Intercontinental genetic divergence of *Castanea* species in eastern Asia and eastern North America

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Castanea is one of the many plant genera with a disjunct distribution pattern between eastern Asia and eastern North America. Five species from three sections of the genus were investigated to examine genetic divergence between eastern Asian and eastern North American species. A total of 62 native populations were sampled for allelic variation at isozyme loci. The Chinese chestnut *C. mollissima* had the highest genetic variability, while the American *C. dentata* had the lowest genetic variability. The highest intracontinental genetic identities were observed between the Allegheny and Ozark chinkapins (0.931) and between *C. mollissima* and *C. seguinii* (0.870), while lower identities were detected between the American *C. pumila* and *C. dentata* (0.720–

0.729). In intercontinental comparisons, genetic identities of 0.505, 0.495 and 0.507 were observed between the American chestnut and the Chinese *C. mollissima*, *C. seguinii* and *C. henryi*, respectively, whereas the Ozark chinkapin *C. pumila* var. *ozarkensis* had lower identities of 0.469, and 0.435 with *C. mollissima* and *C. seguinii*, respectively, but a slightly higher identity of 0.520 with *C. henryi*, the Chinese chinkapin. Divergence times were estimated at 10–13 million years before present between *C. dentata* and *C. mollissima*, and *C. pumila* var. *ozarkensis* and *C. henryi*.

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Introduction

The study of the floristic similarity among temperate forests of North America, Europe and Asia dates back to the 18th century, when Halenius, a student of Linnaeus, first described the phenomenon. Scientists soon recognized that this disjunction spanned all temperate forests of the northern Hemisphere (Boufford and Spongberg, 1983). It is now recognized that the complex history of tectonics, climate fluctuations, repeated emergence and submergence of land bridges, multiple migrations and other factors have led to the current distributions of northern temperate forests, which were contiguous during the Miocene (15 million years before present) when the northern continents were in contact (Wen, 1999; Guo and Ricklefs, 2000).

Castanea Miller is one of at least 65 plant genera with a disjunct distribution pattern between eastern Asia and eastern North America (Wen, 1999). The genus comprises three sections and seven species (Johnson, 1988). Section *Castanea*, characterized by the presence of three nuts per cupule, contains five species of varying economic importance as nut tree crops, and is widely distributed over the temperate zone of the Northern Hemisphere, in eastern and western Asia, eastern North America and southern Europe. Three species, Chinese chestnut

(*C. mollissima* Bl.), Seguin chestnut (*C. seguinii* Dode.) and Japanese chestnut (*C. crenata* Sieb. & Zucc.) occur in eastern Asia. Chinese chestnut has a natural range over much of mainland China except western deserts and plateaus, and Seguin chestnut occurs sympatrically with Chinese chestnut over central and southwest China (Lang and Huang, 1999). Japanese chestnut (*C. crenata* Sieb. & Zucc.) is found on the Japanese islands and Korean peninsula (Jaynes, 1975). American chestnut (*Castanea dentata* (Marsh.) Borkh.) is native to eastern North America, growing from southern Maine south to Georgia, Alabama and Mississippi, and west to southern Ontario and Michigan (Saucier, 1973). European chestnut (*C. sativa* Mill.) is native to western Asia and southern Europe (Villani *et al*, 1994). Section *Balanocastanon*, characterized by one nut per cupule, has a single species *C. pumila* Mill., commonly called American chinkapin. This species comprises two varieties: var. *pumila*, the Allegheny chinkapin, with a wide distribution from southern New Jersey and Pennsylvania, west to Indiana and Missouri, and south to Florida and Texas, and var. *ozarkensis* (Ashe) Tucker, the Ozark chinkapin, with limited and fragmented distribution in the Ozark Mountains of eastern Oklahoma, southwest Missouri, and north-central Arkansas (Johnson, 1988). Section *Hypocastanon* contains only one species, *C. henryi* (Skan) Rehder & Wilson, which is found in a restricted area in southeast China, and is sometimes called Chinese chinkapin since it is characterized by a single nut per cupule (Lang and Huang, 1999). The geographical distribution of *Castanea* species in China and the United States has a similar

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pattern in that geographically restricted species (*C. sequinii* and *C. henryi* in China, and *C. pumila* in the US) overlap widespread species (*C. mollissima* in China and *C. dentata* in the US).

