7 The Systematic Position of *Ehretia* Fossils (Ehretiaceae, Boraginales) from the European Tertiary and Implications for Character Evolution [6]

Abstract

Ehretia fruits contain four seeds arranged in an endocarp, which is either four-parted (*Ehretia* I), two-parted (*Ehretia* II), or undivided (*Ehretia microphylla*). The single parts are called endocarpids, which are one-loculed, two-loculed, or four-loculed, respectively. On the basis of its two-loculed endocarp, the fossil species † *Ehretia clausentia* from the European Eocene is recognised as a representative of the *Ehretia* II clade as are also most other fossils of *Ehretia*. The fossil † *Ehretia hedericarpa* from the European Oligocene and Miocene is the only known fossil of *Ehretia* with one locule per endocarpid and was therefore placed in the *Ehretia* I clade. Morphological and anatomical investigations of the fossils demonstrate some common derived characters of the latter with the *Ehretia* II clade (e.g., diminution of the placenta). In a phylogenetic tree † *Ehretia hedericarpa* is the sister species of the *Ehretia* II clade. The occurrence of † *Ehretia hedericarpa* in Europe agrees with the assumption that it is closely allied to the Laurasian *Ehretia* II clade since the representatives of *Ehretia* I are not known from the former Laurasian Continent.

7.1 Introduction

Ehretia includes some 50 extant species of trees or shrubs of pantropical distribution. Centres of diversity are in Africa and Asia respectively; only three species are native to tropical America (MILLER 1989). Based on molecular data (GOTTSCHLING *et al*. 2001), Ehretiaceae (including parasitic Lennoaceae) are probably closely allied to Cordiaceae. Both, Ehretiaceae and Cordiaceae, are not closely related to Boraginaceae *s.str.* (= Boraginoideae *sensu* GÜRKE 1893), but together with Heliotropiaceae they are recognised as the sister group of Hydrophyllaceae *s.str.* by molecular data.

Although the intraspecific variability of growth form and vegetative traits is high (e.g., MILLER 1989), most species of *Ehretia* have the following characters in common: leaves alternate; inflorescences paniculate; flowers tetracyclic, pentamerous; gynoecium bicarpellate, with four ovules; fruit drupaceous. At maturity, each seed is protected by a sclerenchymatic endocarpic layer and is localised in its own locule. The endocarp is divided into four or two parts (Fig. **7-1**) or undivided (Fig. **7-2**). The single parts are called endocarpids.

The number of endocarpids is an important and consistent character in Ehretiaceae, and three groups can be distinguished in *Ehretia* based on this character (GÜRKE 1893): 1. Species with four one-loculed endocarpids (*Ehretia* I) are distributed in sub-Saharan Africa, tropical Asia, and Australia (Gondwanan); 2. species with two two-loculed endocarpids (*Ehretia* II) are found in East Asia, Australia, and the tropical Americas (originally Laurasian); 3. *E. microphylla* (HILGER & GOTTSCHLING in press) with an undivided endocarp occurs in India and Indonesia. In terms of phylogenetic systematics, 'four endocarpids' is the ancestral condition, while 'two endocarpids' and 'undivided endocarp' are the derived conditions. For a full discussion on character evolution in *Ehretia*, based on molecular data, see GOTTSCHLING & HILGER (2001).

Fossil reproductive units interpreted as endocarpids of *Ehretia* have been found in Europe (Table **6**, appendix). Most of them have two-loculed endocarpids (including † *E. clausentia*) and are placed in the *Ehretia* II clade. † *E. hedericarpa* is the only species with only one locule per endocarpid and has therefore been considered as closely allied to extant species of *Ehretia* I such as *E. laevis* (MAI 1991). In this study, the systematic position of † *E. hedericarpa* is re-evaluated including additional anatomical details.

7.2   Materials and Methods

Three endocarpids out of a total of thirty fossils of † *E. clausentia* found in Nursling (Hampshire, England, UK), CURRY & CHANDLER V.34572 (BM), and two of twenty fossils of † *E. hedericarpa* found in Laussig (Bitterfeld, Saxony, Germany), MAI 2641 (BHUPM), were sectioned.

The fossils were placed into 40% (v/v) hydrofluoric acid for 10 d in order to remove silicates. After that, they were soaked for 7 d in 70% (v/v) ethanol, with phenol crystals, in order to soften them. Finally, they were stored in 70% (v/v) ethanol. They were dried and photographed (CTprecisa, Agfa) with a photo macroscope (Leitz).

A low-viscosity medium based on ERL-4206 (SPURR 1969) was used for embedding. Following infiltration the castings were polymerised at 70°C for 48 h. 10 μm sections were cut with an ultramicrotome Ultracut-E (Reichert-Jung) with glass knives, and photographed (CTprecisa, Agfa) with an Ortholux II and Orthomat (Leitz).

