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Population genetic structure of *Vitex negundo* (Verbenaceae) in Three-Gorge Area of the Yangtze River: The riverine barrier to seed dispersal in plants

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Abstract

Previous studies have demonstrated that large rivers can influence inter- and intra-specific gene flow for many animals. The effects of large rivers on the genetics of plant populations have focused on either hydrochoric impacts of water current on gene flow or genetic differentiation among populations from different watersheds. Few studies have explicitly tested the barrier effects on plant gene flow across banks of large rivers, especially their relative effects on pollen and seed dispersals. The Yangtze River (Changjiang River), one of the major rivers of the world, provides an excellent model to evaluate the impacts of rivers on gene flow in plants. Using RAPD (random amplified polymorphic DNA) and cpDNA (chloroplast DNA) markers, we investigated the genetic structure of 10 populations of *Vitex negundo* in two regions of Three-Gorge Area along the Yangtze River. Each region contained two populations on the north bank, two on the south bank and one island population along the river. The analyses indicated low RAPD between banks, and similar or a little higher differentiation between populations within the same bank. In contrast, a large proportion of chloroplast polymorphism was ascribed to among-bank variation but much lower cpDNA differentiation was among populations within the same bank. These results indicate that the Yangtze River represents a general barrier to the dispersal of seeds but not to the movement of pollen in *V. negundo*. The cpDNA genetic distances or differentiations between the island populations and those on either bank of the river are intermediate to those between the banks across the river, implying that the islands in the Yangtze River may serve as a stepping-stone for seed dispersal. Our results suggest that large rivers may serve as a general barrier, not only for the movement of animals, but also for the dispersal of plants, which should be of great significance for the conservation of biodiversity around the rivers.

Keywords: Gene flow; Riverine barrier; RAPD; Chloroplast DNA; Yangtze River

1. Introduction

Gene flow among populations is one of the important factors maintaining species integrity and the genetic diversity within species (Morjan and Rieseberg, 2004). In addition to geographic isolation, natural barriers such as rivers, shoreline, mountain ranges or even man-made canals or highways may interrupt the habitat and thus affect the population

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genetic structure of a species (Slatkin, 1987; Su et al., 2003). Large rivers have been suggested to influence inter- and intra-specific pattern of genetic diversity and differentiation for many animals (Wallace, 1849; Capparella, 1988, 1991; Ayres and Clutton-Brock, 1992; Pastorini et al., 2003; Telfer et al., 2003; Eriksson et al., 2004; Cheviron et al., 2005). For example, the richness of primates in Amazonian forests arose a ''riverine barrier hypothesis'' for its evolutionary origin (Wallace, 1849), which states that the major rivers in the Amazonian area represent barriers to gene flow, promoting genetic divergence of populations (Colwell, 2000). Although the riverine barrier hypothesis has historical roots, recent molecular studies have provided mixed support for the hypothesis. Capparella (1988, 1991) found that the Amazonian rivers functioned as significant impediments to gene flow among populations of understory species of birds and the degree of genetic divergence among samples was related to river width. However, the genetic breaks of some Amazonian animals (e.g. arboreal spiny rats, a dart-poison frog) were not related to the Amazonian rivers (Patton et al., 1994; Lougheed et al., 1999), but rather to Late Cenozoic palaeogeographical changes in topographical relief (referred to as ridge hypothesis). Therefore, the significance and intensity of major rivers as barriers to gene flow await more investigations for additional river systems and organisms such as plants.

However, the effects of large rivers on plant population genetics have focused on either the hydrochoric impacts of water current on gene flow or the genetic differentiation among populations from different watersheds (e.g. Butcher et al., 2002; Tero et al., 2003; Barrett et al., 2004; Prentis et al., 2004; Liu et al., 2006; Victory et al., 2006). Few studies have been explicitly tested the riverine barrier on plant gene flow across banks of large rivers, especially their relative effects on pollen and seed dispersal, which might be of interest to population geneticists.

The Yangtze River (Changjiang River) is one of the major rivers of the world. In China, only the Yellow River is comparable in dimensions and importance. Originating from the Tibetan Plateau at an elevation higher than 5000 m, the Yangtze flows first south, then north and northeast, and finally east to reach the coast, 6300 km away (Wang, 1997; Chen et al., 2001). Little affected by the quaternary glaciations, the drainage area of the Yangtze River is one of the richest areas in biodiversity in China, and the diversity of genera and families is among the highest globally (Wu et al., 2003). Therefore, the Yangtze River provides a good model to address the effect of a river as a natural barrier on the gene flow of plant species. In addition, the world's largest dam, Three Gorges Dam (TGD) has been constructed in the middle of the Yangtze River in Central China in the past decade. It is 2335 m long and 185 m high. A big reservoir was formed to cover an area of 58 000 km^2, about 16 710 km^2 larger than Switzerland (Wu et al., 2003). As a result, more than 100 mountain-tops became islands and the watercourse widened significantly. As the construction of TGD will be fulfilled in 2009, its potential impacts on population genetics of organisms need to be evaluated.

Vitex negundo L. (Verbenaceae) is a species that is distributed across Japan, China, E. Africa, S and SE Asia and the Pacific Islands (Chen and Gilbert, 1994). In Three-Gorge Area, this species mainly inhabits the degraded forests and is of ecological importance for preventing soil erosion in Central China. The flowers of *V. negundo* produce nectar at dawn and complete by dusk. About 22 species of diurnal insects including wasps, bees and ants visit the flowers during this period according to the observation in India (Reddy et al., 1992). Although little is known about the mode of seed dispersal of *V. negundo*, our field observation showed that *V. negundo* established and expanded its populations mainly through animal-ingest dispersal and thus might be sensitive to the natural or artificial barriers in terms of gene flow and dispersals. Because chloroplast genome transmits mainly through seeds in most angiosperms, chloroplast markers, in conjunction with nuclear markers, have been widely used for distinguishing seed-mediated from pollen-mediated gene flow (McCauley, 1994, 1995; Ouborg et al., 1999). In this study, we sampled 10 populations of *V. negundo* from two regions along the Yangtze River and investigated the population genetic structure and relative levels of gene flow via pollen and seeds in *V. negundo* using biparently inherited RAPD markers and maternally inherited cpDNA sequences. Such information will not only contribute to better understanding of the effect of the Yangtze River on gene flow in plants as a natural barrier, but also may help evaluate the long-term evolutionary outcomes of the construction of the Three Gorges Dam.