Adequate data on basic biology, genetics and inter-specific crossing compatibility, as well as fossil records of *Castanea* species are available largely because of their economic importance as nut tree crops. Relatively few subtle characters (nut numbers per cupule, cupule valves, leaf blade and vestiture types) are useful for distinguishing the species (Graves, 1949; Johnson, 1988). All species are diploid ($2n=2x=24$), wind pollinated and hybridize freely, although some interspecific F_1 's usually suffer from low seed germination and male sterility (Jaynes, 1975).

The historical event of chestnut blight (a fungal disease caused by *Cryphonectria parasitica* (Murrill) Barr), which devastated *Castanea* species in North America since the beginning of the 20th century and in Europe since 1930, remains an unsolved problem for plant breeders and horticulturists worldwide. Blight resistance among the species differs: the American and the European species are susceptible, while the Asian species are blight resistant (Graves, 1950; Huang *et al.*, 1996). The American chestnut is most susceptible (Graves, 1950) and the narrow genetic diversity of the American chestnut as compared to congeneric species and closely related *Quercus* species may have contributed to its demise (Huang *et al.*, 1998). It was hypothesized that the Asian (progenitor) species evolved in the presence of the disease epidemic and retained disease resistance, while the American chestnut lost its resistance as it evolved in the disease-free environment on the North American continent (Jaynes, 1975).

Fossils of *Castanea* have been found throughout the Tertiary in the Northern Hemisphere: Japan (Oligocene); North Korea (Miocene); far eastern Russia (late Eocene, Miocene and early Miocene); Siberia and western Siberia (early Miocene); eastern Russia (late Oligocene) and central Russia (late Eocene, Oligocene, late Miocene and middle Miocene); western Asia (Oligocene); eastern Europe (early Eocene and late Eocene, Oligocene, late Oligocene and early Oligocene, Miocene, middle Miocene and late Miocene, Pliocene and late Pliocene); central Europe (late Eocene, Oligocene, late Oligocene and early Oligocene, late Miocene and middle Miocene, Pliocene); southern Europe (Pliocene); northwestern Europe (Paleocene and Pliocene) (International Organization of Palaeobotany, 1997); northeastern, eastern and southwestern China (late Eocene, Miocene and Pliocene) (International Organization of Palaeobotany, 1997; Zhou, 1999); and North America (Eocene and Miocene) (Crepet and Daghljan, 1980; Zhou, 1999). Although accuracy of the fossil record needs to be considered, *Castanea*, like other genera in Fagaceae, most likely appeared as early as the Paleocene and reached the widest distribution during Miocene (Zhou, 1999). Little is known about the evolutionary and genetic relationship of these morphologically similar but intercontinentally isolated species of *Castanea*.

The evolution of eastern Asia and eastern North American disjunct distributions of flowering plants has recently been reviewed by Wen (1999). While phylogeographic studies did confirm affinities between many of the disjunct taxa, patterns of diversification of species on

one or both continents were found and disjunct species pairs were rarely each other's closest relatives (Wen, 1999; Guo and Ricklefs, 2000). In many woody plant genera, current estimates of divergence time using molecular and fossil data suggest that the disjunct patterns were established in the range of 2–25 mybp during the Miocene (Wen, 1999). Information on Fagaceae has so far been limited or inconclusive (Stanford, 1998). Chloroplast (cp) DNA studies on European chestnut (*C. sativa*) showed low levels of differentiation among populations, which has been related to the impact of human colonization (Fineschi *et al.*, 2000). The purpose of the present investigation was to: (1) determine evolutionary relationships between eastern Asian and North American *Castanea* species using allozyme analysis of a large number of wild populations and (2) compare divergence in this genus with that of other woody angiosperms with eastern Asia–eastern North American disjunct taxa.