7.3   Results

The fossils of † *E. clausentia* (Fig. **7-3**) are ovate in outline and slightly curved. They are 2 to 2.5 mm high and about 1.5 mm wide. There is a conspicuous median groove on the convex abaxial

surface flanked, on each side, by a network of ridges and hollows ('rugose-sulcate ornamentation'). On the slightly concave adaxial surface two conspicuous submarginal furrows are developed (Fig. **7-3**, arrows).

Serial transverse sections of the endocarpids (Fig. **7-4**) show two locules per endocarpid. Above the middle of each endocarpid (position of the arrows in Fig. **7-3**) the two funicular canals can be determined leading to the adaxial surface (red arrows in Fig. **7-4**). Above and beneath this level the locules are completely closed. The longitudinal ridges on the abaxial surface are irregular in proportions and position.

In Figure **7-5** the funicular canal and the adaxial surface are shown in detail. In the median region of the adaxial surface two types of tissues can be distinguished: an opaque tissue, whose cells cannot be distinguished, and a more transparent tissue (green arrow), whose cells have thin cell walls.

The fossils of † *E. hedericarpa* (Fig. **7-6**) are elliptic or obovate and slightly curved. They are 2 to 2.5 mm high and about 1.5 mm wide. The convex abaxial surface bears some parallel ridges ('rugose-sulcate ornamentation'). On the slightly concave adaxial surface, a submarginal furrow is more or less distinctly developed (Fig. **7-6**, arrows): In some cases it is found on both sides, while in other cases it is visible only on one side.

Serial transverse sections (Fig. **7-7**) demonstrate that the fossils contain only a single locule throughout each endocarpid. The collapsed former seed chamber is surrounded by tissue of two types: an inner darker layer (anatomically ventral) and an outer more transparent layer (anatomically dorsal). The submarginal furrow on the adaxial surface is formed by the darker layer (arrow). The longitudinal ridges on the abaxial surface are irregular in proportions and position. Sometimes, they are twice as high as the remaining coat.

The ridge tissue consists of irregularly arranged cells with thin walls (Fig. **7-8**). The darker layer consists of at least one row of cells with thick walls. These cells cover the inside of the seed chamber.

7.4 Discussion

The anatomical features observed confirm the interpretation that the fossils of † *E. clausentia* and † *E. hedericarpa* are indeed endocarpids of extinct *Ehretia* species. Shape and ornamentation do not differ substantially from extant species. The convex part of the fossils corresponds with the abaxial surface of an extant endocarpid (Figs. **7-3**, **7-9**). Furthermore, in all species of *Ehretia* the endocarpic tissue is composed of two layers (Fig. **7-11**): a thin ventral layer of cells with thick walls and a thicker tissue of larger cells, 10 to 15 cells thick (JOHRI & VASIL 1956, KHALEEL 1977) with thinner walls. That arrangement is also found in † *E. hedericarpa* (Fig. **7-8**) and less clearly in † *E. clausentia*. Thus the chambers can be identified as the locules, the soft seeds were

not preserved. While in † *E. clausentia* the position of the funicle can be determined unequivocally (Fig. **7-4**, red arrows), its position remains unclear in † *E. hedericarpa.*

In *Ehretia* three fruit types are found, two of which are relevant in this context. The first type (*Ehretia* I: Figs. **7-1** left, **7-12**) is represented by fruits with four endocarpids including one seed each and an extensive placenta (PITOT 1939a, b), which is surrounded by an outer wing of the endocarpid. The embryo is curved. The second type (*Ehretia* II, Figs. **7-1** right, **7-13**) is represented by fruits with two endocarpids that arise from four mericarpids during ontogeny ('syn-mericarpy'). Morphologically, the fusion takes place beyond the carpel borders, and the placenta is only weakly developed. The embryo is straight and elongated. In the third type (*E. microphylla*, Fig. **7-2**) only one endocarpid is developed by concrescence of two syn-mericarpids, which is irrelevant for the problems discussed here.

† *E. clausentia* is a representative of *Ehretia* II as has been proposed by CHANDLER (1961), who identified extant *E. acuminata* (belonging to *Ehretia* II) as its closest relative. Apart from several superficial similarities such as size, shape, and the 'rugose-sulcate ornamentation' some apomorphies of the *Ehretia* II clade can be found: 'syn-mericarpy', shift of the funicle to an apical position, stretched embryo (GOTTSCHLING & HILGER 2001). Because the fossils are extraordinarily well-preserved, even the remains of the diminutive placenta can be found (Fig. **7-4** and **7-5**, green arrows).

