Character evolution in Apiaceae tribe Scandiceae inferred from ITS molecular phylogenies

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Evolutionary trends of several selected characters in Apiaceae tribe Scandiceae were investigated from the phylogeny which was constructed on the basis of nuclear rDNA ITS sequences. The distribution of cytological, palynological, phytochemical, and fruit morphological characters shows that many of these characters are highly homoplastic, occurring independently multiple times throughout the trees. Therefore, those characters are not useful for the delimitation of genera or subtribes within Scandiceae. In contrast to the variable fruit secondary appendages, the fruit primary appendages show little variation in their shapes. These primary appendage characters are considered to support many of the major clades recognized in the ITS sequence-based phylogeny.

Key words: Apiaceae, phylogeny, evolution

The spiny-fruiting umbels accommodate all taxa of Apiaceae which have spines, hooks, tubercles, or bristly hairs on the primary and/or secondary ridges of their fruits. The complex of umbelliferous plants showing above characters was originally described as tribe Caucalideae by Bentham and Hooker (1867) and Boissier (1872). However, Drude (1897-1898) placed these spiny-fruiting plants into his Scandiceae subtribe Caucalidinae and tribe Dauceae. According to his classification system, some of spiny-fruiting umbels that contain calcium oxalate crystals in the parenchyma cells surrounding the carpophore were treated as closely related to subtribe Scandicinae, which lacks both primary and secondary appendages. Calestani (1905) subsequently divided these spiny-fruiting taxa into three subtribes: Caucalideae, Chaetosciadieae, and Dauceae. He

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placed *Chaetosciadium* in its own subtribe in Ligusticeae due to its unique long bristly hairs on the secondary ridges of the fruit. Koso-Poljansky (1916), relying primarily on anatomical characters of the mericarp, emphasized that such characters are very useful in delimiting genera.

Relatively little systematic work was done at the tribal level until the 1960’s, when Cerceau–Larrival (1962, 1965) distributed these spiny-fruit ed genera into nine new tribes on the basis of pollen characters such as pollen shape, P/E (polar axis/ equatorial diameter), and exine thickness in polar and equatorial views. Guyot *et al.* (1980) expanded Cerceau–Larrival’s (1962, 1965) studies by including several vegetative characters, i.e., trichome, cotyledon, and leaf primordial shapes. They divided Drude’s (1897–1898) Cauca lidineae and Dauceae into five tribes. Chemical compounds from fruits and leaves have been also suggested as useful for elucidating relationships at both generic and species levels within the spiny-fruit ed taxa (Harborne, 1967; Harborne and Williams, 1972; Crowden *et al.*, 1969). On the basis of above information, all taxa of spiny-fruit ed umbellifers were treated into a single tribe, Cauca lideae Heywood and Jury (Heywood, 1982), which comprises up to 21 genera distributed throughout the world in both temperate and tropical regions, expressing its greatest diversity in Mediterranean regions. During the past decade, the implementation of molecular markers has brought a dramatic shift in the classification of the spiny-fruit ed umbel plant groups. For example, relationships among the Cauca lideae and related taxa have been recently examined by the phylogenetic analyses using nuclear rDNA ITS sequences (Lee and Downie, 1999) and chloroplast(cp) DNA restriction sites and rps16 intron sequences (Lee and Downie, 2000). Phylogenetic results based on these molecular data do not support a monophyly of Cauca lideae sensu Heywood (1982) because the relationship between Cauca lideae and Scandiceae, which is considered as a sister group to Cauca lideae, is unclear. Phylogenetic analyses on Drude’s Scandi ceae indicated that two major clades recognized within Cauca lideae and Drude’s Scandiceae were treated as subtribes Daucinae, Torilidinae, and Scandicinae within Scandiceae sensu lato, respectively (Downie *et al.*, 2000). Nomenclature of these three subtribes and a tribe will be used in the subsequent sections. Otherwise, authority to the nomenclature will be given.

The molecular phylogeny has served as a model to evaluate evolution of chemical, morphological and cytological characters in diverse plant groups (Systma *et al.*, 1991; Soltis *et al.*, 1993; Plunkett *et al.*, 1996; Katz–Downie *et al.*, 2000).
While evolutionary trends of non-molecular characters were surveyed on Apiaceae (Plunkett et al., 1996; Katz-Downie et al., 2000), character evolution within Scandiceae could not be assessed in detail because the number of spiny-fruited taxa examined in each study was too few. Currently, phylogenetic information for numerous taxa of Scandiceae are available for interpreting character evolution within the tribe (Lee and Downie, 1999, 2000). In this study, evolutionary patterns of selected cytological, palynological, cotyledonary, phytochemical, and fruit morphological characters are investigated on the basis of the molecular phylogeny.

