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Environmental heterogeneity decides bio-heterogeneity of the *Spiraea japonica* complex (Rosaceae) in China

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Abstract. Amplified fragment length polymorphism (AFLP) fingerprint was the measure used to reveal the patterns of genetic variation within the *Spiraea japonica* L. f. complex, in the context of physical environment differentiation stemming from the Himalaya–Tibet plateau uplift, the most significant geological event in eastern Asia since the late Paleocene. The complex displays obvious genetic heterogeneity among geographic populations, which was greater in south-western China than in eastern China. The uplift of the Himalaya–Tibet plateau, and consequent increase in environmental complexity in south-western China, is presumed to be responsible for this genetic structure. The obvious genetic heterogeneity among populations or varieties of the *S. japonica* complex indicates that the rich biodiversity in south-western China is possibly based on genetic variation, suggesting that the biodiversity centre of south-western China represents a tremendous gene pool. It also supports the idea that the protected area and conservation investment made in south-western China should be further augmented. In addition, the greater genetic heterogeneity displayed by plants in south-western China suggests that conservation plans in this region should be more inclusive than those in eastern China.

Introduction

One of the most significant geographical features of eastern Asia is the sharp increase in elevation from east to west, owing to the uplift of the Himalayas and the Tibetan Plateau. In the east are low plains with elevations ranging from 0 to 500 m asl, whereas in the west, the Himalayas and Tibetan Plateau reach elevations of 4000–5000 m asl. In between these regions exists a narrow transitional belt comprising a mosaic of plateaux, mountains, basins and gorges, with elevations ranging from 500 to 4000 m asl (Committee of the CAS for Physical Geography of China 1985; Fig. 1*a*). The physical environments in the western mountainous plateau and the eastern plains are relatively homogenous compared with the complex mix of habitats in the transitional belt—these habitats are especially diverse in south-western China. This complexity of habitat types is mirrored by climatic complexity; the eastern plains are affected by the south-eastern Pacific monsoon, the western mountainous plateau by the south-western monsoon from the Indian Ocean, whereas the transitional belt (especially in south-western China) is influenced by both monsoons (Committee of CAS for Physical Geography of China 1985; Wang 1994; Fig. 1*a*).

Botanical diversity is greatest in the eastern plains and the transitional belt, with fewer plant species in the high western regions. Moreover, the transitional belt bears almost half of the total plant species in China, especially in south-western China which is an important centre for biodiversity, with more than a third of its species being endemic (Wang 1961; Wu 1980; Wu and Wu 1998). Floristic studies (Wu 1979; Wu and Wu 1998) suggest that the forest area in eastern Asia could be divided into two subkingdoms, the Sino-Japanese forest subkingdom and the Sino-Himalayan forest subkingdom (Fig. 1*b*). The formation of these spatiotemporal patterns of biodiversity has long been suspected to correlate with changes in the physical environment resulting from the uplift of the Himalaya–Tibet plateau (Chang 1983; Tanai 1992; Axelrod *et al.* 1998). However, the nature and extent of this correlation remains unknown, and the underlying genetic characteristics behind the tremendous morphological diversity in south-western China, compared with other regions of China, have not been exemplified. However, China is committing itself to biodiversity conservation. Conservation strategy and its success will mainly depend on understanding the biogeographical processes in specific regions. However, very few population-genetic studies on a regional scale have been directed at the conservation biology of this region. Genetic characteristics on a regional scale largely remain unknown. Intensive biological observations of widespread species at the population level may provide new insights into the biogeography and conservation biology of this region.

Spiraea japonica is a perennial shrubby species complex, widespread across eastern Asia, and has received considerable attention in China for its potential medicinal uses (Wu 1984). Morphologically, the complex is very diverse with nine intraspecific varieties, discriminated by differences in leaf size and shape (Rehder 1913; Yu 1974; Wu and Raven 2003; Table 1). With respect to variation and distribution patterns, the complex seems to reflect some floristic characteristics of the plant flora in eastern Asia. The complex is morphologically most diversified in south-western China, with the majority of varieties occurring in this extremely narrow region. In contrast, only three varieties occupy central and eastern China, Japan and South Korea, a combined area that is much larger than south-western China (Fig. 1*c*). From the perspective of dispersal and variation, the *S. japonica* complex