The results confirm † *E. hedericarpa* belonging to *Ehretia*, but the precise systematic position remains doubtful since the fossils are different from the three types of endocarp found in extant *Ehretia*. MAI (1991) suggested † *E. hedericarpa* as a representative of *Ehretia* I (like extant *E. laevis*) because its endocarpids have one locule each. Since 'four endocarpids' represented by the first fruit type (Fig. **7-1** left) is the ancestral condition in Ehretiaceae, a phylogenetic relationship cannot be inferred from this plesiomorphy. Two reasons contradict a systematic position within *Ehretia* I: Firstly, the seed chamber is slightly but not strongly curved (the latter is a diagnostic feature of *Ehretia* I). Secondly, there is no outer wing of the endocarpid, which could enclose the placenta: It must be assumed that † *E. hedericarpa* had a poorly developed placenta. A well-developed placenta is ancestral within *Ehretia* as demonstrated by outgroup comparison: It is found both in closely related *Bourreria* (PITOT 1939b, VERDCOURT 1991) and in more distantly related *Heliotropium* (HILGER 1992). † *E. hedericarpa* appears to be closely allied to *Ehretia* II since a weakly developed placenta as well as the straight embryo are the derived conditions in *Ehretia*.

A comparison with young endocarpids of *E. acuminata* representing *Ehretia* II (Fig. **7-9** and **7-10**) corroborates that view: The only morphological difference between its endocarpids and the fossilised ones of † *E. hedericarpa* is a concrescence of two mericarpids into one endocarpid with two locules in the former. PITOT (1939b) observed a four-parted endocarp in a clearly immature fruit of *E. tinifolia*, also representing *Ehretia* II (Fig. **7-14**). The transverse section of a

one-loculed endocarpid of this fruit resembles that of † *E. hedericarpa* (which collapsed during fossilisation). The parenchymatic tissue that fills the transversal cleft between two one-loculed endocarpids is sclerified during ontogeny in all representatives of *Ehretia* II. Concluding, sclerification of endocarpids in *Ehretia* II takes place in two phases: firstly a moderate sclerification around the embryo only (in that stage the endocarpic tissue is still quiet smooth), secondly, a concrescence of two one-loculed endocarpids ('syn-mericarpy') leading to a very hard endocarp of two two-loculed endocarpids. With reference to † *E. hedericarpa*, either all endocarpids found were ontogenetically immature (i.e., the sclerification of the second phase had not yet taken place), or mature endocarpids of † *E. hedericarpa* really lacked this sclerification, and the fossils thus represent the sister taxon of *Ehretia* II (including *E. microphylla*). The first hypothesis must be refused, because it is very unlikely that the fairly sclerified cell walls of the first sclerification phase can fossilise, i.e., the fruits must have been mature. If the latter hypothesis is correct, the placental tissue is already reduced (derived condition), but the formation of syn-mericarpids has not yet taken place (ancestral condition). On the basis of these palaeontological data, an evolutionary sequence can be suggested for the fruit characters of *Ehretia* (Fig. **7-15**).

In addition, the position of the funicle should be considered for the systematic position of † *E. hedericarpa*. In *Ehretia* II the small funicle is in apical position (arrows in Fig. **7-10**, derived condition), while in *Ehretia* I the larger funicle is found in the median region (ancestral condition). Unfortunately, the position of the funicle in † *E. hedericarpa* could not be determined unequivocally. Maybe, it was located near the submarginal furrow; in this case, † *E. hedericarpa* would show the ancestral state. This suggests the following sequence of apomorphies: first 'diminution of placenta', later 'syn-mericarpy' and 'apical position of funicle' (Fig. **7-15**).

Most representatives of Ehretiaceae are distributed on the remnants of the former Gondwana continent (exceptions are found in, e.g., *Tiquilia* in the Americas). By outgroup comparison (*Bourreria*, *Halgania*) it is most parsimonious to assume that the stem-species of *Ehretia* lived on the Gondwana continent. In *Ehretia*, only extant representatives of *Ehretia* II are distributed on the former Laurasian Continent. Due to the monophyly of *Ehretia* II, it is plausible to assume that a colonisation of Laurasia took place only once (GOTTSCHLING & HILGER 2001). The disjunction of *Ehretia* II between the Old and the New World may result from the separation of North America and Eurasia (SCOTESE *et al*. 1988). *Ehretia* II subsequently went into extinction in Europe due to the recent climatic history (MAI 1995), but is known from the fossil record (CHANDLER 1961, 1962, 1964, MAI 1991, REID 1923, REID & CHANDLER 1933). The difficulties arising from the assumed occurrence of an representative of the *Ehretia* I clade (MAI 1991, 1995) far from the current stenotropic distribution can be removed by the evidence that † *E. hedericarpa* is really the extinct sister species of the Laurasian clade *Ehretia* II (including *E. microphylla*).