2. Materials and methods

2.1. Sample sites and strategies

Ten populations of *V. negundo* were sampled from two regions (five populations from both the Mudong and Zhongxian regions) along the river in Three-Gorge Area (Fig. 1, Table 1). The two regions are about 142 km apart. Two pairs

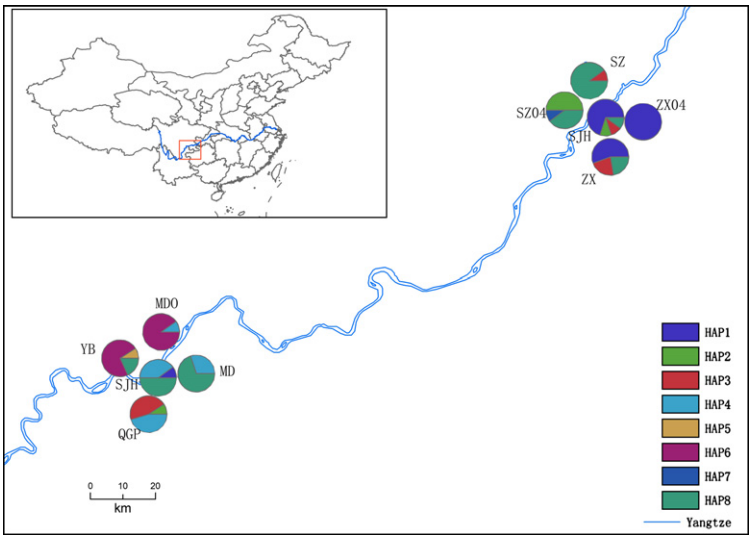

Fig. 1. Population locations and DNA haplotype distribution in *Vitex negundo* populations along the Yangtze River in TGRA.

of populations along the river in each region were sampled. The distances between the populations on the same bank of the river are approximately equal to the width of the river (about 1 km). To test whether higher genetic differentiation between populations parallels wider watercourses of the river, one population in each region was collected in the middle of the Yangtze River, on an island which was formed long before the construction of TGD. Young leaves from individual plants were collected and stored in silica gel at room temperature. For each population, 15–20 individuals were sampled for RAPD analysis and 9–11 individuals were used in chloroplast genotyping. The space between adjacent individuals within a population was about 5 m to minimize sampling related individuals.

2.2. RAPD protocol

Total DNA was extracted from the dried leaves according to CTAB method of Doyle and Doyle (1987). A total of 125 primers (S41-60, S181-220, S341-360, S401-420, S481-500, S2121-2125, Sangon Biotech Company) were used for screening. Three individuals from different populations were taken at random to identify primers that produced 2–10 clear and strong bands. An additional three individuals were used to check the reproducibility of screened primers. After the primer screening, 16 primers (S60, S181, S195, S214, S218, S220, S358, S415, S416, S418, S427, S483, S485, S486, S488, S490) with clear and reproducible bands were chosen for further analysis.

Table 1
Population locations and sample sizes of *Vitex negundo* along Yangtze River in Three-Gorge Area

Region	Population Abbr.	Population location			Sample size (RAPD)	
		Latitude (N)	Longitude (E)	Altitude (m)	RAPD	Chloroplast
Mudong	MD	29°34.52′	106°51.46′	211	14	10
	QGP	29°33.643′	106°50.967′	251	20	11
	SJH[a]	29°35.81′	106°51.87′	279	18	10
	MDO	29°35.09′	106°50.58′	270	20	10
	YB	29°35.646′	106°50.221′	344	20	11
	Total				92	52
Zhongxian	ZX	30°21.13′	108°04.48′	212	15	10
	ZX04	30°21.029′	108°06.252′	308	18	10
	HHC[a]	30°20.34′	108°05.12′	258	15	9
	SZ	30°21.10′	108°05.47′	230	19	10
	SZ04	30°21.756′	108°04.230′	282	18	10
	Total				85	49

[a] Island population.

DNA amplification was performed in a T1 thermocycler (Biometra, Germany), programmed for an initial 240 s at 94 °C, followed by 40 cycles of 15 s at 94 °C, 45 s at 36 °C, 90 s at 72 °C, and a final 4 min at 72 °C. Reactions were carried out in a volume of 20 μl containing 2.0 mmol/L$MgCl_2$, 0.5 μmol/L dNTP, 10× buffer, 2.5 μmol/L primer, 1 UTaq DNA and 20 ng DNA template. Amplification products were analyzed by electrophoresis on 2.0% agarose gel buffered with 0.5× TBE, stained with ethidium bromide, and photographed under ultraviolet light. Molecular weights were estimated using DGL 100 bp DNA Ladder.