Material and Methods

For the spiny-fruited umbel taxa, three molecular data are currently available, ITS sequences of nuclear rDNA, cpDNA restriction site variation, and rps16 intron sequences (Lee and Downie, 1999, 2000). Among these three data sets, the ITS-derived phylogeny was chosen here to determine patterns of character evolution due to availability of more taxa within the spiny-fruited umbels than the other two. In the ITS-derived phylogeny, 43 taxa were investigated while 28 taxa were examined in the cpDNA restriction site mapping-, and 22 in cpDNA rps16 intron sequence-derived phylogenies.

To determine patterns in the evolution of several selected cytological, palynological, phytochemical, and morphological characters within Scandiceae, the distribution of these characters is tabulated next to the strict consensus tree derived from parsimony analysis of ITS sequences (Lee and Downie, 1999). In this way, patterns in the evolution of these characters can be inferred in the independent context of the molecular phylogeny.

Results and Discussions

Cytological character evolution: Extensive cytological studies have shown that the predominant base chromosome number in Apiaceae subfamilies Hydrocotyloideae and Saniculoideae is eight, whereas in subfamily Apioideae it is either eight or eleven (Moore, 1971). Base chromosome numbers of \( x = 6, 7, 8, 9, 10 \) or 11 occur independently within Scandiceae(Fig. 1). It appears that no base chromosome number delimits any of major three subtribes recognized in the ITS tree. The Daucinae contains a variety of base chromosome numbers
Fig. 1. Distribution of selected cytological, palynological and cotyledonary characters on the strict consensus tree derived from parsimony analysis of 58 nuclear rDNA ITS sequences from Apiaceae tribe Scandiceae. Shaded boxes represent the presence of a particular character state while open boxes represent the absence of the character. Base chromosome numbers are indicated by $x=6$ through $x=11$. Pollen and cotyledon (cot.) characters are as follows: 1, subhomboidal pollen; 2, ovoid pollen; 3, subrectangular pollen; 4, equatorially-constricted pollen; L, linear cotyledons; R, round cotyledons.
ranging from \( x=7 \) to \( x=11 \). However, the predominant base chromosome number in this subtribe, \( x=11 \), also occurs in other tribes within Apioidae. On the other hand, the clade comprising most members of the *Torilis* and *Chaetosciadium* is characterized by \( x=6 \) which is not found in other members of the Scandiceae. However, \( x=8 \) and \( x=11 \) are also reported in several taxa of *Torilis*.

Base chromosome numbers may not delimit the generic boundary of either *Daucus* or *Torilis*. *Daucus* contains \( x=9 \), 10, and 11, showing diversity of base chromosome numbers. Chromosome data are rather useful in the delimitation of species within *Daucus* and *Torilis*. The clade of *Daucus maximus* and *Daucus carota* is supported by a base chromosome number of 9. This number has never been reported elsewhere within Daucinae. However, base chromosome numbers of *Laserpitium* and *Cuminum* do not provide infrageneric information showing considerable uniformity of \( x=11 \). On the other hand, intraspecific variation were detected within *Daucus durieuia*, *Torilis nodosa*, and *Turgenia latifolia* reflecting that base chromosome numbers are not useful characters for the delimitation of genera or subtribes within Scandiceae.

Palynological and cotyledonary character evolution: The pollen morphology of Drude’s Scandiceae and Dauceae was well studied by Cerceau–Larrassal (1962, 1965 and 1971) and her colleagues (Gruas–Cavagnetto & Cerceau–Larrassal, 1982). In addition to pollen morphology, Guyot et al. (1980) examined trichome characters, cotyledon, and leaf primordial shapes. As a result of this study, they concluded that Drude’s Caucaalidinae and Daueae should be disposed into five tribes, Artedieae, Agrocharideae, Dauceae, Caucaalidae and Turgeniaceae. However, none of these five tribes proposed by Guyot et al. (1980) were recognized in the ITS tree (Lee and Downie, 1999). Daucinae is considered to be heterogeneous in pollen morphology because it shows all four pollen types. On the other hand, Torilidinae is characterized by the presence of subrectangular and equatorially constricted pollen, and all members of the Scandiciniae clade contain only subrectangular pollen. These subrectangular and equatorially constricted pollen grains, however, also occur in Daucinae. In result, Guyot et al’s (1980) interpretation of cotyledonary shape and pollen evolution finds little support in the ITS trees. These characters occur throughout the trees and trends in their evolution are difficult to be explained. The same conclusion was reached when the evolution of these characters was assessed against a higher-level phylogenies generated using cpDNA *matK* (Plunkett et al., 1996) and ITS
sequences (Katz-Downie et al., 1999), respectively.