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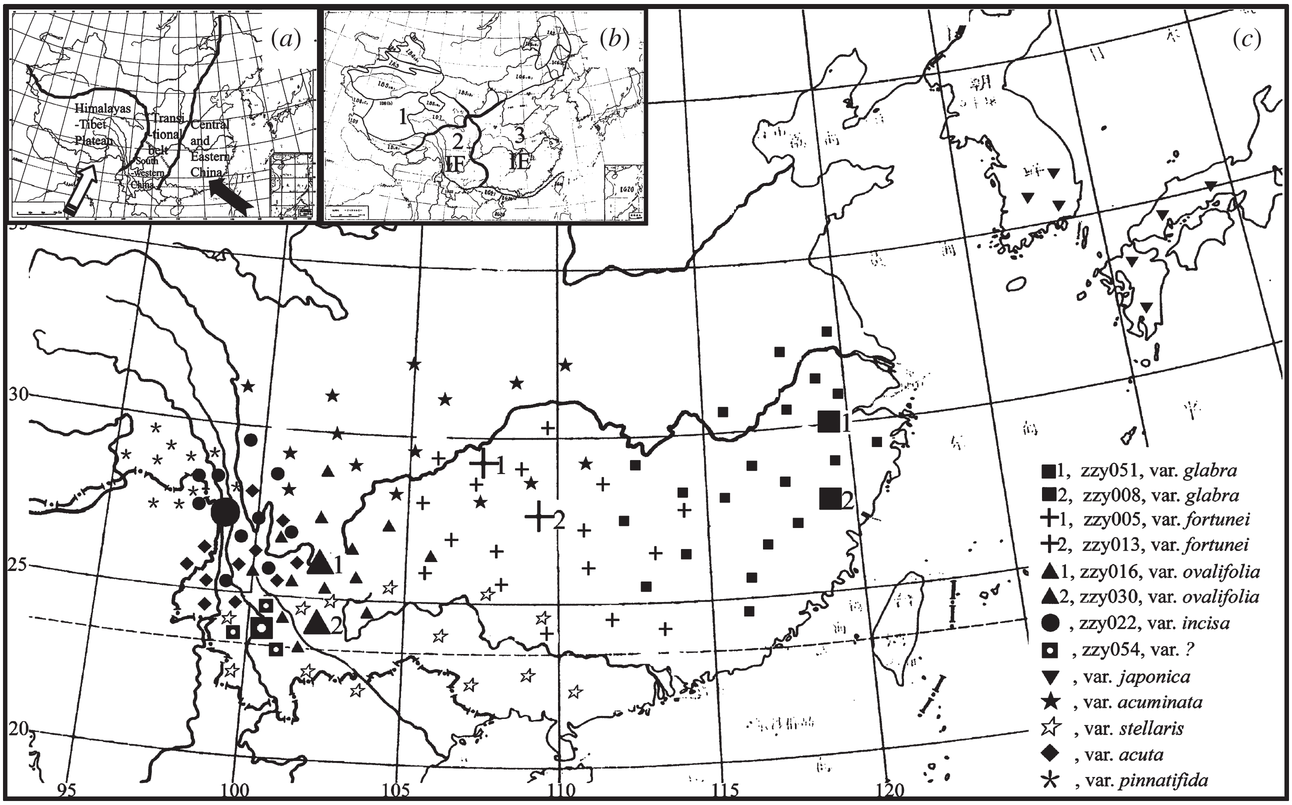

Fig. 1. (*a*) Sketch map showing general physical environmental characteristics of eastern Asia. Arrows represent direction of monsoon. (*b*) Floristic division of eastern Asia and the three environmental gradients. Symbols: 1, Himalaya–Tibet plateau; 2, the transitional belt in south-western China; 3, plain area in eastern China; IF, Sino-Himalayan forest subkingdom; IE, Sino-Japanese forest subkingdom (see Wu (1979) and Wu and Wu (1998) for detailed information of the floristic division of eastern Asia). (*c*) Distribution of the *Spiraea japonica* complex across eastern Asia. Large symbols indicate sample locations for the corresponding varieties.