2.3. Amplification and sequencing of chloroplast non-coding regions

Universal chloroplast markers were employed to identify patterns of population structure that reflect the seed dispersal history of the species, because chloroplast DNA is maternally inherited in most angiosperms including the family Verbenaceae to which *V. negundo* belongs (Tsukaya et al., 2003). Three to six individuals from distinct populations were used for screening variable chloroplast fragments. Most of the universal primers for the screening were described in Demesure et al. (1995), Dumolin-Lapegue et al. (1998), Hamilton (1999), Sang et al. (1997), Taberlet et al. (1991). Additional two pairs of primers were designed by our laboratory (tRNA-leu intron: forward 5′-TCg gTA gAC gCT ACg gAC-3′; reserve 5′-ggA TAg Agg gAC TTg AAC C-3′; tRNA-gly intron: forward 5′-CAT CgT TAg CTT ggA Agg C-3′; reverse 5′-gCg ggT ATA gTT TAg Tgg-3′). Out of the 20 fragments that were amplified and sequenced, four fragments (i.e. *trn*S-*trn*G spacer, *psb*A-*trn*H spacer, *trn*-leu intron and *trn*-gly intron) generated at least one substitution or indel across the individuals surveyed, and were then used in the full survey. DNA amplification was performed in a T1 thermocycler (Biometra, Germany), programmed for an initial 240 s at 94 °C, followed by 30 cycles of 45 s at 94 °C, 30 s at 52–58 °C according to different primer pairs, 90 s at 72 °C, and a final 4 min at 72 °C. Reactions were carried out in a volume of 20 μl containing 2.0 mmol/L$MgCl_2$, 0.5 μmol/L dNTP, 10× buffer, 2.5 μmol/L primer, 1 UTaq DNA and 20 ng DNA template. Amplification products were used for sequencing directly. Sequencing reactions were conducted with the forward or reverse primers using the DYEnamic ET Terminator Kit (Amersham Pharmacia Biotech), following the manufacturer's protocol. Sequencing was done on a Megabase 1000 automatic DNA sequencer (Amersham Pharmacia Biotech) after the sequencing reaction product was purified through precipitation with 95% ethanol and 3% sodium acetate (pH 5.2). The sequences reported in this paper are deposited in GenBank under accession numbers DQ304781–DQ304791 and DQ367403.

2.4. Data analysis

RAPD bands were scored as present (1) or absent (0) for each DNA sample. Ambiguous bands were treated as missing. The neutrality of each locus was estimated based on the Ewens–Watterson test (Manley, 1985) and the loci under selection were excluded from subsequent analyses. The genetic diversity within populations was measured by the percentage of polymorphic bands and Nei's gene diversity (Nei, 1973) using POPGENE (Yeh et al., 1997), assuming Hardy–Weinberg equilibrium. Based on Nei and Li's (1979) genetic distance, dendrograms of individuals on each region were constructed using neighboring-joining method. Divergence among populations was assessed by an analysis of molecular variance (AMOVA, Excoffier et al., 1992). In this analysis, the populations from both Zhongxian and Mudong regions were divided into three groups: two populations in the north bank, two populations in the south bank and the island population. The total variance was partitioned into among-group, among-population within group and within-population components. The analyses were also conducted with only two groups, the north bank group and the south bank group, by excluding the island populations. Because estimates of population differentiation derived from dominant RAPD fingerprints were generally inflated in various methods, Fhi-st (Φ_{ST}) of Excoffier et al. (1992), an analog of F_{ST}, is used as a more appropriate measure of population divergence based on RAPD data (Isabel et al., 1999). The Φ_{ST} values between pairwise populations in each region were estimated with WINAMOVA version 1.55 (Excoffier, 1993). The pairwise Φ_{ST} was divided into three classes: within-bank (paired populations on the same bank), bank-to-bank (paired populations on the opposite banks) and bank-to-island (paired populations on the island and one bank).

Chloroplast sequences were aligned with ClustalX version 1.81 (Thompson et al., 1997). Four fragments were combined into one data matrix because the chloroplast genome is not subjected to recombination and inherited as a single locus (Ouborg et al., 1999). Indels were coded as single mutation following Caicedo and Schaal (2004). The level of genetic variation within populations was measured in terms of haplotype diversity (H_C). Tests of neutrality using

Tajima's D statistic and Fu and Li's Statistic were completed by DNASP (version 4.00.6; Rozas et al., 2003). The population-to-population distribution of haplotypes are presented in Fig. 1. Total genetic variation was subdivided into among-group, among-population within group and within-population components. The pairwise Φ_{ST} within a region was calculated with WINAMOVA (version 1.55; Excoffier, 1993) and also divided into three classes as conducted in RAPD analysis.

3. Results

3.1. Genetic diversity

Despite the sensitivity of RAPDs to experimental conditions, we were able to obtain clear, reproducible RAPD profiles through the control of a consistent amount of DNA per amplification (ca. 20 ng). A total of 82 RAPD bands were produced by 16 primers. The Ewens–Watterson test for neutrality indicated that four RAPD loci were not selectively neutral, and thus were excluded from subsequent analyses and the remaining 78 loci were retained. In the Mudong region, 73 loci (93.6%) were variable within regions and 65.8–81.7% of polymorphic loci were found within populations, resulting in a gene diversity of 0.20–0.30 (H_R). In Zhongxian, the RAPD variation in Zhongxian was very close to that in Mudong (Table 2).

The aligned sequences of *psb*A-*trn*H spacer, *trn*S-*trn*G spacer, *trn*-leu intron and *trn*-gly intron were 369, 559, 457 and 603 base pairs in length, respectively. The combined data set contained 1981 characters including nine polymorphic sites (an 8-bp indel in *trn*-gly intron coded as a single mutation). The combined sequences conformed to the expectation of neutrality by Tajima's criterion ($D = 1.55191$, $P > 0.10$) and Fu and Li's D^* ($D = 1.31782$, $P > 0.10$ and $F^* = 1.65916$, $0.10 > 0.05$) criteria. In total, eight haplotypes were detected, yielding a haplotype diversity of 0.810 at the species level (Table 2). Seven haplotypes were detected, yielding a haplotype diversity of 0.76 in Mudong and five haplotypes were detected with the H_C value of 0.68 in Zhongxian. The two island populations possessed the highest haplotype diversity (0.64 for SJH and 0.67 for HHC) among the populations in each region (Table 2).