Flavonoid character evolution: Since flavones and flavonols possessing oxidation, hydroxylation and methylation patterns are existed in specific taxa of Apiaceae, they can be used to delimit tribal boundaries. Luteolin is widely distributed in Scandiceae, and is a useful flavonoid character separating this tribe from other tribes of subfamily Apioideae (Crowden et al., 1969; Heywood, 1971b; Harborne and Williams, 1972). The flavonoid distribution pattern within the Scandiceae is further distinguished from other Apioideae in containing diverse flavone O-glycosides. These compounds are formed by glycosylation at position 3 of the “C” ring, positions 5 and 7 of the “A” ring, and even position 4’ of the “B” ring (Seigler, 2000). Many monoglycosides (glucosides, glucuronides) and a few diglucosides (rutinosides) have been reported in Scandiceae. However, more complex glycosidic forms, such as luteolin 7-arabinosylglucoside from *Turgenia* and apigenin 7-arabinosyl-rhamnosylglucoside from *Pseudorlavya pumila*, have been also detected.

Evolutionary trends of diverse flavonoid compounds have been interpreted from ITS trees for a variety of plants (Soltis et al., 1993; Käss and Wink, 1995). It has been suggested that many of these compounds have evolved by apparent multiple gains or losses. Within Scandiceae, the commonly occurring luteolin 7-glucoside, as well as numerous other flavonoid compounds, is considered to have been gained or lost multiple times. The incorporation of Drude's Dauceae into Caucaideae characterized by the presence of flavones (Crowden et al., 1969) would be unreliable because both flavones and flavonols are present in most taxa of Dauceae, with the exception of *D. muricatus*, *D. bicolor*, and *D. aureus*, which contain only flavones. In contrast, most representatives of Drude's Caucaideinae, with the exception of *Orlaya*, contain flavones. *Orlaya* contains both flavonols and flavones, as other Dauceae do. The close relationship between Drude's Dauceae and *Orlaya*, as inferred by a similar flavonoid chemistry, is reflected in the results of the parsimony analyses of the three independent molecular data sets (Lee and Downie, 1999, 2000).

A considerable range of flavonols and flavones is present in different genera within Daucineae, and even in different species of *Daucus*. For example, in the fruits of *Daucus* (Harborne and Williams, 1972), nine of the 12 investigated taxa contain flavonols and, in the leaves, four of the 10 investigated taxa produced flavonols, although some taxa contain both. The absence of flavonol compounds in most taxa of Torilidinae (except quercetin 3-glucoside in fruits of *Astrodaucus,*
and quercetin in leaves of *Glochidiotheca*) would indicate that Torilidinae is somewhat distantly related to Daucinae and Scandicinae. With the exclusion of these two genera, no plants in Torilidinae contain flavonols. However, the occurrence of a variety of flavone glycosides does not represent a unique property in Torilidinae because they also occur in Daucinae and Scandicinae. The close relationship between Daucinae and Torilidinae is supported by the shared presence of several derivatives of luteolin (such as luteolin 5'-glucoside, luteolin 4'-glucoside, and luteolin 4'-diglucoside); these flavones were not detected in other tribes of Apioideae. Other flavonoid compounds detected in Daucinae and Torilidinae include chrysosieriol, apigenin, and their derivatives (with the exception of apigenin-apiosylglucoside being restricted only to Apioideae). However, the application of these specific flavonoids for determining higher level taxonomic relationships is of limited value because of their narrow distribution. The flavonoid data currently available have been useful for taxonomic analyses at lower levels (i.e., relationships among genera or species within Scandiceae). For example, luteolin 7'-glucuronosylglucoside, which was detected only in *Cuminum* and *Orlaya* (*O. daucorlaya*, and *O. grandiflora*), supports a close relationship between these two genera. A close relationship between *Torilis* and *Chaetosciadium* is also reflected by a similar distribution pattern of flavones. Both genera contain many of the same compounds, luteolin 7'-glucoside, luteolin 7'-diglucosides, and apigenin 7'-glucoside in fruits.

