probably derived, but further study is needed. *E. hsienmu* would be an excellent candidate for studying the evolution of dioecy in the Malvaceae *s.l.*

RELATIONSHIPS OF *EXCENTRODENDRON*

The relationships of *Excentrodendron* are controversial. As a part of *Burretiodendron*, it has been considered to be related to *Colona* (Kostermans, 1961), or *Craigia* (Chang & Miau, 1978), or *Schoutenia* and *Sicrea* (Zhuge, 1990), but its placement in the traditionally circumscribed Tiliaceae was not questioned until Tang & Gao's (1993) study of pollen. Although the segregation of *Excentrodendron* from *Burretiodendron* has not been accepted by most authors (Zhuge, 1990; Wu, 1991; Brummitt, 1992; APG, 2003; Bayer & Kubitzki, 2003), pollen of *Excentrodendron* is unique in Malvaceae *s.l.* and is distinct from that of *Burretiodendron*. Palynologically, *Excentrodendron* is isolated in Malvaceae *s.l.* In addition to the above-mentioned genera, *Pterospermum* has also been referred to *Excentrodendron* and *Burretiodendron* by recent embryological and molecular phylogenetic studies (Tang, 1998; Alverson *et al.*, 1999; Bayer *et al.*, 1999; Li *et al.*, 2004), although it differs considerably from *Excentrodendron* and *Burretiodendron* in floral morphology (Tang, 1992a). A recent molecular investigation of Malvales revealed a close relationship of *Burretiodendron* with Dombeyeae and placement of *Burretiodendron* in the subfamily Dombeyoideae of the Malvaceae *s.l.* (Alverson *et al.*, 1999; Bayer *et al.*, 1999).

Embryological features have proved useful in studying plant taxa with uncertain affinities (e.g. Maheswari, 1950; Johri, 1984; Tobe, 1989). However, except for economically important plants such as cotton (*Gossypium* spp.), cacao (*Theobroma cacao*) and jute (*Corchorus* spp.), embryological knowledge in other members of Malvaceae *s.l.* is poor. Most of the embryological studies were reported before 1970 and there have been only a few recent reports of embryological investigations (e.g. Tang, 1992b, 1994, 1998; Tang & Pan, 1994). Among the taxa previously referred to *Burretiodendron* and/or *Excentrodendron*, embryological information is available for *Corchoropsis*, *Dombeya*, *Melhania*, and *Pterospermum* (Rao, 1949, 1952a; Tang, 1992b; Tang & Pan, 1994).

COMPARISON WITH *PTERSPERMUM* OF SUBFAMILY DOMBEYOIDEAE

Pterospermum is one of a few genera of Malvaceae *s.l.* for which detailed embryological information is available for three of its species (Sharma, 1938; Rao, 1949, 1952a). It was traditionally placed in the tribe Helictereae of the traditional Sterculiaceae, but differs from other members of the tribe in floral morphology, pollen morphology, basic chromosome numbers and embryological development. A new tribe, Pterospermeae, was therefore erected to accommodate *Pterospermum* (Tang, 1992a). Recent molecular phylogenetic studies suggest a close relationship with Dombeyeae and a placement in Dombeyoideae (Bayer *et al.*, 1999; Bayer & Kubitzki, 2003). *Excentrodendron* shares with *Pterospermum* most characteristics of microsporogen-

esis and male gametogenesis, including a secretory tapetum, simultaneous cytokinesis, tetrahedral tetrads, a fibrous endothecium, a two-celled mature pollen plus the basic type of anther wall development, and five- to six-cell-thick anther walls. In both genera, thickening of the endothecium appears at the time of pollen maturity, and the tapetum is uniseriate in most parts but biseriate in some. The cells of the middle layers get crushed at the time of division of the microspore mother cell. The degeneration of microspore mother cells seems to be characteristic of both genera. Among these common characteristics, the basic type of anther wall development is observed in only around ten families of dicotyledons (Davis, 1966). Within Malvales (*sensu*Alverson *et al.*, 1998 and Bayer *et al.*, 1999), it is observed in *Excentrodendron*, *Muntingia* and *Pterospermum* (Rao, 1952a, b). The common type in the dicotyledons, the dicotyledonous type, is observed in other members of Malvaceae *s.l.* Differences between *Excentrodendron* and *Pterospermum* are found in the number of microsporocytes, the type of microspore tetrad, and the time when the endothecium becomes fibrous. The number of microspore mother cells in each loculus formed by the repeated division of the primary sporogenous cells in *Excentrodendron* differs from that in *Pterospermum*. On the transverse section, usually only four to six were seen in *Excentrodendron*, but eight to 14 in *Pterospermum*. The number, however, might be related to the size of the anther. Most microspore tetrads are tetrahedral, and other types are also observed in *Excentrodendron*, but only tetrahedral tetrads were observed in *Pterospermum* (Rao, 1949, 1952a). In *Pterospermum*, thickening of the endothecium appears at the time of formation of pollen tetrads, but in *Excentrodendron* at the time of pollen maturity.

COMPARISON WITH DOMBEYEAE AND *CORCHOROPSIS* OF SUBFAMILY DOMBEYOIDEAE

Corchoropsis was placed in the traditional Tiliaceae, but transferred to near Dombeyeae of the traditional Sterculiaceae (Tang, 1992b), and to Dombeyoideae of Malvaceae *s.l.* (Bayer & Kubitzki, 2003). It does share many characteristics with Dombeyeae. The embryology of a number of genera of the tribe Dombeyeae and *Corchoropsis* was reported (Rao, 1949; Tang, 1992b; Tang & Pan, 1994). *Excentrodendron* shares most plesiomorphic features with members of Dombeyeae and *Corchoropsis*, including a tetrasporangiate anther, simultaneous cytokinesis, the secretory type of tapetum, a fibrous endothecium, and two-celled mature pollen grains. In addition, a biseriate tapetum in some places in *Excentrodendron* is also observed in *Dombeya*. *Excentrodendron* differs from Dombeyeae

and *Corchoropsis* in the type of anther wall development, the thickness of the anther wall and other features. The development of the anther wall is of the dicotyledonous type in Dombeyeae and *Corchoropsis*, but of the basic type in *Excentrodendron*. The anther wall is four cells thick in *Melhania*, *Dombeya* and *Corchoropsis*, but five or six cells thick in *Excentrodendron*. Thickening of the endothecium in *Dombeya* appears at the time of the formation of pollen tetrads, but in *Excentrodendron* at the time of pollen maturity. Unlike *Excentrodendron*, which has five types of microspore tetrad, only tetrahedral tetrads were observed in *Dombeya*, *Melhania* and *Corchoropsis* (Rao, 1949; Tang, 1992b; Tang & Pan, 1994).

COMPARISON WITH OTHER SUBFAMILIES OF MALVACEAE *S.L.*

Among the remaining subfamilies of Malvaceae *s.l.*, the subfamily Malvoideae probably does not have a close relationship with *Excentrodendron* in consideration of the combined characters of bisexual flowers, monadelphous stamens on staminal column, one-celled and bisporangiate anther, amoeboid taptum and echinate pollen in Malvoildeae (Rao, 1954a; Davis, 1966; Johri, Ambegaokar & Srivastava, 1992; Bayer & Kubitzki, 2003). The subfamily Bombacoideae shares with *Excentrodendron* a five- to seven-cell-thick anther wall, degeneration of some or all of the microsporocytes in an anther loculus, a secretory tapetum that is biseriate in some places, in addition to plesiomorphic characters. The differences are mainly the bisporangiate anthers, more pollen mother cells on the transverse section and pollen morphology in the subfamily (Rao, 1954b; Davis, 1966; Juri, Ambegaokar & Srivastava, 1992; Bayer & Kubitzki, 2003). Apart from tetrahedral tetrads, bilateral tetrads were also observed in Bombacoideae (Rao, 1954b).

The unisexual flowers of the subfamily Sterculioideae recall *Excentrodendron*, but the apetalous flowers, the presence of androgynophore and pollen in Sterculioideae are very different from those found in *Excentrodendron*. Based on embryological information available (Rao, 1949), the anther wall in *Sterculia* and *Excentrodendron* is five to six cells thick. The tapetum is uniseriate throughout its length in *Sterculia*, but is biseriate in some places in *Excentrodendron*. The number of microsporocytes is ten to 12 on the transverse section of the anther of *Sterculia* and four to six in *Excentrodendron*. Whereas only tetrahedral and bilateral tetrads were observed in *Sterculia* (Rao, 1949), five types of tetrad were observed in *Excentrodendron*.

In the subfamily Byttnerioideae, the false periplasmodium type of endothecium observed in *Waltheria*, *Guazuma* and *Melochia* (Rao, 1949, 1951), in which

the inner walls of the tapetal cells break down and their cytoplasmic contents flow out forming a mucilaginous mass around the pollen grains but are eventually absorbed, was not observed in *Excentrodendron*. The anther wall is five cells thick in *Melochia*, but four cells thick in *Guazuma* and *Waltheria*. The degeneration of pollen grains rather than microsporocytes is observed in *Guazuma* and *Waltheria*. The exine of pollen grains of *Guazuma*, *Melochia* and *Waltheria* is smooth and different from the coarse reticulate exine of *Excentrodendron*.

Corchorus and *Triumfetta* of the subfamily Grewioideae differ from *Excentrodendron* in the thickness of their anther walls, which are four cells thick (Rao & Rao, 1952; Dnyansagar & Gaoli, 1964–5). Davis (1966: 9) reported the exclusive occurrence of the basic type of anther wall development in the traditionally circumscribed Tiliaceae, but Rao & Rao (1952) and Dnyansagar & Gaoli (1964–5) revealed only the dicotyledonous type in *Corchorus* and *Triumfetta*. The basic type was observed only in *Muntingia*, previously a member of traditional Tiliaceae or Elaeocarpaceae, but segregated recently as an independent family (Bayer *et al.*, 1998). Thus, *Excentrodendron* differs also from these genera in the type of anther wall development.

No embryological information is available for the subfamily Brownlowioideae. The subfamily Tilioideae consists of only *Tilia* and *Craigia*. Although a subfamily was proposed to accommodate *Craigia*, *Excentrodendron* and *Burretiodendron* (Chang & Miau, 1978), *Craigia* differs from *Excentrodendron* in flower, fruit and pollen morphology (Long *et al.*, 1985; Zhuge, 1989; Tang, Xie & Gao, 2005b), DNA sequence (Bayer *et al.*, 1999; Yuan *et al.*, 2002), and wood anatomy (Tang *et al.*, 2005b). Although the embryological information is not available for *Craigia*, the above studies might suggest only a distant relationship of *Craigia* with *Excentrodendron*.

No information on microsporogenesis and microgametogenesis is available at present for the subfamily Helicteroideae. This subfamily, however, differs considerably in floral and pollen morphology from *Excentrodendron*. Its hermaphroditic flowers, tubular calyx, androgynophore and usually smooth pollen grains indicate a distant relationship with *Excentrodendron*.

CONCLUSION

A flowering and fruiting cycle of around 3–4 years is present in *E. hsienmu*. Unlike many other tree species with mast fruiting, large numbers of flower buds develop in both flowering and nonflowering years. In nonflowering years, the development of microspore mother cells is observed in most flower buds and stages beyond microsporocytes are very rare. The anther wall is more massive in the flowering year than in nonflowering years. Most characteristics of microsporogenesis and microgametogenesis are common to many angiosperms. The early development of microspores and megaspores is observed in pistillate and staminate flowers, respectively. Dioecy is surely derived in Malvaceae *s.l.* Further studies on how staminate and pistillate flowers synchronize their flowering and an evaluation of dioecy in Malvales are needed.

Based on comparisons between *Excentrodendron* and the concerned taxa, it is clear that *Excentrodendron* differs considerably from other subfamilies of Malvaceae *s.l.*, but shares most features of microsporogenesis and microgametogenesis with the genus *Pterospermum* of the subfamily Dombeyoideae. *Pterospermum* also shares most features of megasporogenesis with *Burretiodendron kydiifolium* (Tang, 1998). While the recent nrDNA sequence and wood anatomical studies support the segregation of *Excentrodendron* and *Burretiodendron* (Li *et al.*, 2004; Tang, Xie & Gao, 2005b), they are very closely related, forming a natural group, with *Pterospermum* as its sister group. However, *Pterospermum* is considerably different from *Excentrodendron* in flower, fruit and pollen morphology (Tang, 1992a). *Excentrodendron* also differs from traditionally circumscribed Dombeyeae of Dombeyoideae in a number of characteristics. Placement of this genus in Dombeyeae might increase the heterogeneity of this tribe, which is otherwise a quite natural group. Although molecular studies have also revealed a close relationship of *Pterospermum* with Dombeyeae, this genus is isolated in Malvaceae *s.l.* in the combined characteristics of gross morphology, anatomy, pollen morphology, basic chromosome number, and embryology (Tang, 1992a). The results of the present study indicate that it might be appropriate to treat *Excentrodendron* and *Pterospermum* in separate tribes in the subfamily Dombeyoideae of Malvaceae *s.l.* However, a further study with extensive sampling of representatives of the Dombeyoideae would be necessary before any decisive conclusion can be drawn.

This study also raises the interesting question of how macromorphologically different taxa have evolved quite similar pollen and embryological characters, as seen in *Excentrodendron*, *Burretiodendron* and *Pterospermum*, and how macromorphologically similar taxa have evolved quite different pollen morphology, as in *Excentrodendron* and *Burretiodendron*. A further study of these genera, including more representatives of Dombeyeae, *Eriolaena* and *Pterospermum*, is needed.

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