云 南 植 物 研 究 2009, 31 (4): 289～295
Acta Botanica Yunnanica DOI: 10.3724 SP.J.1143.2009.09075

东亚——北美东部间断透骨草属的扩增片段长度多态性

聂泽龙[1,2]，文 军[1,3,4]，孙 航[1]

(1 中国科学院昆明植物研究所生物多样性与生物地理重点实验室，云南 昆明 650204；2 中国科学院研究生院，北京 100049；3 美国史密森研究院国家自然历史博物馆植物系，华盛顿 20013-7012；4 中国科学院植物研究所系统与进化植物学重点实验室，北京 100093)

摘要：透骨草属 (*Phryma*) 是一个单种属，间断分布于东亚与北美东部。尽管东亚与北美东部居群形态差异非常小，但分子变异却非常明显。本研究进一步运用 AFLP 两对引物来衡量透骨草属的遗传多样性并评估其形态保守性。结果发现透骨草的遗传差异主要存在于两大洲的居群之间。聚类与 PCA 分析显示透骨草分成两大支与其地理分布相吻合，一支全部来自东亚，另一支则是北美东部的居群。我们的结果强烈支持透骨草东亚——北美东部居群存在明显的遗传分化和形态保守。

关键词：AFLP；洲际间断；东亚；北美东部；遗传多样性；透骨草；透骨草科
中图分类号：Q 16 **文献标识码**：A **文章编号**：0253 - 2700 (2009) 04 - 289 - 07

AFLP Analysis of *Phryma* (Phrymaceae) Disjunct between Eastern Asia and Eastern North America

NIE Ze-Long[1,2], WEN Jun[1,3,4], SUN Hang[1*]

(1 *Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650204, China; 2 *Graduate University of Chinese Academy of Sciences*, Beijing 100049, China; 3 *Department of Botany, National Museum of Natural History, MRC* 166, *Smithsonian Institution*, Washington, DC 20013-7012, USA; 4 *Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences*, Beijing 100093, China)

Abstract: Although little morphological differentiation is detected between intercontinental disjunct populations of *Phryma*, this monotypic genus shows distinct molecular divergence corresponding to its distribution in eastern Asia and eastern North America. This study further employs amplified fragment length polymorphism (AFLP) analyses using two selective primer pairs to quantify genetic diversity and evaluate the morphological stasis. Most of the molecular variance is accounted for by variance among populations between regions. Cluster and PCA analyses revealed that *Phryma* constitutes two major groups in line with their geographic distribution, with one genetically distinct group from the eastern Asia and the other with accessions from eastern North America. The results robustly supported the distinct genetic divergence and morphological stasis in *Phryma*.

Key words: AFLP; Intercontinental disjunction; Eastern Asia; Eastern North America; Genetic diversity; *Phryma*; Phrymaceae

Phryma L. (Phrymaceae) is well - known for its unusual morphology with a derived pseudomonomerous gynoecium (bicarpellate with one carpel reduced developmentally) and the classical intercontinental disjunct distribution between eastern Asia and eastern North America (Li, 1952; Wen, 1999). It is also one of the

Foundation item: Xibuzhiguang (Light in Western China) project of the Chinese Academy of Science (to Z.-L. Nie), the Natural Science Foundation of China (NSFC 30625004 and 40771073 to H. Sun), the Yunnan Natural Science Foundation (2008CC013 to H. Sun) and the John D. and Catherine T. MacArthur Foundation (to J. Wen).
Author for correspondence; E-mail: hsun@mail.kib.ac.cn
Received date: 2009 - 04 - 16, Accepted date: 2009 - 06 - 09
作者简介：聂泽龙 (1973-) 男，博士，副研究员，硕导，主要从事植物分子系统发育与生物地理以及细胞地理学研究。

very few monospecific genera with such an intercontinental disjunction. Two varieties (Hara, 1966; Thieret, 1972) or subspecies (Kitamura and Miurata, 1957; Li, 2000) have been recognized within the single intercontinental disjunct species *Phryma leptostachya* L., corresponding to its biogeographic distribution in eastern Asia and eastern North America. A high level of morphological similarity has been reported for the two disjunct subspecies (Hara, 1969; Ramana *et al.*, 1983), but companied with distinct molecular divergence (Nie *et al.*, 2006).

