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GENETIC ISOLATION BY DISTANCE IN THE ENDANGERED PLANT *SINOCALYCANTHUS CHINENSIS* ENDEMIC TO CHINA

JUNMIN LI, ZEXIN JIN* AND JINGJING GU

Institute of Ecology, Taizhou University, Linhai 317000, P R China
**Corresponding author's e-mail: jzx@tzc.edu.cn*

Abstract

Sinocalycanthus chinensis, narrowly endemic to China, is a tertiary relict species. We analyzed the genetic structure pattern of 6 populations of *S. chinensis* using inter-simple sequence repeat (ISSR) markers and the isolation by distance (IBD) pattern was tested in order to understand the relative influences of gene flow and genetic drift on population structure. The genetic diversity at species level was relatively high (P=51.00%, h =0.1397 and I=0.2191, respectively), while that at populations level was relatively low (P=18.00%, h=0.0733 and I=0.1108, respectively). High genetic differentiation was detected among populations (Φ_{ST}=0.6320). Neighbor-joining method of clustering results showed that six populations were clearly separated into eastern and western group. Mantel test showed that there was significant association between genetic distance and geographical distance (r^2=0.8600, P=0.0470). Limited gene flow due to species traits and habitat fragmentation and the consequent genetic drift might be the 2 main causes for the genetic isolation by distance of *S. chinensis* populations.

Introduction

Genetic structure of plant species may be influenced by many factors, such as breeding system, gene flow, environmental selection, population history (Hamrick & Godt, 1996; Schaal *et al.*, 1998; Jin & Li, 2007a; Li & Jin, 2007; Arif *et al*., 2005; Ashraf *et al.*, 2003). Habitat fragmentation of many endangered plant species become worse and worse for the severe human disturbances and have major genetic demographic consequences by reducing effective population size, increasing genetic drift, increasing rate of genetic erosion, elevating inbreeding depression, and limiting gene flow (Li & Jin, 2008; Keller & Waller, 2002). Understanding the pattern of genetic structure and the causes can help to evaluate the endangerment level of an endangered plant species and thus make an appropriate conservation strategy (Yu *et al.*, 2011).

The term "isolation by distance" (IBD) refers to increasing genetic differences among populations separated by increasing geographic distances (Wright, 1943). Plants are prone to genetic differentiation as a result of spatial isolation followed by reduced gene flow within and among populations due to their sessile nature (Michalski & Durka, 2007). Correlation analysis of genetic distances and geographic distances provide a much more effective approach to studying the relative influences of gene flow and genetic drift on regional population structure than simple analysis on genetic diversity (Hutchison & Templeton, 1999). Testing of IBD pattern can provide genetic information, such as gene flow among populations, for the species and help to make decisions about which populations should be considered distinct, and whether or not gene flow should be artificially manipulated (Crispo & Hendry, 2005).

Sinocalycanthus chinensis Cheng et S.Y. Chang is the only representative of *Sinocalycanthus* (family Calycanthaceae), which is an East Asia-North American disjuncted genus, endemic to China. *S. chinensis* is a dense, rounded deciduous shrub that grows 1 to 3m tall. It often distributes under evergreen broad-leaved forest or mixed evergreen and deciduous broad-leaved forest of ravines and mountain slopes at altitudes from 600 to 1,100 m. *S. chinensis* has beautiful flower with high ornamental value but without fragrance. The leaves of *S. chinensis* are used as a remedy for cold, cough and wheeziness (Ni *et al.*, 2003). As a tertiary relict species, *S. chinensis* provides a useful model system for evolutionary and ecological studies in plant biology and genetics and is taken as the national second class protection plant of China (Hu, 2002). Previous studies on *S. chinensis* mainly focused on the morphological anatomy of pollen (Li, 1990), chromosome (Li, 1990), coenology (Zhang *et al.*, 1997; Zhang *et al.*, 2001), reproductive biology (Huang, 1998), plant chemistry (Ni *et al.*, 2003), and the genetic diversity (Zhou & Ye, 2002; Li & Jin, 2006; Jin & Li, 2007b). However, little attention has been paid to the relationship between genetic distances and geographical distances of existed populations.