3.2. Genetic differentiation among populations

AMOVA analysis of RAPD variation indicated that genetic differentiation (Φ_{ST}) among populations was 0.111 and 0.090 in Mudong and Zhongxian, respectively. At the regional level, most variability was attributed to within-population variation (87.79% and 90.97% in Mudong and Zhongxian, respectively, Table 3). No or little variability was found among groups, with only −3.35% (means zero) and −0.10% of total variation residing among groups in Mudong and Zhongxian, respectively. In addition, 15.55% and 9.13% of variation was among populations within groups in Mudong and Zhongxian, respectively. When the island populations (HHC and SJH) were excluded, the results were little changed, with no variation between the north and south banks and 14.19% and 9.55% between populations within banks for Mudong and Zhongxian, respectively.

In contrast to RAPD results, chloroplast-based genetic differentiation (Φ_{ST}) among populations was much higher, up to 0.403 and 0.578 in Mudong and Zhongxian, respectively. AMOVA analyses at the regional level showed that a considerable amount of chloroplast variability was attributed to variation between groups (37.51% and 45.16% for Mudong and Zhongxian, respectively, Table 3). The within-population variation was 59.69% in Mudong and 42.19% in Zhongxian (Table 2). Low levels of variability were found among populations within groups for both Mudong (2.80%) and Zhongxian (12.66%). The genetic differentiation among-banks was still much higher than among groups

Table 2
Genetic variability of *Vitex negundo* in TGRA detected by RAPD and chloroplast sequences

Marker	Region	Mudong					Zhongxian				
	Population	MD	QGP	SJH	MDO	YB	ZX	ZX04	HHC	SZ	SZ04
RAPD	P (%)	65.8	65.8	81.7	68.3	65.8	70.7	74.4	82.9	72.0	68.3
	Gene diversity (H_R)	0.25	0.21	0.30	0.24	0.20	0.25	0.27	0.31	0.26	0.25
Chloroplast sequences	No. of Haplotype	2	3	3	2	3	3	1	3	2	3
	Haplotype diversity (H_C)	0.47	0.64	0.64	0.20	0.47	0.53	0.00	0.67	0.20	0.64

when the island populations were excluded, with 47.46% and 61.04% of variation between the north and south banks and only 3.23% and 9.55% between-population within banks for Mudong and Zhongxian, respectively (data not shown).

Based on RAPD data, the mean pairwise Φ_{ST} for the three classes of paired populations were 0.1611 (within-bank), 0.1021 (bank-to-island), and 0.1368 (bank-to-bank) in Mudong, whereas the values for the three classes in Zhongxian were 0.0878, 0.0834, and 0.0956, respectively (Table 4). Although connection between the opposite bank populations is interrupted by the Yangtze River, the bank-to-bank pairwise Φ_{ST} was lower than within-bank in Mudong and almost equal to that of within-bank in Zhongxian. As shown in Figs. 2 and 3, the neighbour-joining dendrograms constructed for all individuals in the Mudong region further demonstrated that no apparent RAPD differentiation occurred between the populations across the river because individuals from the south bank, north bank and island (marked by three different symbols) were entirely intermingled. The dendrograms constructed for all individuals in the Zhongxian region resulted in similar result (Fig. 3).

In contrast, cpDNA differentiation between the paired populations of bank-to-bank was significantly larger than that of within-bank and bank-to-island (ca. 2.86−11.48 times higher). In the region of Mudong, the mean pairwise Φ_{ST} for the within-bank, bank-to-island and bank-to-bank comparisons were 0.0435, 0.2201 and 0.4996 respectively and these values in Zhongxian were 0.2408, 0.2838 and 0.6892, respectively (Table 4). Because cpDNA is transmitted by seeds, the results suggest that seed flow across the Yangtze River was much lower than that within the same banks. The haplotype distribution also showed that the populations in two opposite banks had different haplotypes in both regions (Fig. 1). In Mudong, haplotypes 3, 4 and 8 were predominant in the populations on the south bank. On the contrary, haplotype 6 was predominant in the populations on the north bank. In Zhongxian, the predominant haplotype was haplotype 1 in the south bank but haplotypes 2 and 8 in the north bank. The island populations shared the haplotypes with those on one or both banks (Fig. 1).

4. Discussion

The RAPD analysis showed that the *V. negundo* populations in Three-Gorge Area along the Yangtze River maintained relatively high levels of genetic diversity that are within the range of other outcrossing plants (45−100%) (Huff et al., 1993; Liu et al., 1994). Results are also consistent with the RAPD data (80.96−92.07% polymorphic loci) reported by Su et al. (2003) on the Great Wall populations of the same species. The number of cpDNA haplotypes (eight haplotypes) in *V. negundo* is quite low compared with other woody species (mean number = 16.9, Petit et al., 2003), this may be due to the haplotypes of *V. negundo* detected in this study was only from two regions within limited areas.

It is expected that most genetic variation resides within populations and low genetic differentiation occurs among populations for outcrossing plants (Hamrick and Godt, 1996). At the regional level, RAPD differentiation of *V. negundo* ($\Phi_{ST} = 0.111$ in Mudong and $\Phi_{ST} = 0.090$ in Zhongxian) conforms to this expectation. In contrast to RAPDs, cpDNA variation is much more structured (Φ_{ST} are 0.403 and 0.578 in Mudong and Zhongxian, respectively). The results are reasonable because maternally inherited cpDNA markers are moved by seeds only and have lower effective population size relative to nuclear markers, which may result in an increased genetic differentiation (Petit et al., 2003; Ingvarsson, 2005).