Table 1. Material resources, vouchers, population codes used in the study and floristic areas in which varieties or populations are distributed

Floristic division follows Wu and Wu (1998)

Taxon	Sample number	Resource	Voucher	Floristic division
Spiraea japonica				
var. *glabra*	14	Linhai, Zhejiang	ZZY008	Sino-Japanese forest subkingdom; eastern China
	7	Hangzhou, Zhejiang	ZZY051	
var. *fortunei*	15	Longshan, Hunan	ZZY013	
	15	Chongqing, Sichuan	ZZY005	
var. *ovalifolia*	12	Shongming, Yunnan	ZZY016	Sino-Himalayan forest subkingdom; south-westernChina
	13	Wuding, Yunnan	ZZY030	
var. *incisa*	16	Weixi, Yunnan	ZZY022	
var. ?	15	Tengchong, Yunnan	ZZY054	

represents a useful model to investigate the correlation between biodiversity patterns and environmental heterogeneity on a regional scale.

We used AFLP as a tool to examine the genetic variation within the *S. japonica* complex and to look for a correlation between the genetic structure and environmental heterogeneity stemming from the uplift of the Himalaya–Tibet plateau, to provide new insights for biodiversity conservation in eastern Asia.

Materials and methods

Taxa and sampling

In all, 107 individuals from eight populations of the *S. japonica* complex were analysed by AFLP. Two populations were from Zhejiang in eastern China, taxonomically belonging to *S. japonica* var. *glabra*; two populations of *S. japonica* var. *fortunei* from Chongqing and Hunan in central China, four populations from Yunnan in south-western China, with two being

S. japonica var. *ovalifolia*, one being *S. japonica* var. *incisa* and one having been dubiously identified as *S. japonica* var. *fortunei*. All studied specimens are listed in Table 1, and vouchers were deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

DNA extraction and AFLP analysis

Total genomic DNA was extracted from dried leaf material according to the CTAB protocol of Doyle and Doyle (1987), treated with RNAase (30 min at 37°C) and adjusted to a concentration of $\sim$25 ng μL^{-1}. The AFLP analysis followed the protocol described by Vos *et al.* (1995), with minor modifications. A quantity of 250 ng per 10 μL of genomic DNA was restricted with the enzyme combination *Mse*I and *Eco*RI and ligated to *Mse*I and *Eco*RI adapters. Pre-amplifications were performed in an ICYCLER thermal cycler (BioRad: Hercules, CA, USA) by using primer pairs with a single selective nucleotide (*Eco*RI–A/*Mse*I-C). Sixteen different primer combinations with three selective nucleotides were tested for the selective amplification. On the basis of consistency of results and the number of scorable bands, two primer combinations were chosen to carry out selective amplification for all samples, namely *Eco*RI–AGC/*Mse*I–CTA and *Eco*RI–AGC/*Mse*I–CTG. The products of final amplification were denatured and loaded on 6% polyacrylamide denaturing gels, with 100 bp DNA Ladder (Promega: Madison, WI, USA) as a standard. The bands were sequentially visualised by silver staining (Bassam *et al.* 1991).

The gels were scanned on an A3-sized scanner. Bands were visually scored as present or absent with the aid of LabWorks Analysis Software version 4.0 (UVP), on the basis of the criterion that only bands that could be scored confidently were recorded. On the basis of the constructed present/absent data matrix, the molecular diversity indices, number of AFLP haplotypes, total number of bands, percentage of polymorphic bands, mean pairwise differences and average gene diversity over loci were calculated by Arlequin 2.01 (Schneider *et al.* 2000). Grouping all the populations into either the south-western group or eastern group, according to their distribution, an analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was also performed by Arlequin, with 1000 permutations and the same general settings as used for the calculation of molecular diversity dices. The structure was defined to partition the total genetic variance into components among geographical groups, among populations and among individuals within populations. Population pairwise F_{st} values were also calculated on the basis of variance components.

Results

Overall genetic variation for the *S. japonica* complex, obtained by analysis with two primer combinations, is given by the number and percentage of AFLP haplotypes, total number of loci, number and percentage of polymorphic loci, mean pairwise differences and average gene diversity over loci (Table 2). In total, 98 reliable loci were recorded with two primer combinations, of which 86 loci were polymorphic at the species level. Genetic variation within populations is very limited. When structure was defined to partition the variance into components among groups, among populations and among individuals within populations, AMOVA analysis attributed 16% and 71% of the total variance among groups and among populations, respectively, whereas variance within populations accounted for only 13% of the total variance (Table 3). Clear geographical differentiation in the level of genetic diversity was also revealed by standard genetic-diversity indices generated by comparing individuals in each population. In total, 88 haplotypes were identified from the 107 individuals analysed. Forty-seven haplotypes were present in the eastern populations, of which 51 individuals were analysed, whereas 56 individuals of the four south-western populations included 41 haplotypes. Genetic diversity within populations of the eastern group was higher than that of the south-western group. The mean number of pairwise differences among all possible combinations of individuals within populations ranged from 3.96 (s.d. = 2.10) to 8.97 (s.d. = 4.40) in the eastern group, and from 2.15 (s.d. = 1.28) to 3.45 (s.d. = 1.86) in the south-western group. Average gene diversity across loci of populations displayed a similar pattern, ranging from 0.03 (s.d. = 0.02) to 0.07 (s.d. = 0.04) in the eastern

Table 2. Genetic biodiversity within populations of the *Spiraea japonica* complex and in the total species, which was reflected by the number and percentage (in parentheses) of haplotypes (AFLP band patterns), number and percentage (in parentheses) of polymorphic loci, mean pairwise distance within population (s.d. in parentheses) and average gene diversity across loci (with 5% allowance of missing data; s.d. in parentheses)

Population	No. of individuals	No. of AFLP haplotypes[A]	No. of polymorphic loci[B]	Mean pairwise distance within population (s.d.)	Average gene diversity across loci (s.d.)	Geographical distribution
ZZY051	7	7 (100%)	21 (21%)	8.48 (4.47)	0.07 (0.04)	Sino-Japanese forest subkingdom; eastern China
ZZY008	14	13 (93%)	30 (31%)	8.98 (4.40)	0.07 (0.04)	
ZZY013	15	14 (93%)	15 (15%)	4.65 (2.41)	0.04 (0.02)	
ZZY005	15	13 (87%)	11 (11%)	3.96 (2.10)	0.03 (0.02)	
ZZY016	12	9 (75%)	10 (10%)	2.86 (1.62)	0.02 (0.02)	Sino-Himalayan forest subkingdom; south-western China
ZZY030	13	10 (77%)	6 (6%)	2.15 (1.28)	0.02 (0.01)	
ZZY022	15	8 (53%)	6 (6%)	2.55 (1.45)	0.02 (0.01)	
ZZY054	16	14 (88%)	8 (8%)	3.46 (1.86)	0.03 (0.02)	
Total	107	88 (82%)	86 (87%)	28.64 (12.63)	0.23 (0.11)	

[A] %AFLP haplotypes = no. of AFLP haplotypes/no. of individuals.

[B] %Polymorphic loci = no. of polymorphic loci/total loci; no. of total loci = 98.

Table 3. AMOVA, showing genetic variance between south-western and eastern group, among and within populations of the *Spiraea japonica* complex

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	321.16	2.80	16.26
Among populations	6	980.39	12.21	71.02
Within populations	99	216.53	2.19	12.72
Total	106	1518.08	17.20	

group and from 0.02 (s.d. = 0.01) to 0.03 (s.d. = 0.02) in the south-western group (Table 2). Population pairwise F_{st} values for the eastern group ranged from 0.52 to 0.79, with an average of 0.70 (s.d. = 0.12), wheras those for the south-western group ranged from 0.75 to 0.94, with an average of 0.90 (s.d. = 0.06) (Table 4). Also, independent AMOVAs indicated that variance among populations is higher in the south-western group than in the eastern group (Table 5).

Discussion

Inbreeding tendency

A noteworthy aspect of the population structure in the *S. japonica* complex is the low level of genetic variation within populations and high genetic differentiation among populations. Dispersal of seeds from the bony follicles of *S. japonica* is mainly effected by explosion of the follicles and gravity, and rarely involves wind and animals. Therefore, the dispersal distance is very limited. Moreover, our germination experiments indicated that very few seeds of *S. japonica* were able to germinate and the species can easily proliferate clonally (Zhang 2003). All these factors may be responsible for the inbreeding tendency in the *S. japonica* complex. Moreover, the genetic variation of the *S. japonica* complex which mostly occurs among populations (varieties), suggests that the morphological diversification of the *S. japonica* complex is genetically based.

Possible biogeography process in eastern and south-western China

Spiraea japonica complex displays a clear geographical differentiation in the level of genetic diversity between eastern and south-western China. Average genetic diversity among populations is higher in the south-western group than in the eastern group, whereas genetic variance within populations is lower in the south-western group than in the eastern group (Table 5). The genetic structure of the *S. japonica* complex correlates with the environmental heterogeneity pattern. As stated previously, eastern and south-western China are very different in environmental heterogeneity, owing to the geological event of the Himalaya–Tibet plateau uplift. Comprising a mosaic of plateaux, mountains, basins and gorges, south-western China is much more complicated than eastern China, with respect to environmental heterogeneity. Arguably, the habitat mosaic in south-western China would cause more geographical barriers, stronger selection pressure for divergence, lower gene flow and more bottleneck events than the relatively homogenous eastern plains. These factors may have contributed to the observed genetic structure of the *S. japonica* complex.

Implications for biodiversity conservation in China

China has established a comprehensive legal framework for managing nature and biodiversity. Protected areas at the national, provincial, prefecture and county levels have been dramatically increased during the past decade, and China has received international recognition for its wetlands, biosphere-reserves and natural- and cultural-heritage preservation programs. However, the main targets in terms of protecting species and habitats are usually defined as a percentage of land area protected. There is a need to ensure that the key natural-habitat types and ecosystems are adequately protected and that they support species recovery plans (Organisation for Economic Co-operation and Development 2006).

South-western China has been recognised as the most important centre of biodiversity in China, with more than a third of its species endemic (Wu 1980; Wu and Wu 1998). The genetic diversity pattern of the *S. japonica* complex suggests that the rich plant biodiversity in south-western China may be based on genetic variation, although environmental modification may be involved in the morphological diversification in this region. This supports the view that the biodiversity centre of south-western China has a tremendous gene pool, and justifies the national nature conservation project (State Environment Protection Administration of China 2000) currently in place in China, in which south-western China is recognised as one of the key areas for building nature reserves. However, as an extremely important gene pool, south-western China was not valued sufficiently. According to the project, only an area of 53 000–66 000 km^2 will be protected in south-western China, whereas in the eastern part, an area of 96 500–101 000 km^2

Table 4. Population pairwise F_{st} values for the *Spiraea japonica* complex based on pairwise difference, showing differentiation level

		ZZY051	ZZY008	ZZY013	ZZY005	ZZY016	ZZY030	ZZY022	ZZY054
Eastern group	ZZY051								
	ZZY008	0.52							
	ZZY013	0.77	0.77						
	ZZY005	0.79	0.77	0.58					
		Averaged pairwise F_{st}: 0.70 (s.d. = 0.12)							
South-western group	ZZY016	0.83	0.78	0.90	0.91				
	ZZY030	0.86	0.80	0.91	0.92	0.75			
	ZZY022	0.89	0.85	0.87	0.85	0.94	0.94		
	ZZY054	0.86	0.82	0.90	0.90	0.91	0.92	0.91	
		Averaged pairwise F_{st}: 0.90 (s.d. = 0.06)							

Table 5. AMOVA, calculated independently for eastern and south-western group, showing genetic variance among and within populations in each group (with 5% allowance for missing data)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Eastern group				
Among populations	3	314.65	8.17	72.73
Within populations	47	144.05	3.06	27.27
Total	50	458.71	11.24	
South-western group				
Among populations	3	665.74	15.82	91.90
Within populations	52	72.48	1.39	8.10
Total	55	738.21	17.21	

will be protected. Considering the tremendous genetic diversity of south-western China, the size of the protected area and conservation investment in south-western China need to be further augmented.

Further, the differences in the characteristics of genetic structure between south-western and eastern China suggest that different conservation strategies need to be adopted in these two regions, especially when the time-consuming nature and financial squeeze associated with conservation are taken into account. For an endangered species in eastern China, the homogenisation effect of environment makes it possible to support a species recovery plan by conserving only a few populations. Whereas for an endangered species in south-western China, because of the greater genetic heterogeneity among populations, the conservation plan should include more populations so that the core germplasm of the species is safely conserved. The genetic diversity pattern displayed by the *S. japonica* complex provides valuable implications for conservation of biogeography. However, further studies are required to assess the extent to which the conclusions can be generalised across different taxa, especially in view of the small sample size in the present study.

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