Hara (1966) pointed out that the intercontinental disjunct populations of *Phryma* were identical in most morphological characters, cytology, and ecological habitats. For example, populations from both continents bear flowers that are erect in bud but later spreading or becoming deflexed; they have the same chromosome number of $2n = 28$; and they share similar habitats of deciduous or mixed forests. Plants from the two different regions only differ slightly in leaf size, shape of upper lip of the corolla, and length of the upper spinulose calyx-lobes (Hara, 1962, 1966, 1969; Li, 2000; Nie *et al.*, 2006).

Although a high level of morphological similarity has been found in *Phryma*, recent molecular studies revealed substantial molecular divergence between the two intercontinental varieties (Beardsley and Olmstead, 2002; Lee *et al.*, 1996; Nie *et al.*, 2006; Xiang *et al.*, 2000). A low level of isozyme identity (0.291) from allozyme loci and high ITS divergence (4.46%) were detected between the disjunct pair in *Phryma* (Lee *et al.*, 1996).

The eastern Asian-eastern North American disjunction is a well-known and classical biogeographic pattern in theNorthern Hemisphere that has received considerable attention in the last 15 years (Donoghue *et al.*, 2001; Meng *et al.*, 2003; Milne, 2006; Milne and Abbott, 2002; Ran *et al.*, 2006; Wen, 1998, 1999, 2001; Wen *et al.*, 1996; Wen *et al.*, 1998; Wen and Stuessy, 1993; Wen and Zimmer, 1996; Xiang *et al.*, 1996, 1998, 2000; Zhou *et al.*, 2006). Most studies on taxa with disjunct distribution in these two regions (especially for the disjunct species) have focused on phylogenetic relationship and biogeographic history at the interspecific level (Nie *et al.*, 2006), yet few have analyzed patterns of genetic variation among the disjunct taxa at the population level. The objectives of this preliminary study are to explore genetic divergence between intercontinental disjunct populations of *Phryma* and examine the phylogeographic structure and biogeographic implications of *Phryma* in the Northern Hemisphere.

1 Materials and Methods

Seven populations were analyzed with three from eastern Asia and four from eastern North America (Table 1). Genomic DNA was extracted from 15 mg silica dried leaf material using the modified CTAB method (Doyle and Doyle, 1987).

Procedures for AFLPs were as described by Vos et al. with some modifications (Vos *et al.*, 1995). 400 - 500 ng DNA of each sample was digested and ligated with 5 U of *Eco*RI and 1 U of *Mse*I in a reaction mixture that contained 1X T4 restriction and ligase buffer with ATP, 0.5 μmol L^{-1} *Eco*RI adapter, 5 μmol L^{-1} *Mse*I adapter, 50 mmol L^{-1} NaCl, 5 ng BSA, 1 U T4-DNA ligase, and water to a final volume of 10 μl. After digestion and ligation, products were diluted 1∶20 with distilled water. In the first round of PCR (pre-selective amplification), each reaction contained 2.0 μl Promega 10X reaction buffer, 2.0 μl dNTPs (2 mM), 10.0 μl water, 2.0 μl Promega Taq Polymerase (5 U μL^{-1}), 0.5 μl *Mse*I + C primer (50 ng μL^{-1}), 0.5 μl *Eco*RI + A primer (50 ng μL^{-1}), and 2.0 μl of the diluted digestion

Table 1 Voucher information of *Phryma* populations sampled in this study

Taxon	Abbreviation	Voucher	Locality
Phryma leptostachya L. var. *leptostachya* (eastern North America)	EN1	Wen 7140 (US)	USA: Illinois
	EN2	Wen 7161 (US)	USA: Illinois
	EN3	Wen 7188 (US)	USA: Alabama
	EN4	Wen 7292 (US)	USA: Wisconsin
Phryma leptostachya var. *asiatica* Hara (eastern Asia)	EA1	Nie 102 (KUN)	China: Yunnan
	EA2	Yue 122 (KUN)	China: Sichuan
	EA5	Wen 5757 (US)	China: Yunnan

