Available online at www.sciencedirect.com
ScienceDirect
Biochemical Systematics and Ecology 35 (2007) 606–613
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Systematic aspects of foliar flavonoids in subsect. *Carpinus* (*Carpinus*, Betulaceae)

Jeong Ill Jeon [a], Chin-Sung Chang [b,*], Zhi-Duan Chen [c], Tae Yoon Park [d]

[a] *Department of Applied Plant Sciences, Shingu College, Seongnam 462-743, Republic of Korea*
[b] *Department of Forest Sciences and The Arboretum, Seoul National University, Seoul 151-921, Republic of Korea*
[c] *State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, The Chinese Academy of Sciences, Xiangshan, Beijing 100093, China*
[d] *Graduate School of Education, Yonsei University, Seoul 120-749, Republic of Korea*

Received 25 October 2006; accepted 15 April 2007

Abstract

Foliar flavonoids from nine taxa of subsection *Carpinus* (*Carpinus*, Betulaceae) were examined. A total of 10 compounds were isolated and identified, mainly mono- and di-glycosides of flavones (apigenin and luteolin) and/or flavonols (myricetin, kaempferol and quercetin). By comparing the results of flavonoid chemistry with the morphological characteristics and geographic distribution patterns, subsection *Carpinus* might be differentiated into two ancestor-based groups, one consisting of *Carpinus tientaiensis*, *Carpinus londoniana*, and *Carpinus betulus*, the other *Carpinus viminea* var. *viminea*, *C. viminea* var. *chiukiangensis*, *Carpinus laxiflora*, and *Carpinus caroliniana*. A major trend in the reduction of the number of compounds from the primitive to the advanced was observed in subsect. *Carpinus*, and the chemical simplification seems to be related to species differentiation within the subsection. The presence of isoflavones in *C. laxiflora* and *C. caroliniana* revealed their advancement chemically, but *C. betulus* and *C. londoniana* showed no chemical differentiation. Overall intercontinental divergence of *Carpinus* was not confirmed by this study. A number of infraspecific taxa in the subsect. *Carpinus* were chemically identical with their original varieties except the *C. viminea* complex. This might be because sect. *Carpinus* is undergoing slower radiation; significant biochemical differentiation among infraspecific taxa seems not to have occurred yet. These flavonoid data reflected intrasectional parallelisms and intersectional radiation in *Carpinus*.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: *Carpinus*; Betulaceae; Flavonoids; Phytogeography; Speciation

1. Introduction

The genus *Carpinus* of Betulaceae comprises approximately 35 woody species, which are widely distributed in Europe, eastern Asia, and North and Central America, although the greatest concentration of species diversity occurs in China (Rehder, 1927; Jones and Luchsinger, 1986; Hillier, 1988).

* Corresponding author. Tel.: +82 2 880 4758; fax: +82 2 873 3560.
E-mail address: quercus@plaza.snu.ac.kr (C.-S. Chang).

0305-1978/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2007.04.004

The taxonomy of *Carpinus* in eastern Asia was considered difficult and largely ignored until Li and Cheng (1979). The major classifications by Spach's (1842) and Winkler's (1904) were discordant in terms of species definitions, and sectional groupings. The two generic groupings, *Carpinus* and *Distegocarpus*, proposed by Spach (1841) and De Candolle (1893) sharply contrasted with the two sections proposed by Winkler (1904) based on floral bracts, infructescences, and scales. However, Winkler's classification remains the most frequently accepted treatment (Rehder, 1927; Li and Cheng, 1979; Furlow, 1990). Li and Cheng (1979) who studied Chinese *Carpinus*, followed Winkler's (1904) sectional treatment, but divided sect. *Carpinus* into three subsections, *Carpinus*, *Monbeigianae* (Hu) P.C. Li, and *Polyneurae* (Hu) P.C. Li. Subsects. *Carpinus* and *Monbeigianae* were mainly defined by the characters of the fruiting bract, while subsect. *Polyneurae* was characterized only by its setiform serrate leaf. Subsect. *Carpinus* has strongly three-lobed fruiting bracts and can be easily distinguished from the other two subsections, which have no distinct lobe at the outer margin of the fruiting bract. The ambiguous relationship between subsects. *Monbeigianae* and *Polyneurae* makes it difficult to confidently establish two monophyletic subsections. Jeon and Chang (2000) considered that these two subsections are not supported due to the presence of intermediate taxa and suggested that they should be united into one as subsection *Monbeigianae*. Yoo and Wen (2002) reported that both subsect. *Monbeigianae* and subsect. *Polyneurae* were not monophyletic in the cladistic analysis using ITS and morphological data.

Although these two sections and three subsections of *Carpinus* are distinguished mainly on the basis of their fruiting bracts, most species are essentially recognized by leaf exomorphological characters. An attempt using ITS sequences (Yoo and Wen, 2002) to the systematics failed to aid the delimitation of the taxa of *Carpinus* because most taxa showed little variation in their ITS profile.