Although several specific flavonoid compounds are considered to be useful in delimiting tribal and generic boundaries, there are problems in using them for phylogenetic purposes (Fig. 2). One major problem is how to use these compounds in terms of characters or units of information (Heywood, 1971b). Spots on chromatograms can be handled as normal presence or absence characters in numerical taxonomic approaches (McNeill *et al.*, 1969), but no quantitative indication is given by such a method. Even if specific flavonoid compounds were synthesized in a plant, they may not be detected because of the small amounts or lack of accumulation. Furthermore, the expression of genes for secondary metabolites may, in some instances, be triggered by different environmental or developmental factors. Thus, there is a possibility that failure in sampling from a diversity of habits and at different times can result in the lack of certain compounds. For studies of these compounds to be valid, therefore, it is desirable that at least some investigation of possible levels of interspecific variability should be conducted (Quicke, 1993).
Fig. 2. Distribution of selected flavonoid characters on the strict consensus tree derived from parsimony analysis of 58 nuclear rDNA ITS1 and ITS2 sequences from Apiaceae tribe Scandiceae. Shaded boxes represent the presence of a particular flavonoid compound while open boxes represent flavonoid compound undetected. The question mark (?) indicates no information available. Character information was obtained from Harborne (1967), Crowden et al. (1969), Harborne (1971), Heywood (1971b), Harborne and Williams (1972) and Okeke (1978).

Fruit flavonoid data ranged from number 1 to 13 are as follows: 1, qu 3-glucoside 2, qu 3-diglucoside 3, qu 3-glucuronides 4, kaempferol (km 3-glucoside, km 3-rutinoside, or km 3-glucuronides) 5, lu 5-glucoside 6, lu 7-glucoside 7, lu 7-diglucoside 8, lu 7-glucuronosylglucoside 9, lu 7-rutinoside 10, lu 4-glucoside 11, lu 4-diglucoside 12, apigenin (ap 7-glucoside, ap 7-rutinoside, ap 7-glucuronosyl glucosides, or ap 7-arabinosylrhamnosylglucoside) 13, chrysoeriol (chr 7-glucoside or chr 7-diglucoside). Leaf flavonoid: 1, quercetin 2, qu 3-glucoside 3, kaempferol 4, luteolin 5, lu 5-glucoside 6, lu 7-glucoside 7, apigenin. qu and lu indicate quercetin and luteolin, respectively.
Morphological character evolution: Although the complexity of fruit morphology in Apiaceae has been well known since Morrison's (1672) work, the uncertainty of tribal or generic delimitations that rely on these characters has made it difficult to establish their evolutionary trends (Heywood, 1971a). Within Scandiceae, the general shape of the fruits, the form and arrangement of primary and secondary appendages, and fruit anatomical and embryological features have been employed in delimiting genera or species. Despite extensive studies on fruit morphology, little is known about the functional or adaptive value of many of these characters. Furthermore, the reassessment of several fruit characters traditionally used in classification of subfamily Apioideae reveals that the extensive homoplasies in these characters occurs during the evolution of the family (Downie and Katz-Downie, 1996; Plunkett et al., 1996; Downie et al., 1998; and Katz-Downie et al., 1999).

All members of Daucinae and Torilidinae, with the exception of Artedidae, have diverse secondary appendages on their fruits (Fig. 3). Long bristles are found in Chaetosciadium, Ammmodaucus and Cuminum setifolium. Spines occur in Daucus, Pseudorlaya, Agrocharis, Orlaya, Astrodaucus, Glochidotheca, Caulis, Turgenia, Yabea and Lisaea. Short tiny and multiple-celled bristles occur in Cuminum cyminum. Tubercles are found in heterocarpic Torilis nodosa, Lisaea heterocarpa and Daucus aureus. Dentate wings occur in Pachyctenium, and spatulate plicate appendages occur in Szovitsia. In Artedidae, appendages are lacking entirely. The diversity of secondary appendages has been useful in identifying species or genera within the spiny-fruitied umbellifers. However, the intergeneric relationships based on these characters are not congruent with those relationships inferred from the ITS sequences. Psammogeton, Chaetosciadium and Ammmodaucus were assumed to be closely related because they each possess long bristles (Davis and Hedge, 1971). However, the ITS-based phylogeny suggests that long bristles evolved independently in Psammogeton, Chaetosciadium and Ammmodaucus. Other characters, such as the number of secondary appendage rows, the degree of confluence in secondary appendage bases, and the presence of a glochidiate apex are less useful in delimiting generic boundaries because they also occur independently in the ITS tree. In contrast to the variable secondary appendages, the primary appendages show little variation in their shape. All taxa of Daucinae and Torilidinae (with the exception of Laser, Polyophium, and Lisaea strigosa) are recognized by the presence of primary appendages on the fruit commissural face (char. 8). The distribution of other characters are also useful.
Fig. 3. Distribution of fruit characters on the strict consensus tree derived from parsimony analysis of 58 nuclear rDNA ITS sequences of Apiaceae tribe Caucalideae. The shaded boxes indicate presence of the character, while the open boxes indicate absence of the character or missing information. The characters are as follows: 1, beak 2, dorsally compressed 3, black or dark brown 4, surface papillate 5, fruit appendage 6, both primary and secondary appendages 7, primary appendages on the dorsal surface 8, primary appendages on the commissural face 9, primary appendages spiny 10, primary appendages curved and stout 11, primary appendages multiple grouping 12, primary appendages stylar-directioned 13, primary appendages with lobed bases 14, secondary appendages narrow bell-shaped 15, secondary appendage surface ornamented by peg-like projections 16, secondary appendages 17, secondary spines 18, secondary bristles 19, secondary wings 20, secondary appendages arranged in one-row 21, secondary appendages arranged in more than two rows 22, secondary appendages with confluent bases 23, secondary appendages with glochidiate apex.
in suggesting generic-level relationships: spiny primary appendages (char. 9) are found in *Turgenia* and *Lisaea*; primary hairs are curved and stout (char. 10) in *Astrodaucus, Glochidotheca* and *Szwitsia*; primary hairs are grouped (char. 11) in *Cuminum* and *Ammoadaucus*; primary hairs are appressed toward the stylar end of fruits (char. 12) in *Torilis* and *Chaetosciadium*; and the primary hair bases are lobed variously (char. 13) in *Daucus, Pseudorlaya* and *Pachyctemum*. These primary appendage characters support many of the major clades recognized in the ITS-based phylogeny.

