Phylogenetic implication of seed coat sculpturing in subtribe Agrimoniinae (Rosaceae)

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ABSTRACT: Seed morphology in the subtribe Agrimoniinae (Rosaceae) was examined using scanning electron microscopy to identify distinct characters and evaluate their evolution in a phylogenetic framework for five genera in the subtribe: Agrimonia L., Aremonia Neck. ex Nestl., Hagenia J.F. Gmel., Leucosidea Eckl. & Zeyh., and Spenceria Trim. All genera have one or two mature achenes in a fruiting hypanthium. In the seed coats, the cell shape, size, wall features, and sculpturing vary across genera. Of most significance is the presence of papillae structures in both Agrimonia and Aremonia. Through the mapping of papillae features onto phylogenetic trees, either one or two changes in seed coats are hypothesized. The phylogenetic tree inferred from four nuclear and six chloroplast regions of sequence data suggests that at least two steps of papillae sculpturing on seed coats are required. On the other hand, in the phylogenetic tree of a low-copy nuclear gene, one independent evolutionary step is postulated to explain the current character states. In the latter hypothesis, the seed coat sculpturing also supports a monophyletic relationship for cosmopolitan Agrimonia and European endemic Aremonia. The seed coat sculpturing provides valuable information for inferring phylogenetic relationships at the generic level in the subtribe Agrimoniinae.

Keywords: Agrimonia, Agrimoniinae, Aremonia, Hagenia, Leucosidea, seed coat, Spenceria

적 요: 장미과 Agrimoniinae(짚신나물아족)의 5속 (Agrimonia L., Aremonia Neck. ex Nestl., Hagenia J.F. Gmel., Leucosidea Eckl. & Zeyh., and Spenceria Trim.)의 종피를 주사전자현미경으로 관찰하여 계통분류학적으로 유용한 형질이 있는지 조사하였다. 또한, 관찰된 종피의 형질들을 이미 수행된 분자계통학적 연구에서 제시된 5속의 계통분류학적 관계를 설명하는 기반에 적용하여 종피 형질의 계통분류학적 진화를 고찰하였다. 짚신나물아족의 5속 모두 하나의 열매화통에 하나 또는 두 개의 성숙한 수과를 가지고 있었고, 종피는 표피세포의 모양, 크기, 세포벽의 돌출 정도, 세포포면의 돌기의 유무 등에서 다양한 형질상태가 관찰되었다. 특히, 세포포면의 유두상 돌기(papillae)는 2속 Agrimonia(짚신나물속)과 Aremonia에서만 관찰되었다. 유두상 돌기가 없는 것이 원시형질이라는 가정하에 유두상 돌기가 나타나는 형질변화를 이미 수행된 분자계

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Subtribe Agrimoniinae (Rosaceae) comprises four narrowly endemic and monotypic genera (*Agrimonia* Neck. ex Nestl., *Hagenia* J.F. Gmel., *Leucosidea* Eckl. & Zeyh., *Spenceria* Trimen) and one widely distributed, speciose genus (*Agrimonia* L.) (Kalkman, 2004; Potter et al., 2007). Most species in the subtribe occur in temperate regions and exhibit dynamic and variable features despite its small size (ca. 25 species) (Kalkman, 2004; Chung et al., 2010). The plants vary in growth form (herbaceous, woody), inflorescence type (racemes, panicles), floral structure (bisexual, unisexual), and pollen form (herbaceous, woody), inflorescence type (racemes, panicles), and pollen and fruit types: Rosoideae (x = 7 (8, 9); achenes, drupelets), Amygdaloideae (x = 8; drupes), Spiraeoideae (x = 9 (17); follicles, capsules), and Maloideae (x = 17; pomes). In addition, variable pollen sculpturing patterns provided useful information for infra-sectional classification. Hebdas and Chinnappa (1994) proposed reclassification in the tribes Dryadeae, Potentilleae, and Sanguisorbeae based on striate, microperforate pollen grains, while Chung et al. (2010) and Lee et al. (2011) postulated inter/infra-generic relationships inferred by pollen sculpturing features in the tribe Sanguisorbeae.