Subsect. *Carpinus* contains six species and three varieties, including *Carpinus tientaiensis* Cheng, *Carpinus londoniana* H.J.P. Winkl., *Carpinus viminea* Wall., and *Carpinus laxiflora* (Siebold et Zucc.) Blume in Asia (Li and Cheng, 1979; Ohwi, 1984; Jeon and Chang, 1997), *Carpinus betulus* L. in Europe, and *Carpinus caroliniana* Walter in North America (Rehder, 1927). The differentiation of the European *C. betulus* is most possibly related to the polyploidy of chromosome number, because until now the known chromosome number of *Carpinus* is $2n = 16$, except for *C. betulus* ($2n = 64$) (Darlington and Ammal, 1945; Goldblatt and Davidse, 1984). Yoo and Wen's (2002) phylogenetic study also showed that subsect. *Carpinus* except for *C. betulus* was supported as monophyletic both in the ITS tree and in the morphological tree.

The purpose of the present study was to clarify species relationships, species boundaries, and phylogenetic patterns within sections and subsections of *Carpinus* comparing flavonoid complements to morphological characteristics. Previous taxonomic success using flavonoids from *Carpinus*, sect. *Distegocarpus* (Chang and Jeon, 2004), indicated that the chemical approach has potential for the circumscription of taxa and the determination of natural groupings. Particular attention was focused on distinguishing several infraspecific taxa because of the high degree of morphological intergradations among populations and the inconsistent circumscription across the various systematic treatments.

2. Materials and methods

Flavonoid profiles were surveyed for nine taxa of subsect. *Carpinus*, i.e., *C. tientaiensis* Cheng, *C. londoniana* H.J.P. Winkl var. *londoniana*, *C. londoniana* var. *lanceolata* (Hand.-Mazz.) P.C. Li, *C. viminea* Wallich var. *viminea*, *C. viminea* var. *chiukiangensis* Hu, *C. laxiflora* (Siebold et Zucc.) Bl. var. *laxiflora*, *C. laxiflora* var. *longispica* Uyeki, *C. betulus* L., and *C. caroliniana* Walter. Plant materials were sampled from specimens of various herbaria [T.B. Lee Herbarium, Seoul National University (SNUA), Arnold Arboretum (A), British Museum (BM), US National Arboretum (NA), Institute of Botany, Academia Sinica (PE), United States National Herbarium (US), Zhejiang Agricultural University in China (ZAU)] and collected by ourselves (Table 1). Since preliminary chemical survey showed seasonal variation in *Carpinus* when tested over the full growing season, fieldwork was mainly conducted approximately between June and August for 6 years (1994–1999).

The extraction of flavonoids from leaves followed the methods of Mabry et al. (1970), Giannasi (1975), and Chang and Giannasi (1991). The flavonoid survey employed two-dimensional paper chromatography using Whatmann 3 MM paper. TBA (tertiary-butanol:acetic acid:water, 3:1:1 by volume) was used as the first solvent and 15% HOAc (acetic acid:water, 15:85 by volume) as the second solvent. Flavonoid profiles were viewed under UV light and colors were recorded before and after fuming with ammonia vapor. Individual spots were circled in pencil and assigned a number. Isolation of flavonoids followed the procedure of Mabry et al. (1970). Compounds were identified using standard

Table 1
Origin and accession number for specimens used for flavonoids surveys of subsect. *Carpinus* of genus *Carpinus*