ligation product. The PCR conditions were: 94℃ for 20 sec, 56℃ for 30 sec, and 72℃ for 2 min, for 19 cycles. The PCR products were diluted 1∶20 with water. Samples used in the bulked DNA study were run individually through the preselective amplification step. Subsequently, 10 μl of the diluted preselective amplification from each of three individuals was combined. In the second round of PCR (selective amplification), each reaction contained 1.5 μl Promega 10X reaction buffer, 1.0 μl dNTPs (2 mM), 11.275 μl water, 1.5 μl Promega Taq Polymerase (5 U μL^{-1}), 0.5 μl *Mse*I + XXX primer (50 ng μL^{-1}), 0.5 μl *Eco*RI + XXX (+ dye) primer (50 ng μL^{-1}), and 1.5 μl of the preselective product. The PCR conditions were: 94℃ for 20 sec, 66℃ for 30 sec, and 72℃ for 2 min, 9 cycles; 94℃ for 20 sec, 56℃ for 30 sec, and 72℃ for 2 min, 30 cycles. All samples were processed in random order and subsamples were rerun for internal control. Selective amplification products were separated using Long Ranger Singel packs (BioWhittaker Molecular Applications, cat. # 50691) running for about 2 hours on the ABI Prism 377 genetic analyzer by combining 0.4 μl of selective amplification product with 1.2 μl of loading buffer containing 0.75 μl formamide, 0.30 μl GeneScan ROX-500 internal size standard, and 0.15 μl loading dye.

We identified and sized the peaks between 50 and 500 bp in the ABI gel images using GeneScan 3.1 (Applied Biosystems) to create sample files. Sample trace files were imported into Genographer (ver 1.6, Montana State University, 2001; http: hordeum.msu.montana.edu genographer) for visualization and scoring. AFLP loci were analyzed using the "thumbnail" option of Genographer, which allows for comparison of signal strength at each locus for all samples. Only polymorphic AFLP markers were scored for each sample and recorded as a binary character with presence as "1" and absence as "0". Monomorphic markers were not scored.

The genetic differentiation between populations of *Phryma* based on all AFLP loci was calculated using Nei's genetic distance (Nei, 1978). A cluster analysis using the unweighted pair-group method with arithmetic averaging (UPGMA) (Sneath and Sokal, 1973) was performed using the software POPGENE 1.32 (Yeh *et al.*, 1997). A genetic distances matrix was also used to perform a hierarchical analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992), using ARLEQUIN version 3.11 (Excoffier *et al.*, 2005), to partition the total genetic variation among individuals within populations, between populations within a region, and between regions of eastern Asia and eastern North America. These are not directly comparable to the traditional F-statistics (Wright, 1951) and are therefore referred to as Φ-statistics (Excoffier *et al.*, 1992). The significance of Φ values was tested by 1000 permutations.

The AFLP data were also subjected to a principal components analysis (PCA), which may help reveal unexpected relationships among a large number of variables into two or three new uncorrelated variables so that they retain most of the original information. Similarity matrices using the Jaccard's similarity coefficient were generated. Eigenvalue and eigenvector matrices were calculated from the similarity matrix. The standardized data were projected onto the eigenvectors of the correlation matrix and represented in a two-dimensional scatter plot. Plots of samples in relation to the first three principal components were constructed with populations designated as either eastern Asian or eastern North American distribution. PCAs were performed using the computer program NTSYSpc, version 2.11h (Rohlf, 2000).

2 Results

The two AFLP primer pairs M-CTC E-ACT and M-CTC E-AGG generated a total of 426 polymorphic markers from 80 individuals for seven populations. Table 2 shows the genetic differentiation [Nei' genetic distance; (Nei, 1978)] between *Phryma* populations. The genetic distance matrix was used to establish the level of genetic divergence between the populations (Table 2). Estimates of genetic distance using AFLP data ranged from 0.027 for the most closely related populations within each region (EN1 and EN2), to 0.811 in the most divergent populations between regions (EA1 and EN3). These results indicate that genetic distance is solely dependent on geographical distance, and clustering based on genetic distances reflects geographical relationships.