Although the genetic differentiation revealed by RAPDs and cpDNA agree well with expectations, the patterns of RAPDs and cpDNA differentiation between populations across the Yangtze River are different. For RAPDs, almost

Table 3
AMOVA analysis of genetic partition[a]

Source of variance	Mudong				Zhongxian			
	RAPD		Chloroplast		RAPD		Chloroplast	
	%	*P*	%	*P*	%	*P*	%	*P*
Among groups	−3.35	0.6374	37.51	0.0330	−0.10	0.5734	45.16	<0.0010
Among populations within groups	15.55	<0.0010	2.80	0.1039	9.13	<0.0010	12.66	<0.0010
Within populations	87.79	<0.0010	59.69	<0.0010	90.97	<0.0010	42.19	<0.0010

P: Variance components significance.
[a] Groups: north bank populations, south bank populations and island population.

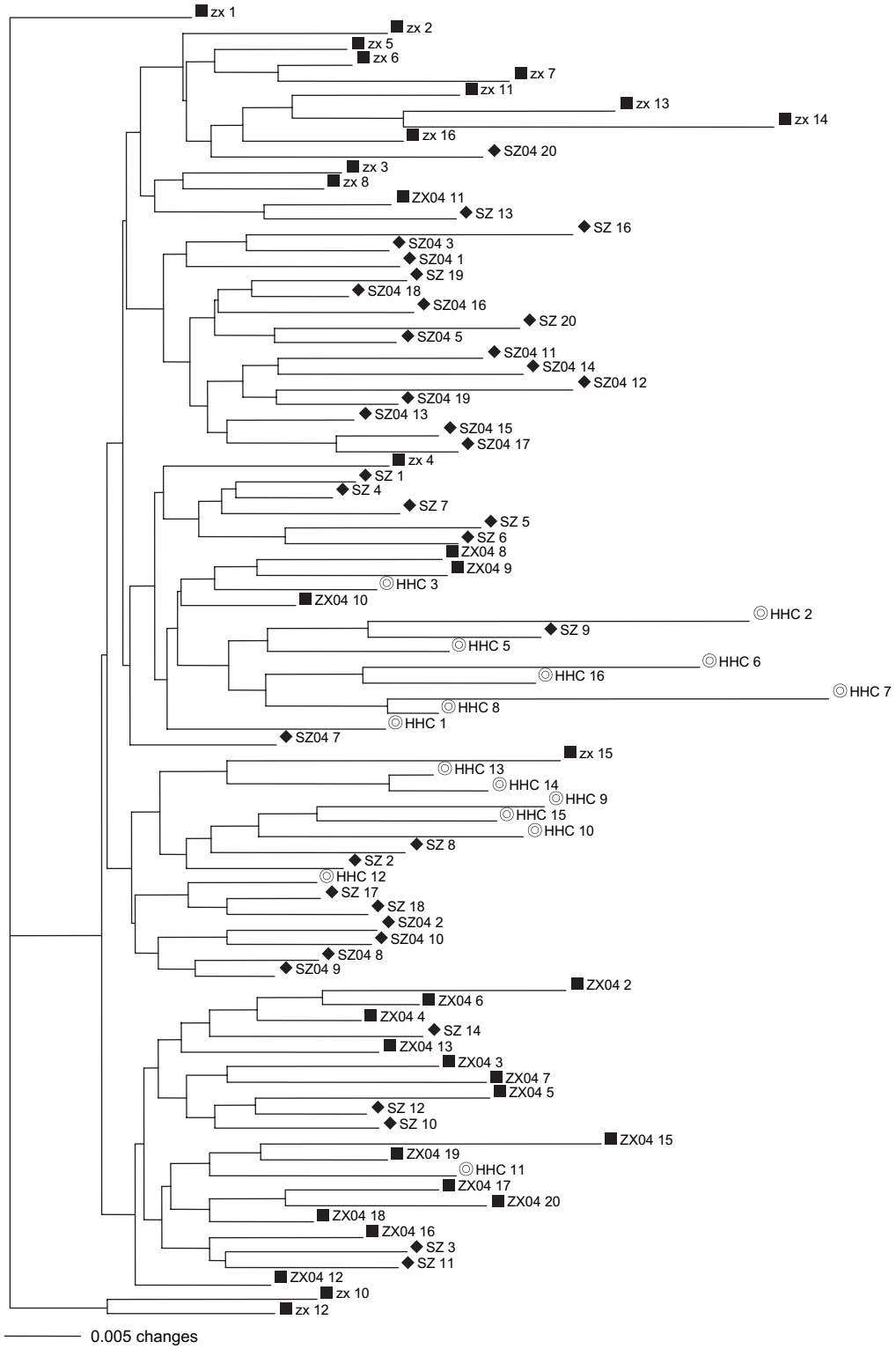

Fig. 2. A neighboring-joining dendrogram of individuals in the region of Zhongxian based on RAPD variation, ■: southern bank; ◆: northern bank; ◎: island.