In flowering plants, seed morphology has provided useful features to delineate infra-generic classification (Wyatt 1984; Buss et al., 2001; An and Hong, 2003; Hassan et al., 2005; Minuto et al., 2006; Muñoz-Centeno et al., 2006; Jacobs et al., 2008; Jin and Park, 2008; Kong et al., 2011), and the great variability of seed surface characters has the potential to explain phylogenetic relationships (Juan et al., 2000; Muñoz-Centeno et al., 2006). Buss et al. (2001) revealed close phylogenetic relationships between *Centropogon* and *Siphocampylus*; *Legenera* and *Downingia*; and *Hippobroma* and *Lobelia* sect. *Tylonium* based on their seed morphologies. The relationships were further supported by the geographic distribution of each group. In the rose family, although diverse fruit types have received much attention from botanists (Kalkman, 1988; Morgan et al., 1994), only a few studies on seed morphology have been conducted. For example, Latif (2004) found that analyses of seed coat patterns using scanning electron microscopy (SEM) are useful to distinguish species within the taxonomically ambiguous genus *Sanguisorba*.

In order to characterize the seed morphology of the genera in Agrimoniinae and utilize it for classification and systematics, we investigated all five genera in the subtribe using SEM. Seed morphological characters were mapped onto the phylogenetic

<table>
<thead>
<tr>
<th>Genus (No. of species)</th>
<th>Habit</th>
<th>Inflorescence</th>
<th>Flower sexuality</th>
<th>Ploidy level</th>
<th>Pollen Exine pattern</th>
<th>Geographic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agrimonia</em> (19)</td>
<td>Herb</td>
<td>Raceme</td>
<td>Bisexual</td>
<td>4x, 6x, 8x</td>
<td>Striate</td>
<td>Temperate N. &amp; S. America, Eurasia</td>
</tr>
<tr>
<td><em>Aremonia</em> (1)</td>
<td>Herb</td>
<td>Raceme</td>
<td>Bisexual</td>
<td>6x</td>
<td>Striate</td>
<td>Central Europe</td>
</tr>
<tr>
<td><em>Hagenia</em> (1)</td>
<td>Tree</td>
<td>Panicle</td>
<td>Unisexual/Dioecious</td>
<td>6x</td>
<td>Microverrucate</td>
<td>Central Africa</td>
</tr>
<tr>
<td><em>Leucosidea</em> (1)</td>
<td>Shrub</td>
<td>Raceme</td>
<td>Bisexual</td>
<td>2x</td>
<td>Striate</td>
<td>Southern Africa</td>
</tr>
<tr>
<td><em>Spenceria</em> (1)</td>
<td>Herb</td>
<td>Raceme</td>
<td>Bisexual</td>
<td>2x</td>
<td>Striate</td>
<td>Central Asia</td>
</tr>
</tbody>
</table>
trees constructed in previous studies (Potter et al., 2007; Chung, 2008). Evolution of seed characters in a phylogenetic framework will contribute to an understanding of character evolution in the subtribe.

**Materials and Methods**

Fifteen species from five genera in Agrimoniinae were sampled: Agrimonia L. (11 species) Hagenia abyssinica J.F. Gmel. (1 species), Leucosidea sericea Eckl. & Zeyh. (1 species), and Spenceria ramalana Trimen (1 species) (Table 2). Achenes were removed from hypanthia and pericarps were peeled off from the achenes under stereomicroscopy. Dried seeds are then mounted on aluminum stubs using double-sided conductive tape. The seeds were then coated with 8 nm of gold/palladium and examined in a Hitachi 2460N SEM at 25 kV, with a 20 mm working distance and consistent spot size. All SEM sample preparations and observations were made at the University of Oklahoma Samuel Noble Electron Microscopy Laboratory.

**Results**

All genera have one or two mature achenes in a fruiting hypanthium; and all seeds are circular on the top and bottom views, and pentagon-like with converged, pointed tips on the side views (Fig. 1A, B, C). The cell shape, size, and wall features are variable across genera and species. In particular,

**Table 2.** Seed coat features in the subtribe Agrimoniina studied.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Cell shape</th>
<th>Cell size (length × width, µm)</th>
<th>Cell wall height (µm)</th>
<th>Papillae sculpturing</th>
<th>Voucher specimen (Herbarium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrimonia L.</td>
<td>Polygonal</td>
<td>ca. 20-30 × 20-70</td>
<td>ca. 2-4</td>
<td>Present</td>
<td>Hagenia abyssinica Nakai, Korea, Chung 2007-5 (OKL); A. eupatoria L., Georgia, M. Merello et al. 2035 (MO); A. grypepale Wallr., USA, Chung 39 (OKL); A. microcarpa Wallr., USA, Nelson 26674 (SUCH); A. nipponica Koidz., Korea, Chung 2007-9 (OKL); A. parviflora Alton, USA, Chung 31 (OKL); A. pilosa Ledeb., Japan, Chung 2607-7 (OKL); A pubescens Wallr., USA, Chung 18 (OKL); A procera Wallr., France, A. K. Skvortsov s.n., (GH); A. rostellata Wallr., USA, Chung 20 (OKL); A. striata Michx., USA, Chung 1 (OKL)</td>
</tr>
<tr>
<td>Aremonia agrimonoides (L.) DC.</td>
<td>Polygonal</td>
<td>ca. 20-30 × 20-70</td>
<td>ca. 1-2</td>
<td>Present</td>
<td>Switzerland, G. Gigo s.n., (DAO)</td>
</tr>
<tr>
<td>Hagenia abyssinica J.F. Gmel.</td>
<td>Polygonal-Circular</td>
<td>ca. 20-30 × 20-30</td>
<td>ca. 2-3</td>
<td>Absent</td>
<td>Tanzania, J. W. Ash 1388 (MO)</td>
</tr>
<tr>
<td>Leucosidea sericea Eckl. &amp; Zeyh.</td>
<td>Circular</td>
<td>ca. 20 × 20</td>
<td>ca. 5-15</td>
<td>Absent</td>
<td>South Africa, Balkwill &amp; Balkwill 9343 (MO)</td>
</tr>
<tr>
<td>Spenceria ramalana Trimen</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Absent</td>
<td>China, C. Schneider 3316 (A)</td>
</tr>
</tbody>
</table>
the seed coat sculpturing is greatly variable among genera (Table 2).

**Agrimonia L.**: Eleven species were observed in the genus: *A. coreana* (Asia), *A. eupatoria* (Europe), *A. gryposepala* (North America), *A. microcarpa* (North America), *A. nipponica* (Asia), *A. parviflora* (North America), *A. pilosa* (Eurasia), *A. procera* (Europe), *A. pubescens* (North America), *A. rostellata* (North America), *A. striata* (North America). All species of the genus have seeds with polygonal, reticulum-shaped cells (ca. 20-30 × 20-70 μm) with protruded cell walls (ca. 2-4 μm high) and prominent papillae structures throughout the cell bodies (ca. 1-2 μm in diameter) (Fig. 1D, E).

**Aremonia Neck. ex Nestl.**: *A. agrimoniodes* was observed from the European monotypic genus *Aremonia*. Its seed shape is similar to *Agrimonia* except having a flattened top. Like that of *Agrimonia*, the seed coat of *A. agrimonioides* is composed of polygonal, reticulum-shaped cells (ca. 20-30 × 20-70 μm) with cell wall protrusions (ca. 1-2 μm high) shorter than those in *Agrimonia*. *A. agrimonioides* also exhibits papillae structures throughout the cell bodies (ca. 1-2 μm in diameter) (Fig. 1F).

**Hagenia J.F. Gmel.**: *H. abyssinica* in this African genus. The seed shape of *H. abyssinica* is similar to *Agrimonia* (Fig. 1C), but its cell shape and sculpturing are greatly different from the features observed in the other taxa. Some cells are polygonal, but most of them are more circular than reticulum (ca. 20-30 × 20-30 μm) with thicker and more protruded walls (ca. 2-3 μm high). Papillae structures are absent on cell surfaces (Fig. 1G).

**Leucosidea Eckl. & Zeyh.**: The other African monotypic genus, *L. sericea*, was also investigated. Overall the seed characteristics (seed and cell size, wall, and sculpturing patterns) of *Leucosidea* overlap those of the other African genus, *Hagenia* (Fig. 1G vs H). However, cells in *Leucosidea* are more circular and smaller in size than those in *Hagenia* (ca. 20 × 20 μm). The thinner cell walls are taller (ca. 5-15 μm high) although they may appear pressed (Fig. 1H). As in *Hagenia*, cells on the surface of *Leucosidea* seeds lack papillae structures (Fig. 1H).

**Spenceria Trimen**: *Spenceria* is a monotypic genus with only one Asian endemic species, *S. ramalana*. The cell shape of *Spenceria* seeds is not evident since their cell walls are greatly pressed with a relatively smooth surface possibly suggesting that cell walls were not well developed. The cell surfaces likewise do not exhibit any papillae structures (Fig. 1I).

**Discussion**

The seed coat surfaces of taxa within the subtribe Agrimoniinae exhibit variability in cell size, shape, wall features, and sculpturing patterns. Although the levels of polyploidy typically exhibit positive correlation with cell sizes in flowering plants, that is polyploidy with higher ploidy levels has larger-sized cells (Stebbins, 1971), the correlation is not found within the genus *Agrimonia* (tetraploidy, 4x: *A. coreana*, *A. eupatoria*, *A. microcarpa*, *A. nipponica*, *A. parviflora*, *A. rostellata*, octoploidy, 8x: *A. gryposepala*, *A. pilosa*, *A. procera*, *A. pubescens*, *A. striata*) nor within the subtribe. Although the diploid genus *Leucosidea* (2x), an endemic African genus, exhibits smaller cells than those in tetraploidy and octoploidy *Agrimonia* (4x and 8x), the cell size of the hexaploid *Hagenia* (6x), another endemic African genus, is similar to the cell size of *Leucosidea* (Fig. 1). Since the strong correlation between ploidy levels and fruit size (the higher ploidy levels, the larger fruits) or distribution patterns has been...
detected in the genus *Agrimonia* (Chung, 2008), a more detailed study on cytological and morphological features is needed to understand the consecutive changes in cellular formation and structuring within the genus.

Of most significance is the presence of papillae structures in both *Agrimonia* and *Aremonia*, which is a key feature in distinguishing them from the other genera in Agrimoniinae. When the seed sculpturing characters are mapped on the phylogenetic trees previously reported (Potter et al., 2007; Chung, 2008), two alternative hypotheses on seed coat sculpturing in Agrimoniinae are postulated (Fig. 2). In the phylogenetic tree inferred from four nuclear and six chloroplast regions of sequence data (Potter et al., 2007), at least two changes of papillae sculpturing on seed coats are required. On the other hand, in the phylogenetic tree of the low-copy nuclear gene, WAXY data (Chung, 2008), one evolutionary step is required to explain the current character states. In the European heathers (Ericaceae, Ericales), Plant Systematics and Evolution 285: 139-148.


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A more detailed morphological study on seeds, especially an anatomical study, will reveal more informative characters for a better and more thorough understanding of this biologically diverse lineage (see Oh et al., 1993; Shepherd et al., 2005; Heo and Suh, 2008; Fagúndez et al., 2010; Jung et al., 2010).

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**Literature Cited**