Table 2 Nei's genetic distance of *Phryma* populations estimated by AFLP analysis (Population codes are as in Table 1)

Population	EA1	EA2	EA5	EN1	EN2	EN3	EN4
EA1	-						
EA2	0.1367	-					
EA5	0.1510	0.1248	-				
EN1	0.6233	0.5133	0.4235	-			
EN2	0.6214	0.5438	0.4379	0.0274	-		
EN3	0.8114	0.6483	0.5826	0.0496	0.1032	-	
EN4	0.6526	0.5948	0.5361	0.0689	0.0535	0.1179	-

The results of the AMOVA based on AFLP variation are shown in Table 3. Most of the total variance was found between regions (60.57%, $P < 0.0001$). The corresponding Φ_{CT} value was 0.61 ($P < 0.0001$). The among-populations within-regions percentage was relatively low (18.2%, $P < 0.0001$) when compared

Table 3 Results of hierarchical analysis of molecular variance (AMOVA) based on AFLP markers between eastern North American and eastern Asian populations of *Phryma*. *P*-values, calculated from a random permutation test (1000 replicates), and Φ-statistics represent the probability of obtaining by chance alone a more extreme variance than the observed values (Excoffier *et al.*, 1992)

Source of variation	d.f.	SS	Variance component	% of variance	Φ-statistics	*P*
Between regions	1	396.084	9.38894	60.57	Φ_{CT} = 0.60574	< 0.0001
Among populations within regions	7	187.236	2.82036	18.20	Φ_{SC} = 0.46152	< 0.0001
Within populations	73	240.216	3.29064	21.23	Φ_{ST} = 0.78770	< 0.0001
Total	81	823.537	15.49993			

to the within-population variance component, close to that of the among-individuals within populations (21.23%, $P < 0.001$). All three Φ-values were significant based on 1000 permutation tests (Table 3).

The UPGMA dendrogram based on the genetic distances between populations showed two main clusters of populations that correlated to their continental distribution (Fig. 1). The PCA of the AFLP-based distance data was performed to examine relationships among Phryma populations. The first and second principal coordinates described approximately 44% and 11% of the total variation, respectively (Fig. 2). Similar to the UPGMA dendrogram, the PCA showed that eastern Asian populations clustered apart from those of eastern North America.

3 Discussion

The AMOVA analyses showed that the largest portion (61%) of genetic variance is contributed by genetic variation between intercontinental regions of eastern Asia and eastern North America and only a small part is due to divergence among populations within regions (18%) and within populations (21%). The results are similar to the pairwise sequence divergence of ITS, *rps*16, and *trnL-F* markers (Nie *et al.*, 2006). For example, the ITS divergence of the two varieties between eastern Asia and eastern North America (3.11%-4.41%) was higher than that among populations within each continent (1.63%-0.65%). The high level of genetic divergence between regions suggests that variations between the two continents display a large proportion of genetic loci of high allelic variations.

The long time of geographic isolation (Lee *et al.*, 1996; Nie *et al.*, 2006; Xiang *et al.*, 2000) may contribute to the high molecular differentiation of *Phryma* between regions observed. Several previous studies estimated the divergence times for the disjunct *Phryma* varieties using various dating approaches. Lee *et al.* (1996) reported the divergence time to be over 20 million years ago (mya) using allozyme data and 12.35 mya using ITS sequences. Xiang *et al.* (2000) estimated the divergence times for the genus with *rbcL* sequence data and a molecular clock calibrated with *Cornus* fossils. They estimated that the two varieties of *Phryma* diverged about 5.85±2.66 mya. Recently we estimated their divergence time as 3.68±2.25 to 5.23±1.37 mya based on combined chloroplast data using both the Bayesian dating and the penalized likelihood methods with relaxed molecular clocks (Nie *et al.*, 2006).