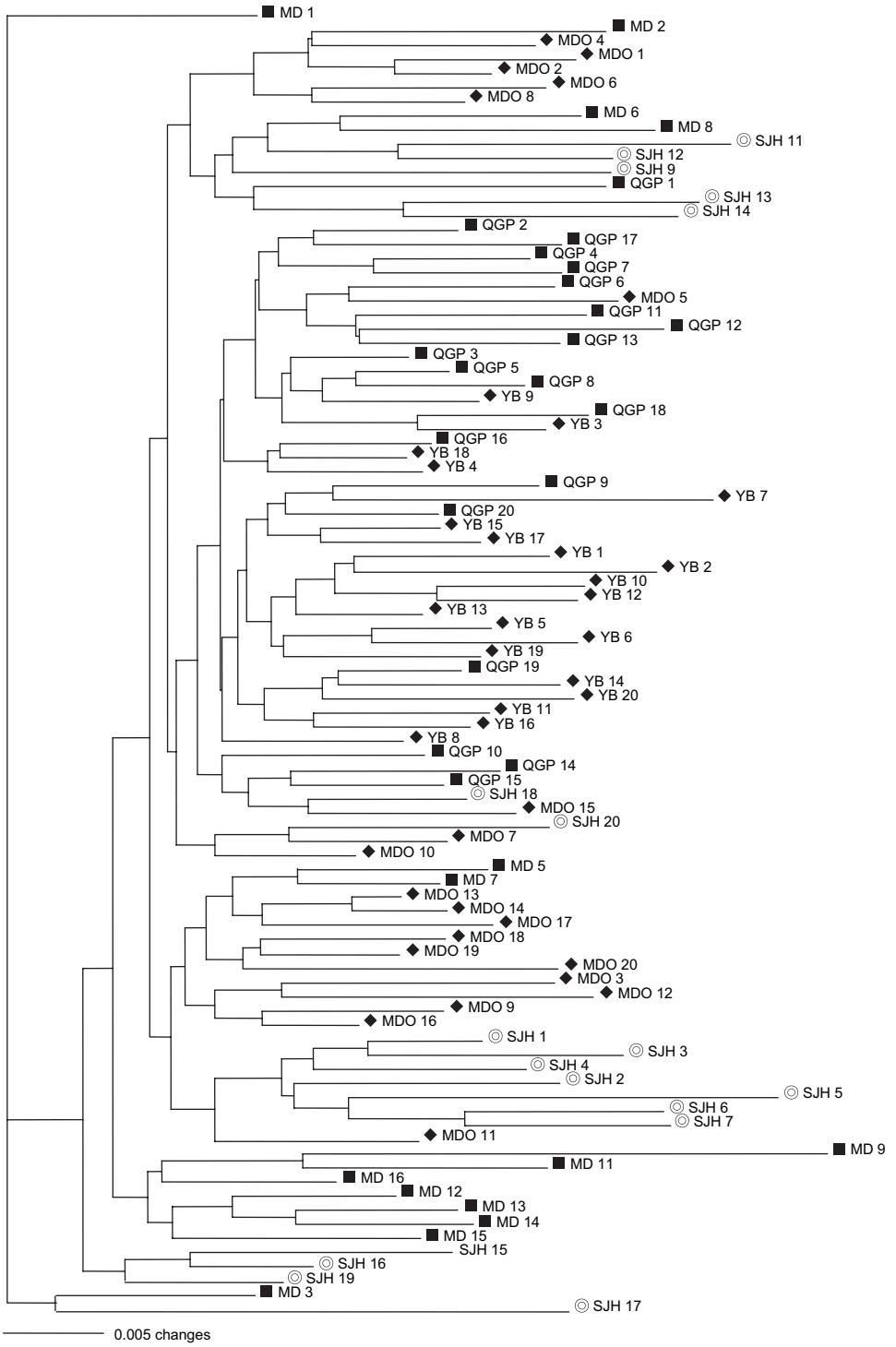

Fig. 3. A neighboring-joining dendrogram of individuals in the region of Mudong based on RAPD variation, ■: southern bank; ◆: northern bank; ◎: island.

Table 4
Mean genetic differentiation (Φ_{ST}) between the paired populations of *Vitex negundo* within regions

Region	Marker	Paired populations on the opposite banks	Paired populations within banks	Paired populations on island and each of banks
Mudong	RAPD	0.1368	0.1611	0.1021
	Chloroplast	0.4996	0.0435	0.2201
Zhongxian	RAPD	0.0956	0.0878	0.0834
	Chloroplast	0.6892	0.2408	0.2838

the same level of differentiation was found between populations from the opposite banks (mean bank-to-bank Φ_{ST}) in both Mudong (0.1368) and Zhongxian (0.0956) regions, as the differentiation between populations on the same banks (mean within-bank Φ_{ST} 0.1611 and 0.0878 in Mudong and Zhongxian, respectively). However, the cpDNA differentiation between populations on the opposite banks is much larger than that between populations within the same bank. The mean cpDNA differentiation between the south and north populations (bank-to-bank) were 0.4996 and 0.6892 in the two regions, respectively, whereas the values between populations on the same banks (within-bank) were only 0.0435 (Mudong) and 0.2408 (Zhongxian). In addition, the predominant cpDNA haplotypes in the populations on the south banks are different from those on the north banks in both regions (Fig. 1), which is contrast to the RAPD profiles where the individuals from the different groups mixed entirely on the neighboring-joining trees (Figs. 2 and 3). Taking the different mode of transmission of the two markers (cpDNA by seeds and RAPD by seeds and pollen) into account, these results suggest that the Yangtze River represents a general barrier to the dispersal of seeds but not to the movement of pollen of *V. negundo*. Furthermore, the island populations in the middle of the river often share haplotypes with one or both of the banks (Fig. 1) but the populations on the opposite banks in each region share very few haplotypes with each other. The mean cpDNA Φ_{ST} of bank-to-island classes are just intermediate to the mean cpDNA Φ_{ST} of within-bank classes and bank-to-bank classes. The results are consistent with one explicit expectation of the riverine barrier hypothesis that increasing divergence should relate positively to the width of watercourse (Patton et al., 1994).

Ouborg et al. (1999) indicated that the majority of seeds dispersed over very short distance and only a very small proportion dispersed over long ranges. For instance, in a seed trap experiment in *Lupinus texensis* Hook., with 95% of the seeds dispersal less than 2 m, less than 0.5% of the seeds dispersed between 3.2 and 3.4 m, and no dispersed seeds were detected beyond 3.5 m (Schaal, 1980). The seed-dispersal mode of *V. negundo* is animal-ingested. However, according to our field investigations, no bird was observed to feed on their fruits and seeds. This might imply that transporting seeds across the river by birds should be very rare and the Yangtze River is an effective barrier for seed dispersal. On the contrary, previous studies demonstrated that gene immigration via pollen was often more than 25% over distances of several hundred metres (Hamrick and Nason, 2000). Relatively long distance pollen flow foraged by bees and other insects has also reported in many plants (Hamrick and Nason, 2000; Ward et al., 2005). Because the watercourse of the Yangtze River in the regions concerned is about 1 km in width, gene flow via pollen is unlikely to be isolated by the river. Therefore, although studies on animal species provided inconsistent results regarding the impact of rivers on gene flow (Capparella, 1988, 1991; Patton et al., 1994; Lougheed et al., 1999), the present study demonstrated that the Yangtze River could disrupt seed dispersal at least for the animal-ingested plants.