Taxa	Origin and accession number
Carpinus betulus L.	Rumania: Mititelu et al. 324 (PE); unknown: Burch and Sponberg 77–37 (SNUA; cultivated at Arnold Arboretum grafted from Royal Botanic Gardens, Kew, England)
C. caroliniana Walter	USA, Georgia: Koyana et al. 6768 (PE); Oklahoma: Hess and Stoynoff 6188 (PE); unknown: Burch and Sponberg 77–38 (SNUA; cultivated at Arnold Arboretum), Logan 289 (SNUA; cultivated at Arnold Arboretum)
C. laxiflora (Siebold et Zucc.) Bl. var. *laxiflora*	Korea: Jeon 10007 (SNUA), Jeon 10009 (SNUA), Jeon 10010 (SNUA), Jeon 10134 (SNUA), Jeon 10140 (SNUA), Jeon 10143 (SNUA), Jeon 10168 (SNUA), Jeon 10737 (SNUA), Jeon 10740 (SNUA), Jeon 10741 (SNUA), Jeon 10743 (SNUA), Japan: Chang 693 (SNUA), Chang 1000 (SNUA), Chang 4067 (SNUA), Maruyama *s.n.* (US 2037337), Shinji *s.n.* (US 2276647), Logan 323 (SNUA, cultivated at Arnold Arboretum)
C. laxiflora var. *longispica* Uyeki	Korea: Jeon 10002 (SNUA), Jeon 10003 (SNUA), Jeon 10005 (SNUA)
C. londoniana H.J.P. Winkl. var. *londoniana*	China, Anhui: Ling 1305; Guangxi: Tsang 27929 (US); Yunnan: Forrest 26622 (US), Yiying Qian 2766, Rock 2760 (US); Zhejiang: Ching 2134 (US), Thailand: RCT 558 (NA), Myanmar (Burma): Rock 2167 (US)
C. londoniana var. *lanceolata* (Hand.-Mazz.) P.C. Li	China, Hainan: anonymous (US 1659931), anonymous (US 1669637), Chen 43372 (PE); Yunnan: anonymous (US 1377869)
C. tientaiensis Cheng	China, Zhejiang: Jeon 10312 (SNUA), Jeon 10367 (SNUA), Jeon 10368 (SNUA), Jeon 10369 (SNUA), Jeon 10411 (SNUA), Chen 2323 (PE), Chen 2441 (PE), Chen 2428 (PE), S. Chen 3892 (A), anonymous (ZAU 010054)
C. viminea Wallich var. *viminea*	China, Guangxi: Tsang 27929 (NA), Tsang 27580 (NA); Hainan: McClure 9548 (NA); Hubei: Huang 2404, Sino-Amer. Exped. 1011 (NA); Jiangxi: Yao 11438 (NA); Sichuan: Zhao 0491 (BM), Zhao 0549 (NA), Zheng 10837 (PE); Zhejiang: Zhang et al. 1366, Zhang et al. 832, Hang-zhou University Chun-An-Dui 1512, Miao 7, Ching 1463 (NA), Ching 1428 (NA), Ching 1513 (NA), anonymous (ZAU 010081), anonymous (ZAU 010086), anonymous (ZAU 010096), anonymous (ZAU 010097)
C. viminea var. *chiukiangensis* Hu	China, unknown: anonymous (NA 0003481); Fujian: Hui Yu Lang 20010, Li 10851 (PE); Gaungxi: anonymous 5994 (PE), Guang-Xi-Dui 683 (PE); Guangdong: To et al. 61 (NA); Guizhou: Steward et al. 424 (US), Steward et al. 673 (US); Hubei: Wan Diao Zheng 1051, Chang and Hwa 728; Hunan: Li 2879 (PE); Jiangxi: Wang 4–66; Sichuan: Cheng and Hwa 1051 (PE), Feng 13804 (PE), Neng and Zhou 91256 (PE); Tibet: anonymous 73–562 (PE 111962), Zhang and Liang 3490 (PE); Yunnan: McLaren 32D (BM), Yu 17770 (PE), Ma 20947 (PE), Yu 19531 (PE); Zhejiang: Zhang et al. 1306, Zhang et al. 467, Zheng 4052, Zheng 4210, Zheng 4210, Hang-zhou University Chun-An-Dui 1645, Hang-zhou University Chun-An-Dui 1742, Hang-zhou University Chun-An-Dui 1810, Hang-zhou University Chun-An-Dui 1832, Ching 2562 (NA), Ching 1527 (NA), Ching 1797 (US), anonymous (ZAU 010056), India: Koelz 20910 (NA), Myanmar (Burma): anonymous (BM 580464), Nepal: Chang et al. 2249 (SNUA), Chang et al. 2354 (SNUA), Beer et al. 10708 (BM), Hara et al. *s.n.* (BM 580447), Ohashi et al. 774796 (BM), Strachey and Winterbottom *s.n.* (BM 580509), anonymous (BM 580526), Hara et al. 69789 (NA), Ohba et al. 8310040 (NA)

All voucher specimens collected by authors were deposited at the herbarium (T.B. Lee Herbarium, SNUA) of The Arboretum, Seoul National University, and each acronym represented herbaria as follows; Herbarium of Arnold Arboretum (A), British Museum of Natural History (BM), Herbarium of US National Arboretum (NA), Herbarium of Department of Biology, Seoul National University (SNU), US National Herbarium (US), Herbarium of Zhe-jiang Agricultural University (ZAU).

UV–visible spectroscopy (Mabry et al., 1970; Giannasi, 1975; Markham, 1982). R_f values of the purified compounds in TBA and HOAc were recorded. Compounds thought to be the same in different taxa were co-chromatographed for verification. Spectral and R_f data were compared with published data (Mabry et al., 1970). Flavonoid glycosidic sugars were identified using trifluoroacetic acid hydrolysis, and co-chromatography with standard sugars in one-dimension, using ethyl acetate:pyridine:water (5:1.6:1, v/v) as the solvent. The sugars were visualized by spraying with *p*-anisidine hydrochloride (Pridham, 1956) followed by heating at 100 °C for 10 min. Aglycones were identified by UV spectroscopy (Mabry et al., 1970) and further confirmed by comparison with compounds identified from previous studies (Chang and Giannasi, 1991; Chang, 2000; Jeon and Chang, 2000; Chang and Jeon, 2004).

3. Results

Ten flavonoid compounds were isolated and identified from the leaves of nine taxa of the subsect. *Carpinus* (seven taxa are shown in Table 2). The flavonoid pattern in *Carpinus* was based on mono- and di-glycosides of flavonols (myricetin, kaempferol and quercetin) and flavones (apigenin and luteolin). Acid hydrolysis yielded glucose, galactose, and rhamnose, and the order of linkage was determined by partial hydrolysis.

The result of flavonoid analyses showed distinct differences among the species of *Carpinus*. The taxa of subsect. *Carpinus* could be divided into two groups based on the presence/absence of flavone compounds. *C. londoniana*, *C. tientaiensis*, and *C. betulus* containing flavonol and flavone compounds constituted the first group which was named the *fangiana*-type (=*fangiana*-type of sect. *Distegocarpus*, Chang and Jeon, 2004). The second group without flavone compounds consisted of *C. viminea* var. *viminea*, *C. viminea* var. *chiukiangensis*, *C. laxiflora*, and *C. caroliniana* as *japonica*-type (*japonica*-type of sect. *Distegocarpus*, Chang and Jeon, 2004).

Within the species of *fangiana*-type, *C. tientaiensis* could be characterized by a presence of kaempferol 3-*O*-galactoside (number 5, see Table 2) and absence of myricetin, compared to *C. londoniana* and *C. betulus*. *C. londoniana* differed from *C. betulus* only in terms of the quercetin 3-*O*-rhamnoglucoside (number 3). The flavonoid profile of *japonica*-type was almost identical to each other. *C. laxiflora* and *C. caroliniana* differed from *C. viminea* by containing a isoflavone (number 9), and *C. laxiflora* and *C. caroliniana* differed by only one unknown compound (number 10).

Infraspecific variation of subsect. *Carpinus* was uniform except for *C. viminea* var. *chiukiangensis*. The flavonoid profiles of *C. laxiflora* var. *longispica* and *C. londoniana* var. *lanceolata* were the same as the original varieties of *C. laxiflora* and *C. londoniana*, respectively. However, *C. viminea* var. *chiukiangensis* differed from *C. viminea* var. *viminea* due to the loss of quercetin 3-*O*-rhamnoglucoside (number 3) and kaempferol 3-*O*-rhamnoglucoside (number 6).

4. Discussion

The relatively simple flavonoid profiles showed distinct differentiation within the subsection *Carpinus*. Two chemical groups could be distinguished by the presence of flavones in the *fangiana*-type (*C. tientaiensis*, *C. londoniana* and *C. betulus*) and the absence in the *japonica*-type (*C. viminea* var. *viminea*, *C. viminea* var. *chiukiangensis*, *C. laxiflora*, and *C. caroliniana*). This chemical dichotomy was also observed in sect. *Distegocarpus* (Chang and Jeon, 2004) as well as subsect. *Monbeigianae* of sect. *Carpinus* (Jeon, 2004). Thus, the flavonoid data tended to cut across sectional treatment within the genus.

Harborne (1977) and Gornall and Bohm (1978) considered that plant groups with flavone and flavonol compounds were more advanced than those with only flavonol compounds. Therefore, the *fangiana*-type with flavonol and flavone compounds might be chemically more advanced than the *japonica*-type.

Table 2
Distribution of flavonoid compounds in subsect. *Carpinus* of genus *Carpinus*

	Taxa	Flavonoid distribution[a]									
		Flavonol						Flavone		IF	Un
		My	Qu		Km			A	L		
		1	2	3	4	5	6	7	8	9	10
japonica-type	*C. viminea* var. *viminea*	×	×	×	×	×	×				
	C. viminea var. *chiukiangensis*	×	×		×	×					
	C. laxiflora	×	×	×	×	×	×			×	
	C. caroliniana	×	×	×	×	×	×			×	×
fangiana-type	*C. londoniana*	×		×				×	×		
	C. tientaiensis		×			×		×	×		
	C. betulus	×	×					×	×		

C. laxifora var. *longispica* and *C. londoniana* var. *lanceolata* were not shown because they had same chemical profiles as their original varieties.

[a] Flavonoid code: IF, isoflavone; My, myricetin; Qu, quercetin; Km, kaempferol; A, apigenin; Lu, luteolin; 1, My 3-*O*-glucoside; 2, Qu 3-*O*-glucoside; 3, Qu 3-*O*-rhamnoglucoside; 4, Km 3-*O*-rhamnoside; 5, Km 3-*O*-galactoside; 6, Km 3-*O*-rhamnoglucoside; 7, A 7-*O*-glucoside; 8, Lu 7-*O*-glucoside; 9, genistein; 10, unknown.

Within the *fangiana*-type, besides flavones, myricetin and quercetin were detected in *C. londoniana* and *C. betulus*, while quercetin and kaempferol in *C. tientaiensis*. Since plants with myricetin compounds were deemed more primitive (Gornall and Bohm, 1978), *C. tientaiensis* confined in Mt. Tientai of Zhejiang province in China is chemically more advanced than both *C. londoniana* and *C. betulus*. However, in spite of disjunct distribution, no chemical resolution was achieved between *C. londoniana* in China and *C. betulus* in Europe.

Among the species of the *japonica*-type, *C. viminea* var. *chiukiangensis*, distributed in southwestern Asia including India, Nepal, and Myanmar, has fewer compounds than *C. viminea* var. *viminea*, *C. laxiflora*, and *C. caroliniana*. It seems plausible that *C. viminea* var. *chiukiangensis* is more derived through the loss of flavonoid compounds.

Character evolution in subsect. *Carpinus* is considered that the lobe of the inner margin is differentiated from the folded state to the unfolded, and that the size of lobe from small to large. Other characters were also incongruent with respect to the divergence of two chemical groups. The leaf petioles of the species of the *fangiana*-type are densely pubescent or villous, whereas those in the *japonica*-type are glabrous, rarely sparsely pubescent. The leaf texture in *C. tientaiensis* and *C. londoniana* was subleathery, whereas in other species it was papery. However, although these morphological segregations matched chemical differentiations, it was more likely that these morphological and chemical characters had evolved independently within sections (Jeon, 2004).

Chemical dichotomy could be observed in other genera. The biosynthetic capacity for flavone *C*- or *O*-glycosides production has existed in some taxa for at least 17–22 million years (*Quercus*, Niklas and Giannasi, 1978; *Acer*, section *Palmata*, Chang and Giannasi, 1991; *Diosporus*, Williams et al., 1993). Likewise, the complete chemical divergence of *Carpinus* presumably occurred in the mid-Tertiary in China. As it is evidenced by the fossil records and the chemical constituents of genus, flavonoid dichotomy might precede morphological divergence, or chemical parallelism might follow morphological divergence.

From the survey of the whole section *Carpinus* (Jeon, 2004), subsect. *Monbeigianae* (*sensu lato*) contained the greatest number of compounds (21), while subsect. *Carpinus* was considered more derived morphologically with reduced flavonoid chemistry (10 compounds). In the cladistic analysis using morphological and ITS data, Yoo and Wen (2002) suggested subsect. *Monbeigianae* is more primitive than subsect. *Carpinus*. Although a significant reduction in flavonoid diversity was present in the most morphologically primitive taxa of sect. *Distegocarpus* (Chang and Jeon, 2004), a major trend in the reduction of the number of compounds from the primitive to the advanced was observed at least for sect. *Carpinus*. According to Harborne (1977) and Gornall and Bohm (1978), it is generally supported that evolution was accompanied by a reduction in structural complexity and flavonoid diversification. Therefore, chemical simplification seemed to be associated with species differentiation within this section (Figs. 1 and 2).

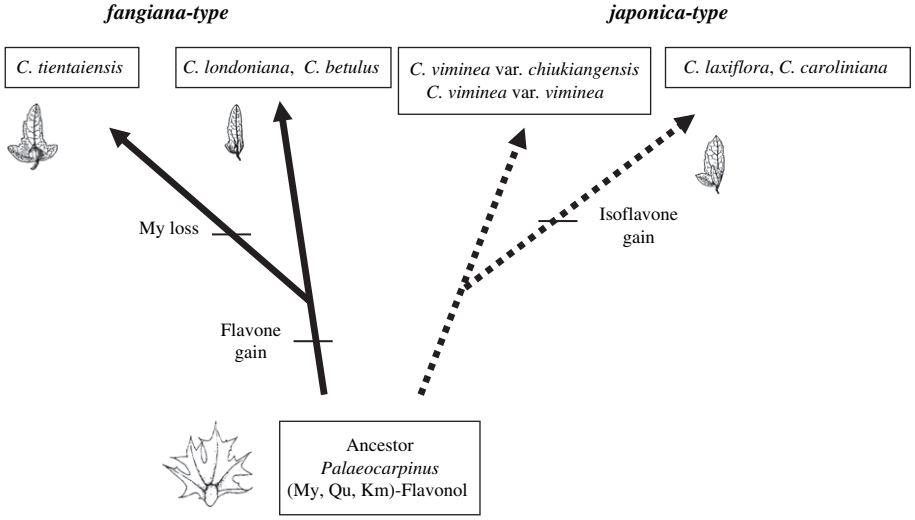

Fig. 1. Biosynthetic relationships and occurrences of flavonoids compounds among taxa of subsect. *Carpinus* of sect. *Carpinus*, genus *Carpinus*. My, myricetin; Qu, quercetin; Km, kaempferol. [Illustrations of bracts: *Palaeocarpinus*, Crane (1989); the others, Li and Cheng (1979)].

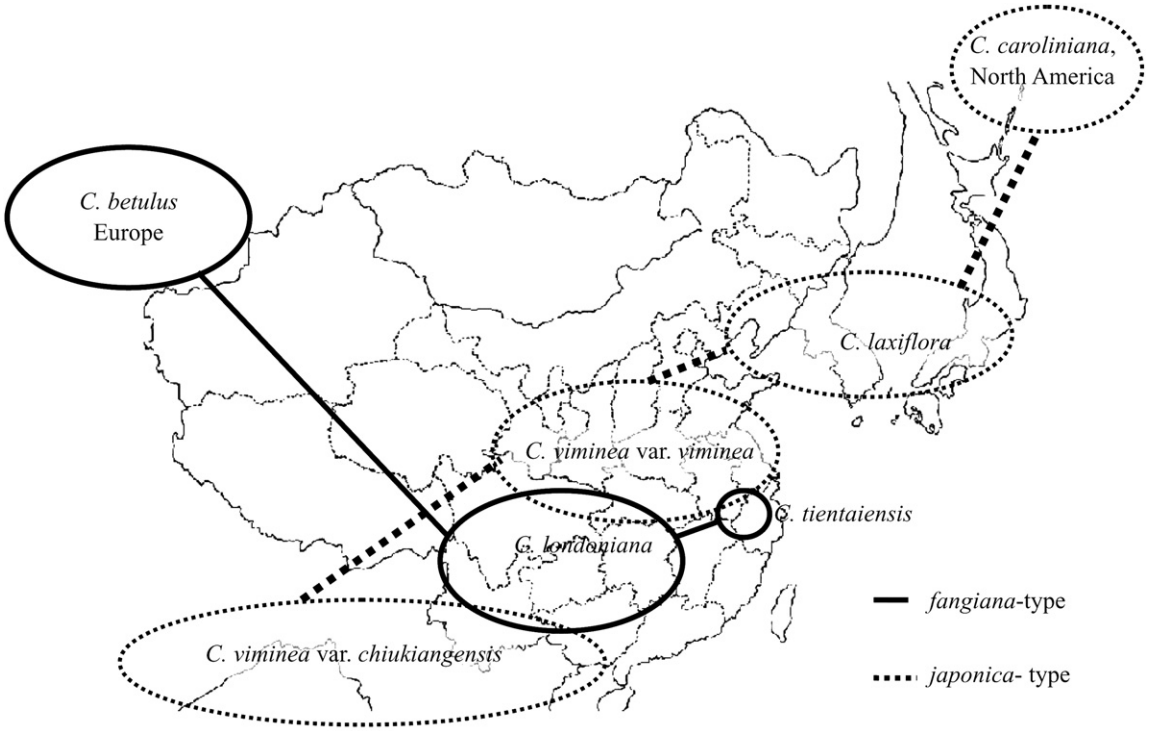

Fig. 2. Distribution and chemical relationship of subsect. *Carpinus* of sect. *Carpinus*, genus *Carpinus*.

In the survey of ca. 20 taxa of subsect. *Monbeigianae* (*sensu lato*; Jeon, 2004), a total of 21 flavonoid glycosides were found. The general classes of flavonoid compounds were flavonols and flavones. Unlike subsect. *Carpinus*, within the flavonols, diverse kaempferol, quercetin, and myricetin glycosides were detected, all as 3-*O*-glycosides. Two distinctive chemical groups, *japonica* and *fangiana*-types were also confirmed in this subsection. However, only a few species including *Carpinus kweichowensis* Hu, *Carpinus henryana* (H.J.P. Winkl.) H.J.P. Winkl., *Carpinus stipulata* H.J.P. Winkl., and *Carpinus sungpanensis* W.Y. Hsia were found to be *fangiana*-type and all the other remaining taxa belonged to *japonica*-type. This *japonica*-type of subsect. *Monbeigianae* contained four unique myricetin 3-*O*-glycosides, six quercetin 3-*O*-glycoses and two kaempferol 3-*O*-glycosides, which were not found from subsect. *Carpinus*. It is quite apparent that subsect. *Monbeigianae* is a distinct subsection.

The primitive group was characterized by a higher flavonoid diversity composed of a wider variety of flavonols, while, in contrast, the derived species was generally characterized by reduced flavonoid profiles with the presence of flavone. Mabry (1974) insisted that within the genus, the more advanced taxa have fewer and structurally simpler compounds than that in the more primitive members. Even though there were many exceptions, Mabry hypothesized that mutation loss occurs more frequently than mutation gain during the course of speciation (Gornall and Bohm, 1978; Wells and Bohm, 1980; Pacheco et al., 1991). Subsect. *Carpinus* was considered highly derived morphologically with reduced flavonoid chemistry, while subsect. *Monbeigianae* contained the greatest number of compounds (10 vs. 21 compounds; Jeon, 2004). In the analysis based on morphology and ITS sequences, Yoo and Wen (2002) also supported that subsect. *Monbeigianae* was more primitive than subsect. *Carpinus*. Regarding the complexity of flavonoid chemistry and phyletic advancement, it appears that the biosynthetic steps were mainly directed towards simplification within this section. Therefore, chemical simplification seems to be associated with species differentiation (Harborne, 1977; Gornall and Bohm, 1978) in section *Carpinus*.

The disjunct biogeographic pattern such as *C. betulus* in Europe vs. *C. londoniana* in China and *C. caroliniana* in North America vs. *C. laxiflora* in eastern Asia, respectively, might be remnants of the morphological adaptation (Fig. 2). The isoflavone in *C. laxiflora* and *C. caroliniana* revealed the early chemical divergence of these taxa, because isoflavone compounds were present at the primitive stage in the biosynthetic pathway (Gornall and Bohm, 1978). On the other hand, the differentiation of the European taxon, *C. betulus* from the ancestor was possibly related to

the polyploidization. The known chromosome number of *Carpinus* was $2n = 16$, but exceptionally $2n = 64$ in *C. betulus* (Darlington and Ammal, 1945; Goldblatt and Davidse, 1984). The fact that diploid (*C. londoniana* and *C. tientaiensis*) and polyploid (*C. betulus*) cytotypes had retained similar chemical profiles suggested that in *C. betulus* polyploidy per se had not contributed to chemical differentiation. Some similar results were described by Crawford (1970) in *Coreopsis mutica*, Doyle (1983) in *Claytonia*, Soltis and Bohm (1986) in *Tolmiea menziesii*, and though these were in contrast to the finding of Levy and Levin (1971, 1974, 1975) and Levy (1976) in *Phlox*, Murray and Williams (1973, 1976) in *Briza*. Based on its uniform flavonoid chemistry, *C. betulus* seemed to be a simple autopolyploid from its diploid ancestor, rather than an allopolyploid derivative of crosses between diploid plants with different patterns (Giannasi and Crawford, 1986). Overall, the intercontinental divergence of *Carpinus* was not confirmed by the present study. It would be surprising if such a long separation combined with climatic and other differences did not result in a selective differentiation of chemistry as well as of ITS profile among taxa.

Most of the infraspecific taxa in subsect. *Carpinus* were found to be chemically identical to their original varieties except that *C. viminea* var. *chiukiangensis* differed from var. *viminea* in the absence of quercetin 3-*O*-rhamnoglucoside and kaempferol 3-*O*-rhamnoglucoside.

In fact, among extant Chinese species of section *Carpinus*, many taxa must be considered descendants of a genetically and morphologically rather weakly differentiated from their ancestors. As Furlow (1987a,b) indicated, species are too finely distinguished in many Asian taxa including subsect. *Monbeigianae*. The absolute congruence between flavonoid data and taxonomic delimitation for subsect. *Monbeigianae* was not supported from our analysis (Jeon, 2004). It seems that the previous taxonomic treatments in this subsection do not represent valid taxonomic groups. Likewise *C. viminea* and *C. laxiflora* in subsect. *Carpinus* are weakly differentiated, so that *C. laxiflora* are recommended to be treated as a variety of *C. viminea*.

Flavonoid and morphological data provide a more efficacious means of identifying probable natural groups of related taxa within the subsection. Subsect. *Carpinus* appears to be heterogeneous with two clear chemical groups, but neither of these groups was found to correlate with any of the published classifications. These flavonoid evolutionary trends reflect intrasectional parallelisms and intersectional radiation.

Acknowledgements

This study was supported by the Research Grant of Seoul National University (98.1-06-2017) to C.-S. Chang, the Research Grant for a Ph.D. student from the Korea Research Foundation to J.I. Jeon, and the Young Scientist Exchange Program between Korea and China of the year 1996 from the Korea Science and Engineering Foundation to J.I. Jeon. We thank the following people for their help in field collection, exchange or loan of specimens. Professor Jun-Qiu Qian, Dr. Zeng-Lai Xu, and Mr. Quan-Jin Ren of Nanjing Botanic Garden, Ms. Quan Xing and Ms. Yu-Dan Tang of Botanic Garden of the Botanical Institute of Beijing, Dr. Hai-ning Qin of Botanical Institute of Beijing, professor Cheng-Xin Fu of Hangzhou University, professor Ren-Qing Wang of Shandong University, Mr. Hai-Duan Sun of Forest Research Institute of Shandong Province, and Mr. Yi Qian of Forest Bureau of Yunnan Province. We owed to herbariums (A, BM, NA, PE, US) for their loan of valuable specimens.

References

Chang, C.S., Giannasi, D.E., 1991. Foliar flavonoids of *Acer* sect. *Palmata* series *Palmata*. Syst. Bot. 16, 225–241.

Chang, C.-S., 2000. Foliar flavonoids of eastern Asian birch (*Betula*) – with respect to Korean plants. Korean J. Plant Taxon. 30, 75–91 (in Korean).

Chang, C.-S., Jeon, J.I., 2004. Foliar flavonoids of *Carpinus*, sect. *Distegocarpus* in eastern Asia. Biochem. Syst. Ecol. 32, 35–44.

Crane, P.R., 1989. Early fossil history and evolution of the Betulaceae. In: Crane, P.R., Blackmore, S. (Eds.), Evolution, Systematics and Fossil History of the Hamamelidae, vol. 2. Systematics and Association Special, vol. 40B. Clarendon, Oxford, pp. 87–116.

Crawford, D.J., 1970. Systematic studies on Mexican *Coreopsis* (Compositae). *Coreopsis mutica*: flavonoid chemistry, chromosome numbers, morphology, and hybridization. Brittonia 22, 93–111.

De Candolle, C., 1893. Sur les bractées florifères. Bull. Herb. Boisser. 1, 124–127.

Darlington, C.D., Ammal, E.K.J., 1945. Chromosome Atlas of Cultivated Plants. George Allen and Unwin, London.

Doyle, J.J., 1983. Flavonoid races of *Claytonia virginica* (Portulacaceae). Am. J. Bot. 70, 1085–1091.

Furlow, J.J., 1987a. The *Carpinus caroliniana* complex in North America. I. A multivariate analysis of geographical variation. Syst. Bot. 12, 21–40.

Furlow, J.J., 1987b. The *Carpinus caroliniana* complex in North America. II. Systematics. Syst. Bot. 12, 416–434.

Furlow, J.J., 1990. The genera of Betulaceae in the Southeastern United States. J. Arnold Arbor. 71, 1–67.
Giannasi, D.E., 1975. The flavonoid systematics of the genus *Dahlia* (Compositae). Mem. NY. Bot. Gard. 26, 1–128.
Giannasi, D.E., Crawford, D.J., 1986. Biochemical Systematics II. A reprise. In: Hecht, M.K., Wallace, B., Prance, G.T. (Eds.), Evolutionary Biology, vol. 20. Plenum Press, New York, London, pp. 25–248.
Goldblatt, P., Davidse, G., 1984. Index to plant chromosome numbers 1979–1981. Monogr. Syst. Bot. Mo. Bot. Gard. 8, 110.
Gornall, R.J., Bohm, B.A., 1978. Angiosperm flavonoid evolution: a reappraisal. Syst. Bot. 3, 353–368.
Harborne, J.B., 1977. Flavonoids and the evolution of the Angiosperms. Biochem. Syst. Ecol. 5, 7–22.
Hillier, J., 1988. Manual of Trees and Shrubs. Hillier Nurseries (Winchester), Ltd., Ampfield House, Ampfield, Romsey.
Jeon, J.I., Chang, C.-S., 1997. Reconsideration of *Carpinus* L. (Betulaceae) of Korea primarily based on quantitative characters. Korean J. Plant Taxon 27, 157–187 (in Korean).
Jeon, J.I., Chang, C.-S., 2000. Foliar flavonoids of genus *Carpinus* in eastern Asia - primarily based on native taxa to Korea. Korean J. Plant Taxon 30, 139–153 (in Korean).
Jeon, J.I., 2004. Systematic Implication of Foliar Flavonoids in *Carpinus* L. (Betulaceae). Ph.D. dissertation, Seoul National University, Seoul, 218 pp.
Jones, S.B., Luchsinger, A.E., 1986. Plant Systematics. McGraw-Hill, Inc., New York.
Levy, M., Levin, D.A., 1971. The origin of novel flavonoids in *Phlox* allotetraploids. Proc. Nat. Acad. Sci. USA 68, 1627–1630.
Levy, M., Levin, D.A., 1974. Novel flavonoids and reticulate evolution in the *Pholox pilosa–P. drummondii* complex. Am. J. Bot. 61, 156–167.
Levy, M., Levin, D.A., 1975. The novel flavonoid chemistry and phylogenetic origin of *Phlox floridana*. Evolution 29, 487–499.
Levy, M., 1976. Altered glycoflavone expression in induced autotetraploids of *Phlox drummondii*. Biochem. Syst. Ecol. 4, 249–259.
Li, P.-C., Cheng, S.-H., 1979. Betulaceae. In: Kuang, K.Z., Li, P.C. (Eds.), Flora Reipublicae Popularis Sinicae, vol. 21. Science Press, Beijing, pp. 44–137 (in Chinese).
Mabry, T.J., 1974. Chemistry of disjunct taxa. In: Bendz, G.J.S., Runnstrom-Reio, V. (Eds.), Chemistry in Botanical Classification. Nobel Symposium, vol. 25. Academic Press, New York, pp. 63–66.
Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York.
Markham, K.R., 1982. Techniques of Flavonoid Identification. Academic Press, New York.
Murray, B.G., Williams, C.A., 1973. Polyploidy and flavonoid synthesis in *Briza media* L. Nature (London) 243, 87–88.
Murray, B.G., Williams, C.A., 1976. Chromosome number and flavonoid biosynthesis in *Briza* L. (Gramineae). Biochem. Genet. 14, 897–904.
Niklas, K.J., Giannasi, D.E., 1978. Angiosperm paleobiochemistry of the Succor Creek flora (Miocene), Oregon, USA. Am. J. Bot. 65, 943–952.
Ohwi, J., 1984. Flora of Japan. Smithsonian Institute, Washington, DC.
Pacheco, P., Crawford, D.J., Stuessy, T.F., Silva, M.O., 1991. Flavonoid evolution in *Dendroseris* (Compositae, Lactuceae) from the Juan Fernandez Islands, Chile. Am. J. Bot. 78, 534–543.
Pridham, J.B., 1956. Determination of sugars on paper chromatograms with *p*-anisidine hydrochloride. Anal. Chem. 28, 1967–1968.
Rehder, A., 1927. Manual of Cultivated Trees and Shrubs, Hardy in North America. MacMillan Publishing Co., Inc., New York.
Soltis, D.E., Bohm, B.A., 1986. Flavonoid chemistry of diploid and tetraploid cytotypes of *Tolmiea menziesii* (Saxifragaceae). Syst. Bot. 11, 20.
Spach, E., 1841. Revisio Betulacearum. Ann. Sci. Nat., Bot. Sér. 2 (15), 182–212.
Spach, E., 1842. Notes sur les *Carpinus*. Sci. Nat., Bot. Sér. 2 (16), 248–254.
Wells, E.F., Bohm, B.A., 1980. Chemotaxonomic studies in the Saxifragaceae S.I. 15. The flavonoids of subsection *Villosae* section *Heuchera* in the genus *Heuchera*. Can. J. Bot. 58, 1459–1463.
Williams, C.A., Richardson, J., Greenham, J., Eagles, J., 1993. Correlations between leaf flavonoids, taxonomy and plant geography in the genus *Disporum*. Phytochemistry 34, 197–203.
Winkler, H., 1904. Betulaceae. In: Engler, A. (Ed.), Das Pflanzenreich, IV, 61. W. Engelmann, Leipzig, pp. 1–149.
Yoo, K.O., Wen, J., 2002. Phylogeny and biogeography of *Carpinus* and subfamily Coryloideae (Betulaceae). Int. J. Plant Sci. 163, 641–650.