Daucinae and Torilidinae are morphologically distinctive within Apioideae because of its diverse shapes of fruit appendages. These plants have successfully colonized open areas which are often sandy and of low vegetation. In order to facilitate fruit dispersal under dry environmental conditions, the weak and relatively simple primary appendages do not function well for the dispersal. On the other hand, the larger and more diverse secondary appendages (e.g., spines, bristles, dentate wings, etc.) serve better as the major functional tool. Several genera have evolved appendages specialized for wind dispersal. These include compressed fruits bearing broad and thin secondary wings (e.g., *Laserpitium*). Other genera of Scandiceae, which have secondary spiny appendages, have fruits that are dispersed passively by animals.

**Conclusion**

Scandiceae is a complex of umbelliferous plant groups which develope diverse types in palynological, cytological, phytochemical and fruit morphological features. This diversity is very unique in Scandiceae as compared to other tribes in Apiaceae, supporting that these spiny-fruited umbellifers are the most advanced taxa within the family. Most of these selected characters tabulated next to the ITS-derived tree do not reveal useful in delimiting subtribal or generic boundaries within Scandiceae by occurring multiple times on the ITS tree. Specialization in fruit morphology is considered as a response of these spiny-fruited umbel plants to adapt more efficiently to locally changing environmental conditions. Under dried climates, they have been evolved independently for more successful fruit dispersal and for better defense systems by containing more diverse phytochemical compounds than other tribes within Apiaceae do. In order to determine evolutionary trends in more detail within Scandiceae sensu Downie *et al.* (2000), phylogenetic analyses including newly added taxa
into the tribe (i.e., Drude’s Laserpitieae) are now in progress (Lee et al., unpublished data).

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핵산 리보좀 DNA ITS 염기서열의 계통학적 연구 결과를 이용한 산형과 사상자족 식물군들의 형질 진화

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산형과 사상자족 식물군의 형질의 진화경향성이 핵산 리보좀 18S와 26S DNA 사이에 위치한 ITS의 염기서열에 의한 계통분류학적 연구를 기초로 조사되었다. 절약분석 결과 생성된 계통수에 의한 세포학적, 화분학적, 식물화학적, 외부형태학적 형질들은 상동형질들이 아닌 것으로 밝혀졌다. 따라서, 자업의 형태, 화분, 염색체 기반수 등에서 나타나는 형질들은 사상자족내 속이나 아족들의 범주를 정하는데 중요한 형질이 아닌 것으로 밝혀졌다. 다양한 변이를 보이는 염색체의 2차 부속물에 관계된 형질들과는 반대로 1차 부속물에서 발견되는 형질들이 그 형태에 있어서 변이를 덜 보여 주었다. 이러한 1차 부속물에 관련된 형질들이 ITS 염기서열에 기반한 계통수에서 생성되는 주요 분류군들의 단일계통을 지지하는 것으로 밝혀졌다.

주요어: 산형과, 계통, 진화

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