Based on 117-120 RAPD loci, Su et al. (2003) found that the Great Wall, a man-made defence building constructed by ancient Chinese, could disrupt the gene flow of *V. negundo* and five other plants. However, our RAPD study of 10 *V. negundo* populations in Three-Gorge Area did not detect significant interruption of gene flow by the Yangtze River. The following two reasons may account for the discrepancy. First, most parts of the Great Wall were built on the top of steep mountains, or even on top of cliffs. The Great Wall might alter the micro-environment along its two sides significantly and thus may lead to genetic differentiation to some extent (Hsiao and Lee, 1999). Second, selection might be another factor underlying the differentiation between populations in divergent habitats such as the opposite slopes of a mountain. Ecological habitat adaptations have been shown experimentally to limit localized gene flow (Bockelmann et al., 2003). Micro-environment differs much more greatly on mountains than along rivers. The individuals may under stronger disruptive selection in mountain populations than in riverine populations, which may lead to higher genetic differentiation of plants around the Great Wall.

The landscape of Three Gorges Reservoir Area (TGRA) is becoming more fragmented than before (Wu et al., 2003) for the construction of TGD. With the increase of watercourse width in TGRA, the exchange of seeds between

two opposite banks and between island and banks is expected to decrease rapidly according to the conclusions of this study. This may potentially impact the local ecosystem, the population structure and dynamics of plant species in TGRA, especially for plants with low seed-dispersal ability. As discussed above, the genetic distances or differentiations between the island populations and those on either bank of the river are intermediate to those between the banks across the river, implying that the islands in the Yangtze River may serve as a stepping-stone for gene flow and seed dispersal. Several dozens to more than 100 mountain-tops will become islands after the formation of TGRA in 2009 (Wu et al., 2003). The 'stepping-stones' may be of importance for seed dispersal of plants across the river and so of practical importance to the management and conservation of biodiversity in this area.

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References

Ayres, J.M., Clutton-Brock, T.H., 1992. River boundaries and species range size in Amazonian primates. Am. Nat. 140, 531–537.

Barrett, S.C., Cole, W.W., Herrera, C.M., 2004. Mating patterns and genetic diversity in the wild daffodil *Narcissus longispathus* (Amaryllidaceae). Heredity 92, 459–465.

Bockelmann, A.-C., Reusch, T.B.H., Bijlsma, R., Bakker, J.P., 2003. Habitat differentiation vs. isolation-by-distance: the genetic population structure of *Elymus athericus* in European salt marshes. Mol. Ecol. 12, 505–515.

Butcher, P.A., Otero, A., McDonald, M.W., Moran, G.F., 2002. Nuclear RFLP variation in *Eucalyptus camaldulensis* Dehnh. from northern Australia. Heredity 88, 402–412.

Caicedo, A.L., Schaal, B.A., 2004. Population structure and phylogeography of *Solanum pimpinellifolium* inferred from a nuclear gene. Mol. Ecol. 13, 1871–1882.

Capparella, A.P., 1988. Genetic variation in neotropical birds: implications for the speciation process. Acta Congr. Intern. Ornith. 19, 1658–1664.

Capparella, A.P., 1991. Neotropical avian diversity and riverine barriers. Acta Congr. Intern. Ornith. 20, 307–316.

Chen, S.-L., Gilbert, M.G., 1994. Verbenaceae. In: Wu, C.-Y., Raven, P.H. (Eds.), Flora of China, vol. 17, pp. 28–32.

Chen, Z., Gupta, A., Yu, L., 2001. The Yangtze River: an introduction. Geomorphology 41, 73–75.

Cheviron, Z.A., Hackett, S.J., Capparella, A.P., 2005. Complex evolutionary history of a Neotropical lowland forest bird (*Lepidothrix coronata*) and its implications for historical hypotheses of the origin of Neotropical avian diversity. Mol. Phylogenet. Evol. 36, 338–357.

Colwell, R.K., 2000. A barrier runs through it … or maybe just a river. Proc. Natl. Acad. Sci. USA 97, 13470–13472.

Demesure, B., Sodzi, N., Petit, R.J., 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol. Ecol. 4, 129–131.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Dumolin-Lapegue, S., Pemonge, M.-H., Petit, R.J., 1998. Association between chloroplast and mitochondrial lineages in oaks. Mol. Biol. Evol. 15, 1321–1331.

Eriksson, J.H., Boesch, G.C., Vigilant, L., 2004. Rivers influence the population genetic structure of bonobos (*Pan paniscus*). Mol. Ecol. 13, 3425–3435.

Excoffier, L., 1993. WINAMOVA 1.55 (Analysis of Molecular Variance). Department of Anthropogy and Ecology, University of Geneva, Carouge, Switzerland.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Hamilton, M.B., 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol. Ecol. 8, 521–522.

Hamrick, J.L., Godt, M.J.W., 1996. Effects of life history traits on genetic diversity in plant species. Phil. Trans. R. Soc. B 351, 1291–1298.

Hamrick, J.L., Nason, J.D., 2000. Gene flow in forest trees. In: Young, A., Boshier, D., Boyle, T. (Eds.), Forest Conservation Genetics: Principles and Practice. CSIRO Publishing, CABI Publishing, Collingwood, Australia, Wallingford, United Kingdom, pp. 81–89.