Materials and methods

Plant material

In North America, 12 populations of *C. dentata* were sampled across the native range along Appalachia from Alabama to central New York, during 1994 and 1995; nine populations of *C. pumila* var. *ozarkensis* were sampled in the centre of its native habitat of the Ozark mountains in Arkansas, during 1996–1997; 11 populations of *C. pumila* var. *pumila* were sampled in the southeastern states during 1998–2000 (Table 1, Figure 1a).

In People's Republic of China, 21 wild populations of *C. mollissima* were sampled in 1997 from northern, central and southwestern China in the native range of the species, representing the entire diversity of the species; six populations of *C. seguinii* and three populations of *C. henryi* were sampled in 1997 in central China, the centres of the species' range, except one population of *C. seguinii* was sampled from Guangxi, southern China (Table 1, Figure 1b).

Samples of most populations were collected as seeds from which cotyledonary (diploid) tissues yielded the highest quantity and quality of isozymes, and produced the most consistent and reproducible banding patterns. For *C. dentata* populations and a few populations of other species from which seeds could not be obtained, 1-year-old dormant twigs with mature buds were taken from trees, kept in zip-lock bags at 4°C until electrophoresis was conducted. Enzymes extracted from cotyledonary tissues of seeds or mature buds of one-year-old twigs had been tested earlier for identity and reliability (Huang *et al.*, 1994a).

Allozyme analysis

Procedures for allozyme electrophoresis, genetic analysis and allele designation of the loci have been described in detail (Huang *et al.*, 1994a,b; Dane *et al.*, 1999; Dane and Huang, 2002). The isoelectric focusing polyacrylamide slab gel system (pH 4–9) or precast agarose gels (pH 4–6) were used. Gels were assayed for acid phosphatase (ACP; EC 3.2.3.2), alcohol dehydrogenase (ADH; EC 1.1.1.1), aconitase (AK; EC 4.2.1.3), diaphorase (DIA; EC 1.8.1.41), esterase (EST; EC 3.1.1), formate dehydrogenase (FDH; EC 1.2.1.2), glutamate dehydrogenase (GDH; EC

Table 1 Populations of *Castanea* sampled for allozyme divergence between eastern Asia and eastern North America

Species	Population	Size	No loci	Code	Location
<i>C. dentata</i>	Am-94-1	20	18	AL	Macon Co, AL, USA
	Am-94-2	21	18	GA	Black Rock Mt. State Park, GA, USA
	Am-94-3	21	18	NC1	Nantahala National Forest, NC, USA
	Am-94-4	29	18	NC2	Thomas Ridges, Great Smoky Mt, NC, USA
	Am-94-5	17	18	VA1	Glade, Smyth Co, VA, USA
	Am-94-6	28	18	VA2	Plummer, Smyth Co, VA, USA
	Am-94-7	25	18	PA1	York Co, PA, USA
	Am-94-8	21	18	PA2	Southern PA, USA
	Am-94-9	31	18	OH	Carroll Co, OH, USA
	Am-94-10	20	18	MI	Southeastern MI, USA
	Am-94-11	16	18	NY	Troy, NY, USA
	Am-94-12	11	18	CT	Essex, CT, USA
<i>C. pumila</i> var. <i>ozarkensis</i>	Oz-97-1	15	14	OZ1	Sylamore Ranger District, AR, USA
	Oz-97-2	46	14	OZ2	Sylamore Ranger District, AR, USA
	Oz-97-3	29	14	OZ3	Sylamore Ranger District, AR, USA
	Oz-97-4	38	14	OZ4	Sylamore Ranger District, AR, USA
	Oz-98-1	36	14	OZ5	Sylamore Ranger District, AR, USA
	Oz-98-2	35	14	OZ6	Sylamore Ranger District, AR, USA
	Oz-98-3	7	14	OZ7	Sylamore Ranger District, AR, USA
	Oz-98-4	8	14	OZ8	Sylamore Ranger District, AR, USA
	Oz-98-5	27	14	OZ9	Newton Co, AR, USA
<i>C. pumila</i> var. <i>pumila</i>	Pu-98-1	96	15	AL-U	Uchee, AL, USA
	Pu-98-2	36	15	FL-E	Eglin Air Force Base, FL, USA
	Pu-98-3	36	15	FL-G	Eglin Air Force Base, FL, USA
	Pu-98-4	17	15	FL-I	Eglin Air Force Base, FL, USA
	Pu-98-5	82	15	FL-L	Eglin Air Force Base, FL, USA
	Pu-98-6	70	15	OH-p	Carroll Co, OH, USA
	Pu-98-7	206	15	VA	Smyth Co, VA, USA
	Pu-98-8	126	15	VB	Smyth Co, VA, USA
	Pu-99-1	21	15	MS	Leake Co, MS, USA
	Pu-99-2	65	15	FL-B	Eglin Air Force Base, FL, USA
	Pu-99-3	60	15	FL-D	Eglin Air Force Base, FL, USA
<i>C. mollissima</i>	Ch-97-1	24	20	BJ-c	Changping Co, Beijing, China
	Ch-97-2	24	20	BJ-h	Huairou Co, Beijing, China
	Ch-97-3	24	20	HB-f	Fangxian Co, Hubei, China
	Ch-97-4	24	20	SNJ	Shengnongjia Mt, Hubei, China
	Ch-97-5	24	20	HB-y	Yichang Co, Hubei, China
	Ch-97-6	24	20	HB-z	Zigui Co, Hubei, China
	Ch-97-7	24	20	HB-w1	Wufeng Co, Hubei, China
	Ch-97-8	24	20	HB-w2	Wufeng Co, Hubei, China
	Ch-97-9	24	20	HB-1	Luotian Co, Hubei, China
	Ch-97-10	24	20	HB-t	Tongcheng Co, Hubei, China
	Ch-97-11	24	20	HN-y	Yongshun Co, Hunan, China
	Ch-97-12	24	20	GZ-b	Bijie Co, Guizhou, China
	Ch-97-13	24	20	GZ-y	Yuping Co, Guizhou, China
	Ch-97-14	24	20	GX-n	Nandan Co, Guangxi, China
	Ch-97-15	24	20	GX-x	Xingan Co, Guangxi, China
	Ch-97-16	24	20	GX-z	Ziyuan Co, Guangxi, China
	Ch-97-17	24	20	GX-d	Donglan Co, Guangxi, China
	Ch-97-18	24	20	YU-11	Luquan Co, Yunnan, China
	Ch-97-19	20	20	YU-12	Luquan Co, Yunnan, China
	Ch-97-20	25	20	YU-y	Yiliang Co, Yunnan, China
	Ch-97-21	24	20	YU-m	Malipo Co, Yunnan, China
<i>C. seguinii</i>	Ch-97-22	24	20	HB-ys	Yichang Co, Hubei, China
	Ch-97-23	24	20	HB-zs	Zigui Co, Hubei, China
	Ch-97-24	24	20	HB-t1	Tongcheng Co, Hubei, China
	Ch-97-25	24	20	HB-t2	Tongcheng Co, Hubei, China
	Ch-97-26	24	20	HB-ls	Luotian Co, Hubei, China
	Ch-97-27	22	20	GX-xs	Xingan Co, Guangxi, China
<i>C. henryi</i>	Ch-97-28	21	20	HB-yh	Yichang Co, Hubei, China
	Ch-97-29	24	20	HB-fh	Fangxian Co, Hubei, China
	Ch-97-30	24	20	GX-zh	Ziyuan Co, Guangxi, China

Figure 1 (a) Location of natural populations of *Castanea dentata*, *C. pumila* var. *ozarkensis* and *C. pumila* var. *pumila* collected in North America. (b) Location of natural populations of *C. mollissima*, *C. seguinii* and *C. henryi* collected in People's Republic of China.

1.4.1.2), isocitrate dehydrogenase (IDH; EC 1.1.1.41), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.40), phosphoglucosomerase (PGI; EC 5.3.1.3), phosphogluconate dehydrogenase (PGD; EC 1.1.1.44), phosphoglucomutase (PGM; EC 5.4.2.2), peroxidase (PRX; EC 1.11.1.7), shikimate dehydrogenase (SKD; EC 1.1.1.25), superoxide dismutase (SOD; EC 1.12.1.1) and triosephosphate isomerase (TPI; EC 5.3.1.1). The following loci were scored when possible: *Acp-1*, *Acp-2*, *Acp-3*, *Adh-1*, *Ak-2*, *Dia-1*, *Dia-2*, *Dia-3*, *Dia-4*, *Dia-6*, *Est-1*, *Est-2*, *Est-4*, *Est-5*, *Fdh*, *Gdh*, *Idh*, *Mdh-2*, *Me*, *Pgi-1*, *Pgi-2*, *Pgd*, *Pgm*, *Prx-1*, *Prx-2*, *Prx-3*, *Prx-4*, *Skd-1*, *Skd-2*, *Skd-3*, *Skd-4*, *Sod-1* and *Tpi* (Table 1).

Data analysis

Allele frequencies for isozyme loci were estimated (Nei, 1987), and a set of measures of intra- and interpopulation genetic statistics were generated using a computer program developed by BIOSYS-1 (Swofford and Selander, 1981) and NTSYS Version 2.1 (Rohlf, 2000). Nei's (1978) genetic distance and identity were calculated for all pairwise combinations of populations using the maximum number of shared loci. A dendrogram was constructed based on the matrix of Nei's distance using unweighted pair-group mean analysis (UPGMA). Divergence times were estimated using the rate of 1 unit of Nei's genetic distance per 15–20 mybp (Sarich, 1977).

Results

Castanea species in eastern Asian and eastern North America showed continent-specific loci, although the five species shared most loci and most high-frequency alleles. While *Skd-1* and *Skd-2* were observed in the American species, *Skd-3* and *Skd-4* were detected in the Chinese species and interspecific hybrids between *C. mollissima* and *C. dentata* showed all four *Skd* loci. *Pgi-2b* and *Pgi-2d* alleles were only detected in *C. mollissima*, with *Pgi-2b* unique to the Shennongjia population in central China. *C. henryi* could be distinguished from *C. mollissima* and *C. seguinii* by the absence of *Me-1c*. *Dia-6b* and *c* alleles were only detected in *C. pumila* var. *ozarkensis*.

Table 2 Allozyme variability measures averaged for all populations of each species in eastern Asia and eastern North America *Castanea* species

Species	Genetic diversity parameters							
	<i>P</i>	<i>A</i>	<i>H</i> _{obs}	<i>H</i> _{exp}	<i>H</i> _T	<i>G</i> _{ST}	<i>F</i>	<i>I</i>
<i>C. mollissima</i> ^a	90	2.2	0.284	0.311 (0.253–0.341)	0.321	0.075	0.087	0.963 (0.896–0.999)
<i>C. seguinii</i> ^a	82.4	2.1	0.192	0.186 (0.130–0.188)	0.187	0.109	–0.032	0.980 (0.947–0.999)
<i>C. henryi</i> ^a	76.5	2.1	0.218	0.261 (0.192–0.247)	0.282	0.221	0.165	0.935 (0.929–0.946)
<i>C. dentata</i> ^b	60	1.7	0.184	0.167 (0.096–0.196)	0.214	0.087	–0.091	0.972 (0.902–0.999)
<i>C. pumila</i> var. <i>pumila</i> ^c	72.7	1.9	0.206	0.296 (0.115–0.295)	0.296	0.137	0.187	0.980 (0.962–0.997)
<i>C. pumila</i> var. <i>ozarkensis</i> ^d	63.3	1.8	0.272	0.228 (0.191–0.265)	0.341	0.147	–0.117	0.977 (0.934–1.000)

P = percent polymorphic loci; *A* = number of alleles per locus; *H*_{obs} = observed heterozygosity; *H*_{exp} = Hardy-Weinberg expected heterozygosity; *H*_T = total genetic diversity; *G*_{ST} = relative magnitude of genetic differentiation among populations; *F* = fixation index; *I* = Identity.

^aLang and Huang (1999)

^bHuang et al (1998)

^cFu (2001)

^dDane et al (1999).

Table 3 Average genetic identity (above diagonal) and distance (below diagonal) between five *Castanea* species in eastern Asia and eastern North America

Species	<i>C. mollissima</i>	<i>C. seguinii</i>	<i>C. henryi</i>	<i>C. dentata</i>	<i>C. pumila</i> var. <i>pumila</i>	<i>C. pumila</i> var. <i>ozarkensis</i>
<i>C. mollissima</i>						
<i>C. seguinii</i>	0.139 ^(19a) (0.071–0.249)					
<i>C. henryi</i>	0.180 ⁽¹⁸⁾ (0.067–0.310)	0.870 (0.779–0.931)				
<i>C. dentata</i>	0.684 ⁽¹⁵⁾ (0.480–0.919)	0.167 ⁽¹⁸⁾ (0.099–0.259)	0.835 (0.733–0.935)			
<i>C. pumila</i> var. <i>pumila</i>	0.674 ⁽¹⁰⁾ (0.393–1.0)	0.709 ⁽¹³⁾ (0.521–0.832)	0.846 (0.772–0.906)	0.505 (0.399–0.619)		
<i>C. pumila</i> var. <i>ozarkensis</i>		0.749 ⁽¹⁰⁾ (0.547–1.0)	0.680 ⁽¹³⁾ (0.540–0.780)	0.492 (0.435–0.594)	0.510 (0.368–0.675)	
			0.712 ⁽¹⁰⁾ (0.552–1.0)	0.507 (0.548–0.583)	0.473 (0.368–0.579)	0.435 (0.368–0.552)
				0.328 ⁽¹¹⁾ (0.154–0.598)	0.491 (0.368–0.576)	0.520 (0.423–0.584)
					0.720 (0.550–0.857)	0.729 (0.551–0.865)
						0.930 (0.757–1.0)
					0.072 ⁽¹⁵⁾ (0–0.279)	

^aNumber of shared loci used for estimates.

The mean genetic variability for each species is given in Table 2. *C. mollissima* showed the highest population genetic variability, while *C. dentata* had the lowest genetic variability. The expected genotype frequencies at most loci in most populations of these two widespread species in eastern Asia and eastern North America, conformed to Hardy–Weinberg expectation ($P < 0.05$), with marginally positive fixation indices in *C. mollissima* (0.087) and negative fixation indices in *C. dentata* (–0.091). However, significant heterozygosity deficiencies ($P < 0.05$) were found in the narrowly distributed *C. henryi* ($F = 0.165$), and heterozygosity excess was detected in the restricted *C. pumila* var. *ozarkensis* ($F = -0.117$). High genetic variability was observed in *C. henryi* and *C. pumila* var. *ozarkensis*, even though *C. pumila* var. *ozarkensis* populations were sampled from a limited area in Arkansas and geographically isolated from *C. pumila* var. *pumila* populations (Table 2).

Nei's (1978) genetic identity and distance were calculated for all pairwise population comparisons and the results of averaged values using the maximum number of loci shared by the different species are presented in Table 3. In intracontinental species comparisons, high genetic identities were observed among the three Chinese species, ranging from 0.835 for *C. mollissima* and *C. henryi* to 0.870 between *C. mollissima* and *C. seguinii*. Among the American species, the highest identity (0.930) was found between two varieties of the American chinkapin, while the lowest (0.720) was between *C. dentata* and *C. pumila* var. *pumila*. In intercontinental comparisons, the North American *C. dentata* had genetic identities of 0.505, 0.495 and 0.507 with the Chinese *C. mollissima*, *C. seguinii* and *C. henryi*, respectively. The Ozark chinkapin, *C. pumila* var. *ozarkensis* had lower identities of 0.469, and 0.435 with *C. mollissima*, and *C. seguinii*, respectively, but higher identity of 0.520 with *C. henryi* (Table 3). These intracontinental and intercontinental relationships were reflected on the UPGMA dendrogram (Figure 2).

Discussion

The genetic variability found in *C. mollissima* is the highest among the *Castanea* species examined here, and higher than that previously reported for European chestnut (*C. sativa*, $H_{exp} = 0.24–0.27$) (Pigliucci *et al*, 1991; Villani *et al*, 1994). High levels of genetic variability were detected in *C. mollissima* populations distributed in the Changjian river area, particularly in Shennongjia. This region is well known for its variable topography, different climatic ranges influenced by mountain ranges. Warm climates during the last glaciation period have provided an ideal refuge area for many ancient endemic plant and animal species (such as Dawn redwood, *Metasequoia glyptostroboides*, and Panda, *Ailuropoda melanoleucus*). As a consequence central and southwestern populations maintained higher levels of genetic variability than northern populations at the margin of the species range. Many plant species were forced southward during the glacial maximum to refugia and migrated northward after the glaciers' retreat (Zheng, 1983).

The low genetic variability detected in *C. dentata*, which is as widespread as *C. mollissima*, might be the consequence or cause of the rapid decline of the species

Figure 2 UPGMA dendrogram of allozyme divergence based on Nei's genetic distance among 62 populations of five species in genus *Castanea*.

because of to chestnut blight (Huang *et al*, 1998). In contrast, relatively high genetic variability was found in more restricted species in both North America and China (*C. pumila* var. *pumila* and *C. sequinii*). The difference in genetic diversity between restricted (*C. henryi*) and regionally distributed species (*C. sequinii*) might reflect their evolutionary history. High genetic diversity was found in the narrowly distributed *C. pumila* var. *ozarkensis*, similar to that in regionally distributed var. *pumila* in North America.

Genetic divergence has been examined for several woody genera with disjunctions between eastern Asia and eastern North America, indicating a wide range in interspecific identities based on allozyme data: from $I=0.273$ between *Merispermum dauricum* and *M. canadense* (Lee *et al*, 1996) to $I=0.434$ between *Liriodendron chinense* and *L. tulipifera* (Parks and Wendel, 1990), and

$I=0.431$ between *Liquidambar formosana* and *L. styraciflua* (Hoey and Parks, 1991) to $I=0.712$ between *Magnolia officinalis* var. *biloba* and *M. tripetala* (Qui *et al*, 1995) and $I=0.809$ between *Magnolia officinalis* var. *biloba* and *M. hypoleuca* (Qui *et al*, 1995). In this study, the Ozark chinkapin exhibits higher divergence from the Chinese chestnut species (*C. mollissima* and *C. sequinii*) than the American chestnut and Allegheny chinkapin (Table 3), but lower divergence from the Chinese chinkapin (*C. henryi*) as compared to the Allegheny chinkapin. This is consistent with the hypothesis of Johnson (1988), who considered the Ozark chinkapin as ancestral and less highly evolved than the Allegheny chinkapin. The American *Castanea* species exhibit the highest divergence from *C. sequinii*, but similar divergence from *C. mollissima*, or *C. henryi*. *C. sequinii* and *C. henryi* appear derived from *C. mollissima*, with a reduction in the number of

nuts per cupule in *C. henryi* and development of precocious growth habit in *C. seguinii*. Reduction in the number of nuts per cupule might have evolved independently on both continents. Burs with two to three nuts instead of one have been observed at low frequency on *C. pumila* trees. Lack of significant morphological changes in a species over a long period of time or morphological stasis has been observed in eastern Asian and eastern North American disjunct tree genera such as *Liriodendron* (Parks and Wendel, 1990), *Liquidambar* (Hoey and Parks, 1991), *Magnolia* (Qui *et al*, 1995), *Gleditsia* and several other taxa (Wen, 1999). Evolutionary constraints and stabilizing selection are proposed to be major mechanisms for morphological stasis (Wen, 1999).

Estimation of divergence time using Nei's genetic distance translates to similar divergence time of 10–13 mybp between *C. dentata* and *C. mollissima*; *C. pumila* var. *pumila* and *C. mollissima*, *C. dentata* and *C. henryi*, *C. pumila* var. *ozarkensis* and *C. henryi*. Even though these estimates are in accordance with divergence times in several woody genera (between 2–25 mybp) with disjunct patterns using molecular and fossil data (Wen, 1999), the problem with the electrophoretic protein clock is that reliable independent estimates of species or population divergence are largely lacking (Nei, 1987).

In the case of the genus *Castanea*, it is reasonable to assume that extinct *Castanea* species existed from Paleocene to Pliocene in a much wider distribution of the Northern Hemisphere, and that species exchange remained via the Bering land bridge during the late Miocene to maybe early Pliocene, which is supported by fossil records (International Organization of Palaeobotany, 1997). Sargent (1896) mentioned the existence of *Castanea* in northern Greenland, and in Alaska and leaf impressions in Miocene rocks of Oregon, and Colorado. The ancient western American population was probably destroyed by the geological uplift of the Rocky Mountains and concomitant climate deterioration. Contemporary patterns of diversity suggest that the effects of climate changes in the late Tertiary were less severe in eastern Asia and promoted diversification, but were more severe in North America and may have caused extinction (Guo and Ricklefs, 2000). Stanford's (1998) cpDNA sequencing study of the *matK* region indicated two major *Castanea* clades with the three Chinese species supported as a single clade, whose sister group contained the four other species of the genus with the Japanese species (*C. crenata*) most closely related to *C. dentata*. *C. pumila* appeared most closely related to the European chestnut (*C. sativa*), suggesting multiple migrations.

The quaternary history of *C. sativa* has been well documented, indicating that the species retreated back to western Asia and survived as a relict in a few locations of south-eastern Europe and Turkey (Huntley and Birks, 1983; Villani *et al*, 1994; Fineschi *et al*, 2000). It is the late expansion, which occurred after the end of Pleistocene about 5000 year ago, that formed the present distribution patterns in eastern Asia-Europe. In eastern North America, the American species were driven southward to refugia in the Gulf Coastal region and Florida during the Wisconsin glacial maximum 18 000–20 000 years ago and migrated northward along Appalachia after the glacier's retreat (Davis, 1983; Huang *et al*, 1998).

The pattern of eastern Asian and eastern North American disjunct taxa in *Castanea* could be a combination of results of geographical and evolutionary historical events involved in both the early and later Tertiary, via both Bering and North Atlantic land bridges. Fossils, geological, molecular and phylogenetic evidence in other taxa similarly suggests complex origins of disjunct taxa between eastern Asia and eastern North America. The pattern is the product of vicariance, dispersal, extinction and speciation (Wen, 1999). Collaborations among phylogeneticists, paleobotanists and geologists are needed to gain better insights into the origin and evolution of these complicated eastern Asian–eastern North American disjunct patterns.

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