Genetic divergence within the eastern North American populations is less than that within the eastern Asian populations (Table 2), although only a few populations sampled in this study. A similar situation is found with the DNA sequences with more extensive sampling from our previous study (Nie *et al.*, 2006). The eastern Asian populations are reported to be more heterogeneous in ITS sequence variation than those in the eastern North America (maximum divergence of 1.63% vs. 0.65%). As suggested by Nie *et al.* (2006), the more genetic variation in the eastern Asian *Phryma* may be due to higher level of geographic isolation with the more heterogeneous and pronounced topographies in eastern Asia (Qian and Ricklefs, 2000; Wen, 1999; Xiang *et al.*, 2004).

Morphological stasis in *Phryma* is also supported by genetic analysis at the population level. Little morphological variation has been observed from the disjunct pairs of *Phryma* (Holm, 1913; Li, 2000). Multivariate analysis based on 23 quantitative morphological characters also revealed that no significant variations were found between them (Nie *et al.*, 2006). However,

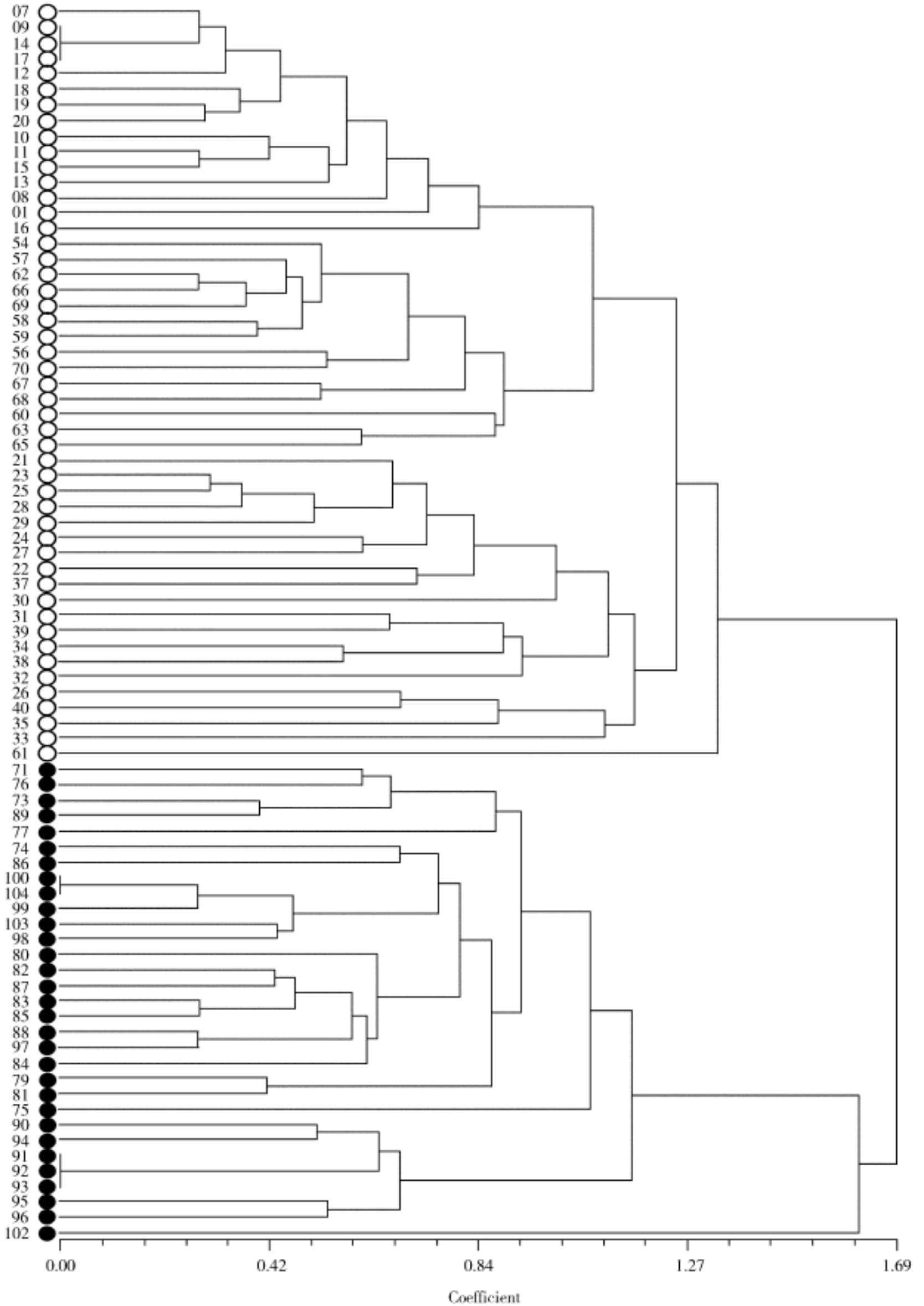

Fig. 1 UPGMA dendrogram based on pairwise genetic distance showing relationships between *Phryma* populations from AFLP data. (● = EN samples; ○ = EA samples)

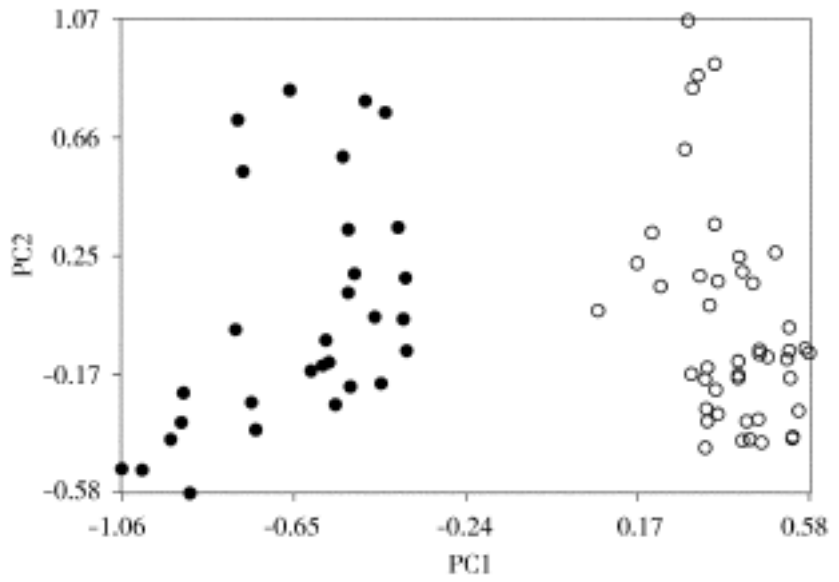

Fig . 2 Principal component analysis of AFLP data . Accessions are plotted according to the values of the first (x-axis) and the second (y-axis) components and with different symbols according to geographical origin (● = EN samples; ○ = EA samples)

AFLP analysis revealed a clear geographical pattern of genetic variation, consistent with our findings from the sequence data (Nie *et al.*, 2006) . The results showed that *Phryma* is a genetically diverse entity, and AFLP markers can be effectively employed to assess genetic diversity and to measure genetic relationship among populations .

Morphological stasis was suggested to explain the discordance of the genetic and morphological rates of evolution (Nie *et al.*, 2006) . Among various possible explanations for stasis in morphology, a relatively constant environment with the concomitant action of stabilizing selection, might be the most plausible (Nie *et al.*, 2006; Wen, 1999) . The intercontinental populations of *Phryma* occupy similar habitats in rich mesic to moist, deciduous or mixed deciduous and evergreen forests in both eastern Asia and eastern North America . These types of forests covered most of the temperate regions of the Northern Hemisphere in the Tertiary, but can be found today only in southeast North America and in eastern to central China and central to southern Japan (Milne, 2006; Milne and Abbott, 2002; Nie *et al.*, 2008; Wen, 1999) .

In present study, our sampling is limited with only three eastern Asian populations from SW China and four populations from eastern North America . Nevertheless, our AFLP results at the population level corroborate our findings in the sequence analyses (Nie *et al.*, 2006) . Both the AFLP (Table 2) and DNA sequence data (Nie *et al.*, 2006) suggested that the eastern Asian populations have much higher genetic and molecular divergence than that of eastern North America . Comparative phylogeographic analysis (Avise, 2000) of the disjunct taxa is very important to our understanding of mechanisms responsible for their phylogenetic relationships and disjunct distribution . Future studies are planned to extensively sample the populations on both continents to better understand the relationships among genetic divergence, morphological differentiation, and the development of the intercontinental disjunct pattern . We intend to employ phylogeographic analysis, as implemented in Gao *et al*. (2007) and Meng *et al*. (2007), but analyze the *Phryma* populations intercontinentally and intracontinentally .

Acknowledgements: Laboratory work was conducted in and partially supported by the Pritzker Laboratory for Molecular Systematics and Evolution of the Field Museum .

References:

Avise JC, 2000 . Phylogeography: the history and formation of species [M] . Cambridge, MA: Harvard University Press

Beardsley PM, Olmstead RG, 2002 . Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma* [J] . *American Journal of Botany*, 89: 1093—1102

Donoghue MJ, Bell CD, Li JH, 2001 . Phylogenetic patterns in Northern Hemisphere plant geography [J] . *International Journal of Plant Sciences*, 162: S41—S52

Doyle JJ, Doyle JL, 1987 . A rapid DNA isolation procedure from small quantities of fresh leaf tissues [J] . *Phytochemical Bulletin*, 19: 11—15

Excoffier L, Laval G, Schneider S, 2005 . Arlequin ver . 3.0: an integrated software package for population genetics data analysis [J] . *Evolutionary Bioinformatics Online*, 1: 47—50

Excoffier L, Smouse PE, Quattro JM, 1992 . Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data [J] . *Genetics*, 131: 479—491

Gao LM, Moeller M, Zhang XM *et al.*, 2007 . High variation and strong phylogeographic pattern among cpDNA haplotypes in *Taxus wallichiana* (Taxaceae) in China and North Vietnam [J] . *Molecular Ecology*, 16: 4684—4698

Hara H, 1962 . Racial differences in widespread species, with special reference to those common to Japan and North America [J] . *American Journal of Botany*, 49: 647—652

Hara H, 1966 . The Flora of Eastern Himalaya [M] . Tokyo, Japan: University of Tokyo

Hara H, 1969. Remarkable examples of speciation in Asiatic plants [J]. *American Journal of Botany*, 56: 732—737

Holm T, 1913. *Phryma leptostachya* L., a morphological study [J]. *Botanical Gazette*, 56: 305—318

Kitamura S, Miurata G, 1957. New names and new conceptions adopted in our colored illustrations of herbaceous plants of Japan (Sympetalae) [J]. *Acta Phytotaxonomy and Geobotany*, 17: 5—13

Lee NS, Sang T, Crawford DJ *et al*., 1996. Molecular divergence between disjunct taxa in eastern Asia and eastern North America [J]. *American Journal of Botany*, 83: 1373—1378

Li HL, 1952. Floristic relationships between eastern Asia and eastern North America [J]. *Transactions of the American Philosophical Society*, 42: 371—429

Li ZY, 2000. Taxonomic notes on the genus *Phryma* L. from Asia [J]. *Acta Phytotaxonomica Sinica*, 38: 386—391

Meng L, Yang R, Abbott RJ *et al*., 2007. Mitochondrial and chloroplast phylogeography of *Picea crassifolia* Kom. (Pinaceae) in the Qinghai-Tibetan Plateau and adjacent highlands [J]. *Molecular Ecology*, 16: 4128—4137

Meng SW, Douglas AW, Li DZ *et al*., 2003. Phylogeny of Saururaceae based on morphology and five regions from three plant genomes [J]. *Annals of the Missouri Botanical Garden*, 90: 592—602

Milne RI, 2006. Northern Hemisphere plant disjunctions: a window on tertiary land bridges and climate change? [J]. *Annals of Botany*, 98: 465—472

Milne RI, Abbott RJ, 2002. The origin and evolution of tertiary relict floras [J]. *Advances in Botanical Research*, 38: 281—314

Nei M, 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals [J]. *Genetics*, 89: 583—890

Nie ZL, Sun H, Beardsley PM *et al*., 2006. Evolution of biogeographic disjunction between eastern Asia and eastern North America in *Phryma* (Phrymaceae) [J]. *American Journal of Botany*, 93: 1343—1356

Nie ZL, Wen J, Azuma H *et al*., 2008. Phylogenetic and biogeographic complexity of Magnoliaceae in the Northern Hemisphere inferred from three nuclear data sets [J]. *Molecular Phylogenetics and Evolution*, 48: 1027—1040

Qian H, Ricklefs RE, 2000. Large-scale processes and the Asian bias in species diversity of temperate plants [J]. *Nature*, 407: 180—182

Ramana RV, Rao PSP, Narayana LL, 1983. A contribution to the floral anatomy of *Phryma leptostachya* L. [J]. *Current Science*, 52: 922—924

Ran JH, Wei XX, Wang XQ, 2006. Molecular phylogeny and biogeography of *Picea* (Pinaceae): Implications for phylogeographical studies using cytoplasmic haplotypes [J]. *Molecular Phylogenetics and Evolution*, 41: 405—419

Rohlf FJ, 2000. NTSYSpc-Numerical Taxonomy and Multivariate Analysis System, version 2.1 [M]. New York: Applied Biostatistics Inc

Sneath PHA, Sokal RR, 1973. Numerical Taxonomy [M]. San Francisco: Freeman

Thieret JW, 1972. The Phrymaceae in the southeastern United States [J]. *Journal of the Arnold Arboretum*, 53: 226—233

Vos P, Hogers R, Bleeker M *et al*., 1995. AFLP: a new technique for DNA fingerprinting [J]. *Nucleic Acids Research*, 23: 4407—4414

Wen J, Stuessy TF, 1993. The Phylogeny and Biogeography of *Nyssa* (Cornaceae) [J]. *Systematic Botany*, 18: 68—79

Wen J, Zimmer EA, 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA [J]. *Molecular Phylogenetics and Evolution*, 6: 167—177

Wen J, Jansen RK, Zimmer EA, 1996. Phylogenetic relationships and DNA sequence divergence of eastern Asian and eastern North American disjunct plants [A]. In: Nei M, Takahata N eds. Current Topics in Molecular Evolution [M]. Hayama, Japan: Penn. State Univ., USA, and Grad. Sch. for Adv. Stud., 37—44

Wen J, 1998. Evolution of the eastern Asian and eastern North American disjunct pattern: insights from phylogenetic studies [J]. *Korean Journal of Plant Taxonomy*, 28: 63—81

Wen J, Shi SH, Jansen RK *et al*., 1998. Phylogeny and biogeography of *Aralia* sect. *Aralia* (Araliaceae) [J]. *American Journal of Botany*, 85: 866—875

Wen J, 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants [J]. *Annual Review of Ecology and Systematics*, 30: 421—455

Wen J, 2001. Evolution of eastern Asian-Eastern North American biogeographic disjunctions: a few additional issues [J]. *International Journal of Plant Sciences*, 162: S117—S122

Wright S, 1951. The genetical structure of populations [J]. *Annals of Eugenics*, 15: 323—354

Xiang QY, Brunsfeld SJ, Soltis DE *et al*., 1996. Phylogenetic relationships in Cornus based on chloroplast DNA restriction sites: implications for biogeography and character evolution [J]. *Systematic Botany*, 21: 515—534

Xiang QY, Soltis DE, Soltis PS, 1998. The eastern Asian and eastern and western North American floristic disjunction: congruent phylogenetic patterns in seven diverse genera [J]. *Molecular Phylogenetics and Evolution*, 10: 178—190

Xiang QY, Soltis DE, Soltis PS *et al*., 2000. Timing the eastern Asian-Eastern North American floristic disjunction: molecular clock corroborates paleontological estimates [J]. *Molecular Phylogenetics and Evolution*, 15: 462—472

Xiang QY, Zhang WH, Ricklefs RE *et al*., 2004. Regional differences in rates of plant speciation and molecular evolution: a comparison between eastern Asia and eastern North America [J]. *Evolution*, 58: 2175—2184

Yeh FC, Yang RC, Boyle TBJ *et al*., 1997. POPGENE, the user-friendly shareware for population genetic analysis [M]. Alberta: Molecular Biology and Biotechnology Centre, University of Alberta

Zhou ZK, Yang XF, Yang QS, 2006. Land bridge and long-distance dispersal-old views, new evidence [J]. *Chinese Science Bulletin*, 51: 1030—1038