Hsiao, J.Y., Lee, S.M., 1999. Genetic diversity and microgeographic differentiation of Yushan cane (*Yushania niitakayamensis*; Poaceae) in Taiwan. Mol. Ecol. 8, 263–270.

Huff, D.R., Peakall, R., Smouse, P.E., 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. Theor. Appl. Genet. 86, 929–934.

Ingvarsson, P.K., 2005. Nucleotide polymorphism and linkage disequilibrium within and among natural populations of European aspen (*Populus tremula* L., Salicaceae). Genetics 169, 945–953.

Isabel, N., Beaulieu, J., Theriault, P., Bousquet, J., 1999. Direct evidence for biased gene diversity estimates from dominant random amplified polymorphic DNA (RAPD) fingerprints. Mol. Ecol. 8, 477–483.

Liu, Y., Wang, Y., Huang, H., 2006. High interpopulation genetic differentiation and unidirectional linear migration patterns in *Myricaria laxiflora* (Tamaricaceae), an endemic riparian plant in the Three Gorges Valley of the Yangtze River. Am. J. Bot. 93, 206–215.

Liu, Z.-W., Jarret, R.L., Duncan, R.R., Kresovich, S., 1994. Genetic relationships and variation among ecotypes of seashore paspalum (*Paspalum vaginatum*) determined by random amplified polymorphic DNA markers. Genome 37, 1011–1017.

Lougheed, S.C., Gascon, C., Jones, D.A., Bogart, J.P., Boag, P.T., 1999. Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog (*Epipedobates femoralis*). Phil. Trans. R. Soc. B 266, 1829–1835.

Manley, B.F.J., 1985. The Statistics of Natural Selection. Chapman and Hall, London.

McCauley, D.E., 1994. Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: Implications for the study of gene flow in plants. Proc. Natl. Acad. Sci. USA 91, 8127–8131.

McCauley, D.E., 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends Ecol. Evol. 10, 198–202.

Morjan, C.L., Rieseberg, L.H., 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Mol. Ecol. 13, 1341–1356.

Nei, M., 1973. Analysis of gene diversity in subdivided population. Proc. Natl. Acad. Sci. USA 70, 3321–3323.

Nei, M., Li, W.-H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76, 5269–5273.

Ouborg, N.J., Piquot, Y., Van Groenendael, J.M., 1999. Population genetics, molecular markers and the study of dispersal in plants. J. Ecol. 87, 551–568.

Pastorini, J., Thalmann, U., Martin, R.D., 2003. A molecular approach to comparative phylogeography of extant Malagasy lemurs. Proc. Natl. Acad. Sci. USA 100, 5879–5884.

Patton, J.L., Silva, M.N.F.D., Malcolm, J.R., 1994. Gene genealogy and differentiation among arboreal spiny rats (*Rodentia*: Echimyidae) of the Amazon Basin: a test of the riverine barrier hypothesis. Evolution 48, 1314–1323.

Petit, R.J., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Muller-Starck, G., Demesure-Musch, B., Palme, A., Martin, J.P., Rendell, S., Vendramin, G.G., 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. Science 300, 1563–1565.

Prentis, P.J., Vesey, A., Meyers, N.M., Mather, P.B., 2004. Genetic structuring of the stream lily *Helmholtzia glaberrima* (Philydraceae) within Toolona Creek, south-eastern Queensland. Aust. J. Bot. 52, 201–207.

Reddy, T.B., Rangaiah, K., Reddi, E.U.B., Reddi, C.S., 1992. Consequences of nectar robbing in the pollination ecology of *Vitex negundo* (Verbenaceae). Res. Commun. 62, 690–691.

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DNASP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19, 2496–2497.

Sang, T., Crawford, D.J., Stuessy, T.F., 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Am. J. Bot. 84, 1120–1136.

Schaal, B.A., 1980. Measurement of gene flow in *Lupinus texensis*. Nature 284, 450–451.

Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science 236, 787–792.

Su, H., Qu, L.-J., He, K., Zhang, Z., Wang, J., Chen, Z., Gu, H., 2003. The Great Wall of China: a physical barrier to gene flow? Heredity 90, 212–219.

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.

Telfer, P.T., Souquiere, S., Clifford, S.L., Abernethy, K.A., Bruford, M.W., Disotell, T.R., Sterner, K.N., Roques, P., Marx, P.A., Wickings, E.J., 2003. Molecular evidence for deep phylogenetic divergence in *Mandrillus sphinx*. Mol. Ecol. 12, 2019–2024.

Tero, N., Aspi, J., Siikamaki, P., Jakalaniemi, A., Tuomi, J., 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. Mol. Ecol. 12, 2073–2085.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTALX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Tsukaya, H., Fukuda, T., Yokoyama, J., 2003. Hybridization and introgression between *Callicarpa japonica* and *C. mollis* (Verbenaceae) in central Japan, as inferred from nuclear and chloroplast DNA sequences. Mol. Ecol. 12, 3003–3011.

Victory, E.R., Glaubitz, J.C., Rhodes, O.E., Woeste, K.E., 2006. Genetic homogeneity in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. Am. J. Bot. 93, 118–126.

Wallace, A.R., 1849. On the monkeys of the Amazon. Proc. Zool. Soc. Lond. 20, 107–110.

Wang, P.X., 1997. The last fifty million years–on origin of the modern natural macro-environmental pattern. Sin. Sci. 49, 18–22.

Ward, M., Dick, C.W., Gribel, R., Lowe, A.J., 2005. To self, or not to self. A review of outcrossing and pollen-mediated gene flow in neotropical trees. Heredity 95, 246–254.

Wu, J., Huang, J., Han, X., Xie, Z., Gao, X., 2003. Three-Gorges Dam: experiment in habitat fragmentation? Science 300, 1239–1240.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., Mao, J.X., 1997. POPGENE, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada.