Proc. Indian Acad. Sci. (Plant Sci.), Vol. 93, No. 1, February 1984, pp. 43–51

Reproductive morphology of *Hoppea fastigiata* C B Clarke

K SANKARA RAO*
Department of Biology, The University of Calgary, Calgary, Alberta, Canada T2N 1N4
* Present address: Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

MS received 19 June 1982; revised 4 October 1983

Abstract. The reproductive morphology of *Hoppea fastigiata* has been studied and described. Evolutionary trends in the subtribe Erythraeinae are highlighted.

Keywords. *Hoppea fastigiata*; reproductive morphology; Gentianaceae.

1. Introduction

Although Gentianaceae have been the subject for many morphological and developmental studies, relatively little information is available on the subtribe Erythraeinae members of which are of considerable ecological and morphological interest. Studies so far carried out encompass the halophytic *Enicostemma littorale* (Srinivasan 1941), saprophytic *Obolaria virginica* (Johow 1885; Holm 1897), xerophytic *Cicendia filiformis* (Guérin 1926) and a few mesophytic taxa of mostly grassland vegetation viz., *Erythraea centaurium* (Stolt 1921; Guérin 1926; Créte 1949a, b), *Chlora perfoliata* (Créte 1955), *Hoppea dichotoma* (Arekal 1961; Sankara Rao 1978), *Canscora diffusa, C. decussata* (Maheswari Devi 1962), *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969), *Erythraea roxburghii* (Maheswari Devi and Satyanarayana 1971), *Canscora decurrens* (Maheswari Devi and Lakshminarayana 1977; Sankara Rao 1979). In planning a further study of the reproductive morphology of Erythraeinae, *Hoppea fastigiata* C. B. Clarke, a hygrophytic species endemic to India has been selected (Gamble 1928). Features of evolutionary significance in Erythraeinae have been summarised in the light of available data.

2. Material and methods

Hoppea fastigiata C. B. Clarke, a tiny herb with quadrangular stem, opposite small sessile leaves and pale yellow flowers, was collected from the National Park, Bannerghatta, Karnataka state. Voucher specimens were deposited with the herbarium, Centre for Taxonomic Studies, Bangalore (JCB). Flower buds and fruits in different stages of development were fixed in formalin-acetic -ethanol, dehydrated and embedded from an n-butanol series. Paraffin sections, 7–12 μm thickness, were stained in haematoxylin, and counter-stained in erythrosin. Sections of mature seeds were stained separately with bromophenol blue, periodic acid-Schiff's reagent and sudan black-B to determine the nature of stored food in the endosperm (Jensen 1962).

3. Observations

3.1 *Microsporangium*

Flowers are protandrous with a single fertile stamen. The anther is tetrasporangiate. The development of the microsporangial wall follows the dicotyledonous sequence (figures 1–4). The cells of the sporogenous layer undergo division and enlargement and function as microsporocytes (figures 2–4). Part of the tapetum bordering the inner face of the sporogenous tissue is derived from the ground tissue near the connective and becomes continuous with the tapetum on the outer face (figures 3, 4). Thus, the tapetum is of dual origin. The tapetal cells remain uninucleate and become vacuolate at the time of microsporogenesis (figures 3, 4). They undergo radial elongation and protrude into the anther locule, especially from the connective side (figure 4). Following microsporogenesis, the glandular tapetum degenerates.

In the mature anther, the epidermis is persistent and the endothecium is fibrillar. The middle layer is crushed during the development of anther.

3.2 *Microsporogenesis and male gametophyte*

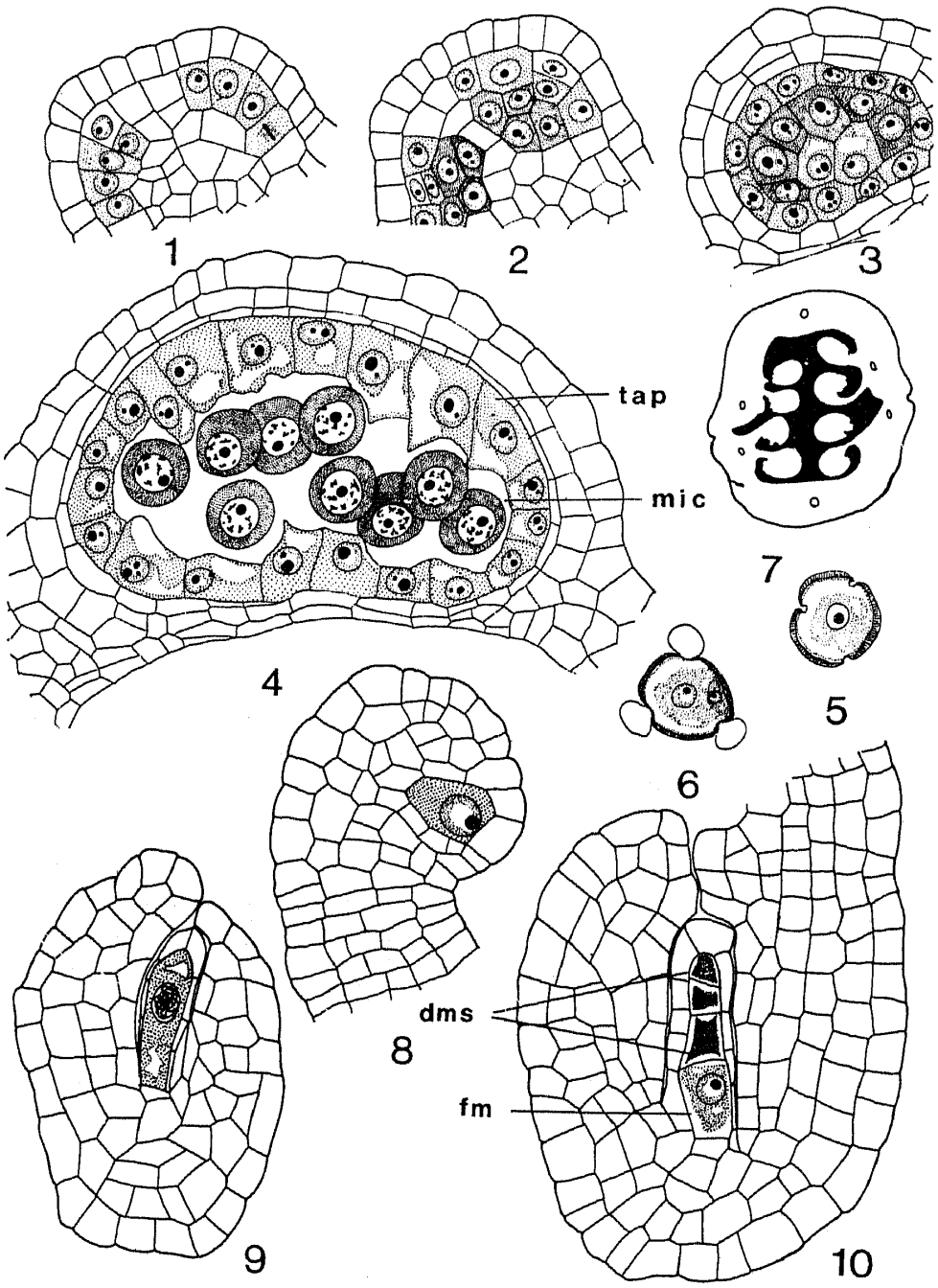
The microsporocytes by meiosis and simultaneous cytokinesis produce tetrahedral microspore tetrads. The microspores separate from the tetrad when they are uninucleate (figure 5). The division of microspore nucleus results in a male gametophyte with a large vegetative cell and smaller lenticular generative cell. At about this time, certain cytoplasmic hyaline capitate processes, one at each germ pore region appear on the pollen grain and fall off before anther dehiscence (figure 6). Pollen grains are isopolar, 3-zonocolporate and oblate-spheroidal ($30 \times 34\,\mu m$). Exine surface is striato-reticulate.

3.3 *Megasporogenesis and female gametophyte*

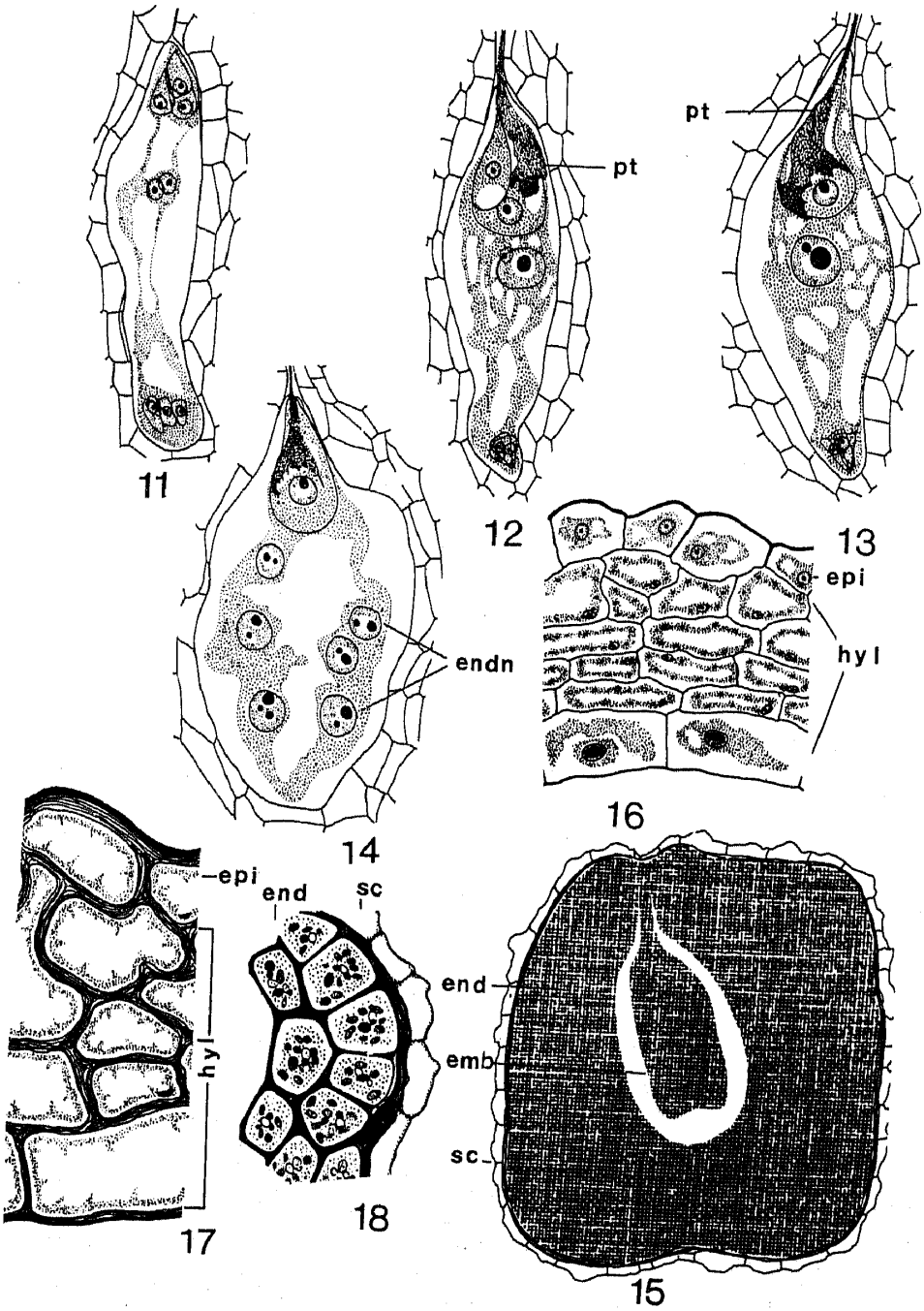
The two placentae are marginal and forked at the base (figure 7). The ovules are numerous, anatropous, tenuinucellar, unitegmic and borne in vertical rows on the placentae (figures 7–10). They develop as small protuberances and start curving at an early stage. Growth of the integument around the nucellus is complete shortly after the megasporocyte is formed in the nucellus (figure 9). In the mature ovule, the integument is five or six cells thick and the micropyle is long and narrow (figure 10).

The hypodermal archesporium functions directly as the megasporocyte (figures 8, 9). Through meiosis, the megasporocyte forms a linear tetrad of megaspores. The functional chalazal megaspore in the tetrad undergoes three successive free-nuclear divisions producing an eight-nucleate female gametophyte of the Polygonum type. The eight nuclei in the gametophyte organise themselves into an egg apparatus at the micropylar end, three antipodals at the chalazal end and two polar nuclei (figure 11). In the mature female gametophyte which is more or less spindle-shaped, the two synergids as well as the large egg are pear-shaped. The polar nuclei fuse at the center and the resulting secondary nucleus moves close to the egg apparatus. The antipodals are organized into cells with their pointed ends directed towards the chalaza. These cells remain persistent till the endosperm becomes two-nucleate (figures 12, 13). During the early phase of female gametophyte formation, the epidermal cells of the nucellus are crushed and absorbed.

The entry of the pollen tube is porogamous and enters the gametophyte through one



Figures 1–10. **1.** TS of young anther showing the archesporial primary parietal and primary sporogenous cells × 600. **2–3.** TS of young anther lobes showing wall layers and sporogenous cells × 600. **4.** TS of anther lobe showing wall layers and microsporocytes × 600. **5.** Microspore × 800. **6.** Two-celled pollen grain × 800. **7.** TS of ovary × 100. **8.** L S young nucellus showing hypodermal archesporial cell × 600. **9.** L S young ovule showing megasporocyte × 600. **10.** L S ovule showing functional megaspore × 600. (*fm*, functional megaspore; *dms*, degenerating megaspores; *mic*, microsporocytes; *tap*, tapetum).



Figures 11–18. **11.** Organized female gametophyte × 600. **12–13.** Stages showing fertilization of female gametophyte × 600. **14.** Zygote and nuclear endosperm × 600. **15.** L S seed (outline) × 120. **16.** Cross-section of ovary wall × 250. **17.** Cross-section of pericarp × 250. **18.** Cross-section of seed coat × 250. (*emb*, embryo; *end*, endosperm; *endn*, endosperm nuclei; *epi*, epidermis; *hyl*, hypodermal layers; *pt*, pollen tube; *sc*, seed coat).

of the synergids and discharges its contents. Double fertilization occurs. The synergids degenerate soon after fertilization (figures 12, 13).

3.4 *Endosperm*

The development of the endosperm is the Nuclear type. The division of the primary

endosperm nucleus precedes that of the zygote. By a series of successive divisions, a large number of free nuclei are formed (figure 14). With increase in number of nuclei in the endosperm, the central vacuole breaks up into several smaller ones. This is followed by an increase in density of the cytoplasm in which the nuclei remain uniformly distributed. Cell walls are laid in the cytoplasm simultaneously at this stage. Only a part of the endosperm tissue is consumed by the developing embryo and therefore, the mature seed is endospermic with protein crystals as reserve food (figures 15, 18).

3.5 *Embryogeny*

The zygote divides transversely to form a terminal cell, *ca* and a basal cell, *cb* (figures 19, 20). The terminal cell *ca* in this two-celled proembryo divides transversely to form two cells, *cc* and *cd* (figure 21). The basal cell also undergoes a similar division forming two superposed cells (figure 22). Thus, the proembryonal tetrad is linear. The cells *cc* and *cd* by one more transverse division each, form four cells that are arranged in a linear row above the basal cells (figures 23, 24). The two daughter cells of the tier, *cc* are designated as *ce* and *cf* and those of *cd* as *m* and *ci*. In each of the three tiers of cells, *ce*, *cf* and *m*, two vertical divisions take place at right angles to one another resulting in the formation of three superposed tiers of four cells each (figures 25–28).

Periclinal divisions take place in the tier *cf* delimiting the dermatogen, *de*, from a group of inner cells which soon divide transversely resulting in two tiers of cells (figures 29–32). Cells in these inner tiers of *cf* divide transversely and periclinally and the derivatives undergo elongation and differentiation into periblem, *pe* and plerome, *pl* of the hypocotyledonary part of the embryo, *phy* (figures 33–37). The cells of the tier, *ce* contribute to the stem tip, *pvt* and cotyledons, *pco* (figures 30–37). Meanwhile, the derivatives of the cell *m* contribute to all parts of the root, *iec* and *co* (figures 32–37). Cells derived from *ci* and *cb* form a suspensor, *s* (figures 24–37).

Thus, in *Hoppea fastigiata*, cell *ca* of the two-celled proembryo contributes to the development of the entire dicotyledonary embryo and part of the suspensor while the basal cell *cb* contributes to the formation of only a part of the suspensor. Further, in the destination of the cells of the proembryonal tetrad, the embryogeny corresponds to the Physalis II variation of Solanad type (Johansen 1950).

3.6 *Pericarp and seed coat*

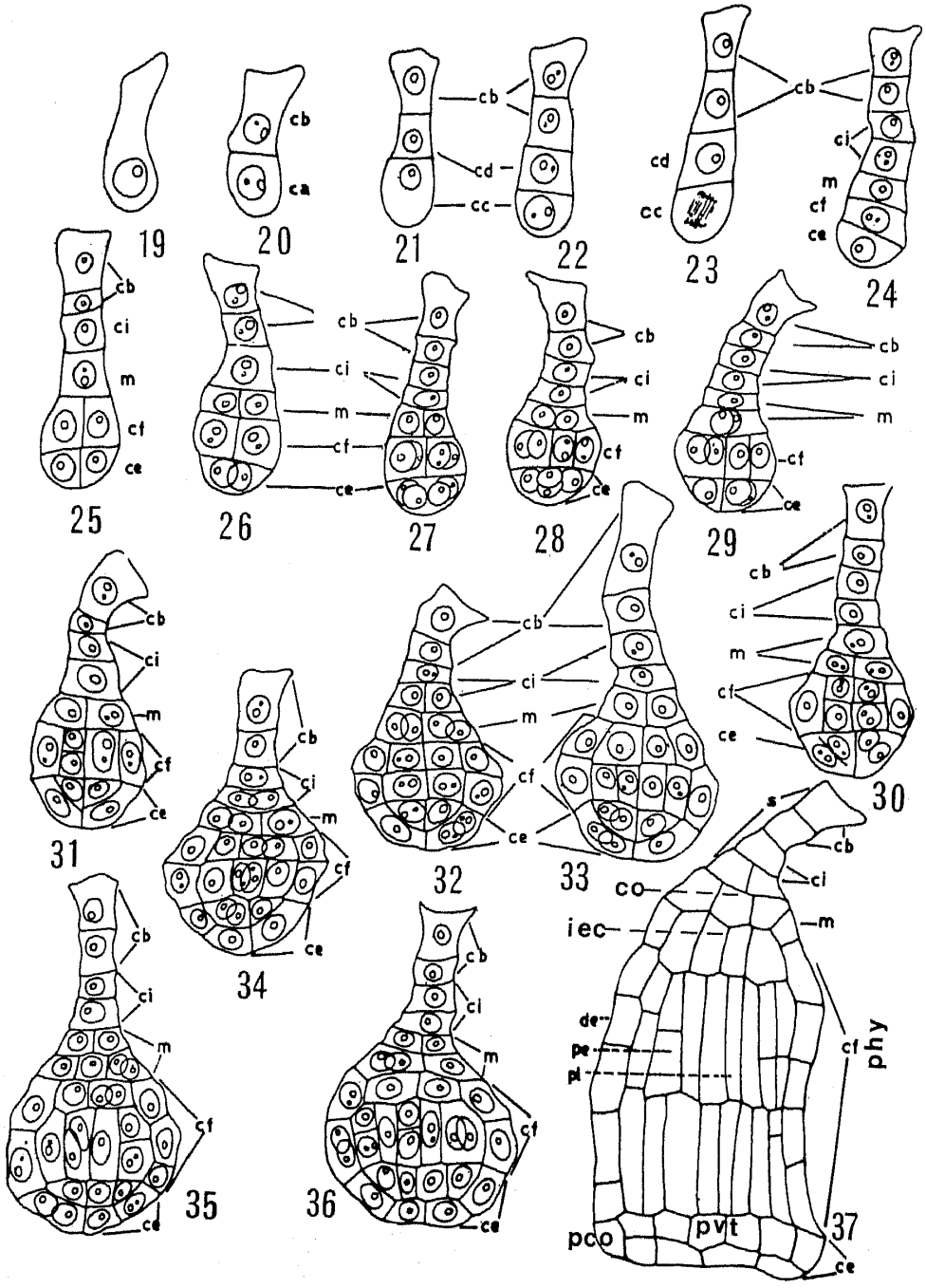
The ovary wall is five to seven cells thick. The outer tangential walls of the ovary epidermis are cutinized. Cytoplasm in all the cells of the ovary wall is highly vacuolate (figure 16). The epidermis of the pericarp is also thickly cutinized and the innermost layer of cells shows tangential elongation. Cells of the hypodermal layers become thick-walled (figure 17).

The epidermis of the integument becomes the seed-coat, the cells of which are thin-walled. The outer tangential walls of the outermost endosperm cells develop lamellar thickenings. This lamellated layer lies directly below the seed coat (figures 15, 18).

4. Discussion

Erythraeinae show considerable variation in their tapetal ontogeny and morphology.

In a relatively simple type of development as recorded in *Enicostemma littorale*



Figures 19–37. **19.** Zygote. **20.** Two-celled proembryo. **21.** Three-celled proembryo. **22.** Proembryonal tetrad. **23–24.** Five to seven-celled proembryos. **25–30.** Proembryo showing the formation of tiers *ce*, *cf*, *m*, *ci*. **31–36.** Advanced stages of proembryos showing the demarcation of histogens. **37.** Heart-shaped embryo (all × 600). (*ca*, apical cell; *cb*, basal cell; *cc*, upper daughter cell of *ca*; *cd*, lower daughter cell of *ca*; *ce*, upper daughter cell of *cc*; *cf*, lower daughter cell of *cc*; *ci*, daughter cell of *cd*; *de*, dermatogen; *m*, daughter cell of *cd*; *pe*, periblem; *pl*, plerome; *co*, root cap; *iec*, initials of root cortex; *pco*, cotyledons; *phy*, hypocotyl; *pvt*, stem tip; *s*, suspensor).

(Srinivasan 1941), *Canscora diffusa*, *C. decussata* (Maheswari Devi 1962), *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) and *Erythraea roxburghii* (Maheswari Devi and Satyanarayana 1971), the tapetum is derived entirely from the inner parietal layer of the microsporangium and forms a homogenous layer of cells at the periphery of the locule.

Tapetal ontogeny of the type described for *Alectra thomsoni* (Vijayaraghavan and Ratnaparkhi 1973) has been observed in *Hoppea dichotoma*, *Canscora decurrens* (Sankara Rao 1978; Sankara Rao and Chinnappa 1983b) and *Hoppea fastigiata*. The tapetum is of dual origin and develops partly from the inner parietal layer and partly from the elements of the connective tissue. The tapetal cells in these species, unlike in *Alectra thomsoni*, remain uninucleate and do not accumulate starch.

Of special interest is the tapetal development described in *Canscora pauciflora* (Sankara Rao and Chinnappa 1983b). Strips of sterile tissue of sporogenous origin and a peripheral tapetal layer of parietal origin, together constitute the tapetal complex in this species. Steffen and Landmann (1958) referred to a similar tapetal complex in *Gentiana cruciata* as Balken or trabeculate tapetum.

The tapetum is glandular in *Chlora perfoliata*, *Erythraea centaurium*, *Cicendia filiformis* (Guérin 1925), *Enicostemma littorale* (Srinivasan 1941), *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969), *Erythraea roxburghii* (Maheswari Devi and Satyanarayana 1971), *Hoppea dichotoma* (Sankara Rao 1978) and *H. fastigiata* in the present study while in *Canscora diffusa*, *C. decussata* (Maheswari Devi 1962) and *C. decurrens* (Sankara Rao 1979), it is plasmodial.

Pollen grains are in monads. They are tri- or tetrazonocolporate. Considerable intraspecific variation of pollen occurs in *Centaurium erythraea*. A small percentage of grains in *C. erythraea* ssp. *grandiflorum* are syncolpate. Further, in three of the *Centaurium* species viz., *C. pulchellum*, *C. exaltatum* and *C. littorale*, pericolporate grains are formed in addition to tricolporate pollen (Sankara Rao and Chinnappa 1983a). It should also be noted that the pollen grains of *Canscora*, *Hoppea*, *Sabbatia*, *Enicostemma* and *Centaurium* (Sankara Rao 1978, 1979; Sankara Rao and Chinnappa 1983b) develop from their germ pores certain cytoplasmic hyaline capitate protrusions. These protrusions, however, are ephemeral and detached from the grains before anthesis.

In the entire group, the ovules are unitegmic. However, the absence of an integument and consequent absence of a micropyle has been reported in the saprophytic *Obolaria virginica* (Johow 1885; Holm 1897). According to Oehler (1927), the apparent ategmic condition of the ovule in this species is due to the fusion of the scanty nucellus with the integument.

The development of the female gametophyte conforms to the Polygonum type as in the other Erythraeinae. The antipodals in the group show variation in their behaviour. They are generally weakly-developed uninucleate cells which degenerate at the time of fertilization and belong to the group Ia of the scheme of antipodal classification of Stolt (1927). In *Hoppea dichotoma* (Arekal 1961), *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) and *H. fastigiata*, on the other hand, the antipodal cells which are normally-developed and uninucleate, remain persistent upto 2-nucleate stage of endosperm. They conform to group II of the antipodal classification.

Though the nuclear type of endosperm development is a feature common to all Erythraeinae, species show variation in the mode of wall formation that eventually

follows. It is centripetal in *Canscora diffusa*, *C. decussata* (Maheswari Devi 1962) and *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) whereas in *Hoppea dichotoma* (Arekal 1961), *Canscora decurrens*, *C. pauciflora* (Sankara Rao 1979) and *H. fastigiata* it is simultaneous.

Protein granules observed as food reserves in the endosperm cells of *Hoppea fastigiata* have also been recorded in *Erythraea roxburghii* (Maheswari Devi and Satyanarayana 1971), *Canscora decurrens*, *C. pauciflora* (Sankara Rao 1979). In *Canscora diffusa* and *C. decurrens*, however, starch grains in addition to protein granules appear in the endosperm cells (Maheswari Devi 1962).

Judging from the data available, it can be seen that the reproductive morphology of the group is essentially gentianaceous with bisexual hypogynous protandrous flowers, tetrasporangiate anthers and their dicotyledonous wall development, glandular or plasmodial tapetum, simultaneous cytokinesis in the microsporocytes, tricolporate pollen, anatropous unitegmic tenuinucellar ovules lacking integumentary tapetum, Polygonum type of female gamatophyte development, nuclear endosperm, Solanad type of embryogeny and endospermic seeds. They are specialized in so far as they show: (a) a combination of herbaceous habit and saprophytic nutrition as in *Bartonia* and *Obolaria*: (b) halophytic and xerophytic adaptations in *Enicostemma littorale* and *Cicendia filiformis*; (c) aggregation of small flowers into dense inflorescences as in *Faroa* and *Enicostemma*; (d) zygomorphy in the flowers of *Canscora*, *Hoppea* and *Schinziella*; (e) gradual reduction in the male complement of flowers to varying degrees; (f) syngenesious condition of anthers as in *Tapeinostemon*; (g) the three-traced condition of open carpels with parietal placentae (Gopala Krishna and Puri 1962); (h) ategmic ovules as in *Obolaria*; (i) precocious degeneration or delayed division of the upper dyad cell during megasporogenesis in *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) and *Canscora diffusa* (Maheswari Devi 1962); (j) persistent antipodal cells in *Hoppea* (Sankara Rao 1978 and present study) and *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) and (k) polyembryony in *Erythraea centaurium* (Crété 1949a). These specializations are trends of evolutionary significance in the Erythraeinae.

Acknowledgement

The author is thankful to Dr M Nagaraj and Dr C C Chinnappa for encouragement.

References

Arekal G D 1961 Contribution to the Embryology of *Hoppea dichotoma* Willd. (Gentianaceae); *Can. J. Bot.* **39** 1001–1006

Crété P 1949a Polyembryony in *Erythraea centaurium* Pers.; *Bull. Soc. Bot. Fr.* **96** 113–115

Crété P 1949b Embryogénie des Gentianacées. Dévelopement de l'embryon chez l'*Erythraea centaurium* Pers.; *C.R. Hebd. Seances Acad. Sci.* **228** 1448–1449

Crété P 1955 Embryogénie des Gentianacées. Développement de l'embryon chez le *Chlora perfoliata* L.; *C. R. Hebd. Seances Acad. Sci.* **241** 1825–1828

Gamble J S 1928 *Flora of the Presidency of Madras* (2-Repro. Bot. Survey of India: Calcutta)

Gopala Krishna G and Puri V 1962 Morphology of the flower of some Gentianaceae with special reference to Placentation; *Bot. Gaz.* **124** 42–57

Guérin P 1925 L'anthere des Gentianacées. Développement du sac polliniques; *C. R. Hebd. Seances Acad. Sci.* **180** 852–854

Guérin P 1926 Le développement de l'anthere chez les Gentianacées; *Bull. Soc. Bot. Fr.* **73** 5–18
Holm T 1897 *Obolaria virginica*: A morphological and anatomical study; *Ann. Bot.* **11** 369–383
Jensen W A 1962 *Botanical Histochemistry* (San Francisco: Freeman)
Johansen D A 1950 *Plant Embryology* (Massachusetts: Waltham)
Johow F 1885 Die chlorophyllfrein Humusbewohner Westindiens biologisch-morphologisch dargestellt; *Jahrb. f. wiss. Bot.* **16** 415–449
Maheswari Devi H 1962 Embryological studies in Gentianaceae (Gentianoideae and Menyanthoideae); *Proc. Indian Acad. Sci. (Plant. Sci.)* **B60** 52–65
Maheswari Devi H and Satyanarayana P 1971 A contribution to the embryology of *Erythraea roxburghii* G. Don.; *Plant Sci.* **3** 61–67
Maheswari Devi H and Lakshminarayana 1977 Embryological studies in Gentianaceae; *J. Indian Bot. Soc.* **56** 182–188
Oehler E 1927 Entwicklungsgeschichtlich-zytologische Untersuchungen an einigen saprophytischen Gentianaceen; *Planta* **3** 641–733
Sankara Rao K 1978 Gametophytes in *Hoppea dichotoma* Willd.; *Curr. Sci.* **47** 786–788
Sankara Rao K 1979 *Embryological studies in Gentianaceae*; Ph.D. Thesis, Bangalore University
Sankara Rao K and Chinnappa C C 1983a Pericolporate pollen in Gentianaceae; *Can. J. Bot.* **61** 174–178
Sankara Rao K and Chinnappa C C 1983b Studies in Gentianaceae. Microsporangium and pollen; *Can. J. Bot.* **61** 324–336
Srinivasan A R 1941 Cytomorphological features of *Limnanthemum cristatum* Griseb. and *Enicostemma littorale Blume; Proc. Indian Acad. Sci. (Plant. Sci.)* **B14** 529–542
Steffen K and Landmann W 1958 Entwicklungsgeschichtliche und cytologische Untersuchungen am Balkentapetum von *Gentiana cruciata* und *Impatiens glandulifera*; *Planta* **50** 423–460
Stolt K A H 1921 Zur Embryologie der Gentianaceen und Menyanthaceen; *K. Sven. Vetenskapsacad Handl.* **61** 1–56
Stolt K A H 1927 Uber die Embryologie von *Gentiana prostrata* und die Antipodalen der Gentianaceen; *Bot. Not.* **80** 225–242
Vijayaraghavan M R and Usha Padmanaban 1969 Morphology and Embryology of *Centaurium ramosissimum* Druce and affinities of the family Gentianaceae; *Beitr. Biol. Pflanzen.* **46** 15–37
Vijayaraghavan M R and Ratnaparkhi S 1973 Dual origin and Dimorphism of the Anther Tapetum in *Alectra thomsoni*; *Ann. Bot.* **37** 355–359