The size of the wild population of *S. chinensis* has contracted due to habitat destroy and overploitation and only a few populations limitedly distributed in Linan City and Tiantai County in Zhejiang province (Zhang *et al.*, 2001; Zhou & Ye, 2002). Till 2008, Chen *et al*., (2008) reported that new *S. chienesis* population was found in Anhui City near Zhejiang Province. No genetic study has been focused on this new population. Here, we analyzed the genetic structure pattern of 6 populations of *S. chinensis* including Anhui population using ISSR markers and the IBD pattern were tested in order to understand the relative influences of gene flow and genetic drift on population structure.

Materials and Methods

Population sampling: According to the field survey information, 6 typical wild populations of *S. chinensis* were sampled from Zhejiang Province and Anhui Province (Table 1; Fig. 1). Fresh tender leaves were collected randomly from 30 adult trees in each population. The distances between sampled trees were from 30m to 50m, which depended on the population size. The sampled leaves were kept at 4ºC in sealed bags and stored at -70ºC until DNA extraction.

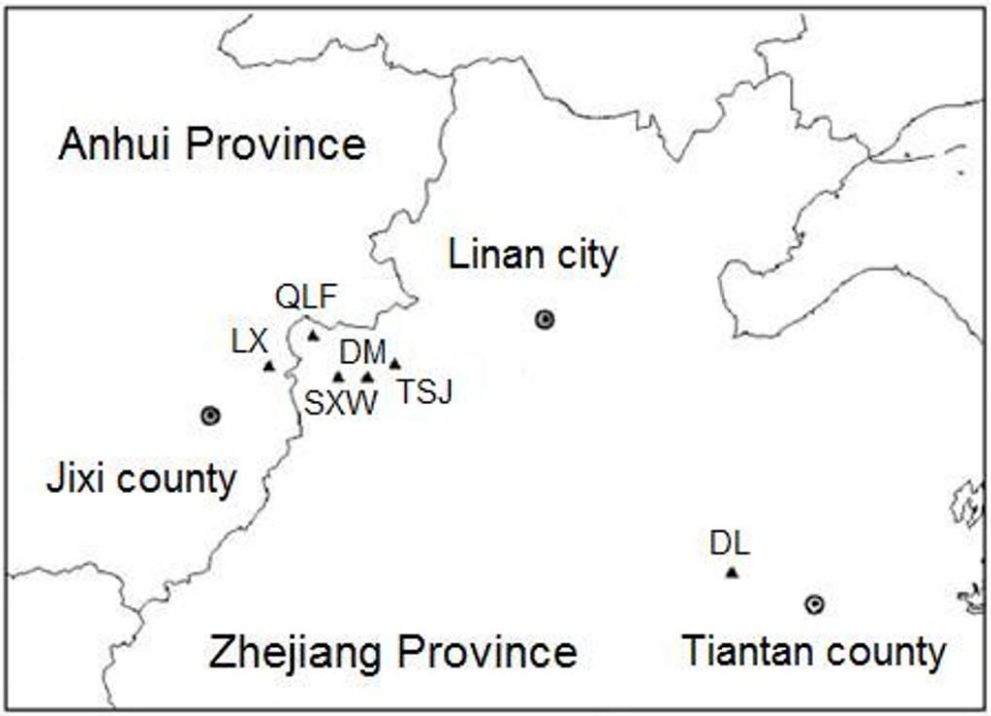

Fig. 1 Map showing the geographic distribution of six populations of *S. chinensis*.

Table 1. Basic conditions of populations of *S. chinensis* sampled.

Population	Abbreviation	Locality	Latitude, longitude	Altitude (m)	Slope aspect	Community type	Habitat
Dalei Mountain I	DL	Jiwoping, Longxi village, Tiantai county, Zhejiang	28°59.257′N, 120°45.766E	782	NE25°	Chinese fir forest	Among shrub on valley
Daming Mountain	DM	Xikeng, Lin'an city, Zhejiang province	30°02.450′N, 118°59.371′E	854	NE30°	Evergreen broad-leaved forest	Under forest beside brook
Shuxiwu	SXW	Shunxiwu, Qingliangfeng town, Lin'an city, Zhejiang province	30°01.676'N, 118°56.087'E	717	SE55°	Evergreen broad-leaved forest	Under forest
Tashajiang	TSJ	Tashajiang, Lin'an city, Zhejiang province	30°03.354'N, 119°00.855′E	928	SE60°	Evergreen broad-leaved forest	Under forest
Qingliangfeng	QLF	Maxiao, Qingliangfeng town, Lin'an city, Zhejiang province	30°07.981'N, 118°55.226'E	725	NE50°	Mixed evergreen and deciduous broad-leaved forest	Under forest on valley
Longxu Mountain	LX	Yunzhou villiage, Jixi county, Anhui province	30°03.937′N, 118°41.768′E	912	NE30°	Evergreen broad-leaved forest	Under forest

Total DNA extraction and ISSR amplification: Frozen leaves were ground in liquid nitrogen and genomic DNA was extracted from 0.1g powder following the optimized SDS (Sodium dodecyl sulfate) method (Li & Jin, 2006). DNA concentration was determined by comparing the sample with a reference DNA in 0.8% agarose gel. DNA was diluted to 10 ng·μl^{-1} and stored at -20 ºC for ISSR amplification. The optimal reaction conditions for ISSR of *S. chinensis* were as follows: 1 × Taq polymerase buffer [10 mmol·L^{-1} Tris·HCl (pH 9.0), 50 mmol·L^{-1}KCl, 0.1% Triton X-100, 1.5 mmol·L^{-1} $MgCl_2$], 0.75 U Taq DNA polymerase (Huamei Inc., Shanghai, China), 20ng template DNA, 6 pmol primer, 0.15 mmol·L^{-1} of each dNTPs in a total reaction volume of 10μl.

Amplification reaction was performed in a PTC 220 Thermal Cycler (Bio-Rad, Inc., Hercules, California, USA). The touchdown cycle program included an initial 5 min denaturation at 94ºC, followed by 10 cycles of 1 min at 94ºC, 1 min at 56ºC (touchdown for 1ºC every cycle) and 1.5 min at 72ºC, followed by 25 cycles of 1 min at 94ºC, 1 min at 50ºC and 1.5 min at 72ºC, and 5 min final extension at 72ºC. PCR product was electrophoresed in

1.6% agarose gel at 100V for 2h, stained with ethidium bromide. The electrophoresis buffer was 0.5×TBE. Images were taken with Gel Doc XR image analysis system (Bio-Rad, Inc., Hercules, California, USA).

The negative control was run by replacing template DNA with ddH_2O. For every primer, triplicate amplification was conducted. A subset of 8 primers (Table 2) was chosen from 100 primers (UBC primer set No. 9, Biotechnology Laboratory, University of British Columbia) for further analysis since they provided: consistent, strong amplification products, uniform and reproducible fragments, lack of amplification in negative control.

Data analysis: Amplified bands were scored in a size range from 0.2 to 2 kb. ISSR amplified fragments were scored manually for presence (1) or absence (0). The percentage of polymorphic loci (*P*), Shannon's information index (*I,* Lewontin, 1972) and Nei's (1973) gene diversity (*h*) were calculated using POPGENE ver 1.31 software (Yeh & Boyle, 1998). Gene flow was calculated from $Nm = 0.5(1 - G_{ST})/G_{ST}$.

An analysis of molecular variance (AMOVA) was done with the ARLEQUIN ver 3.01package (Excoffier *et al.*, 2006) for measuring variance within populations and among populations. Variance components, the sum of all squared differences, and analogues of *F*-statistics (Φ) based on Euclidean distance between individuals, which is the equivalent of the Wright's F_{ST} index, were calculated to estimate the population differentiation. The RAPDDIST program (Black, 1996) was used to generate bootstrap datasets. Neighbor-Joining cluster analysis was performed to produce phylogenetic trees using NEIGHBOR clustering program in PHYLIP package (Felsenstein, 1995), and the majority-rule (extended) consensus trees were generated from bootstrap trees, using the program CONSENSE in the PHYLIP package. The phylogenetic trees were viewed and drawn using TREEVIEW program (Page, 1996).

Nei's genetic distance (Nei, 1972) was applied to evaluate the genetic relationships between populations. Geographical distances were calculated by Earth Explorer 4.0 program. Mantel tests were used to test for a significant association between genetic and geographical distance, and reduced major axis regression was used to estimate regression statistics. The computer program IBD ver 1.52 (Bohonak, 2002) was used to calculate Mantel tests (5 000 randomizations) and the slope, y-intercepts and coefficient of determination (r^2).

Results

Genetic structure: Genetic diversity at population level was relatively low (*P*=18.00%, *h* = 0.0733 and *I* =0.1108, respectively) with highest at DM population and lowest at DL population (Table 3). However, the genetic diversity at species level was relatively high (*P*=51.00%, *h* = 0.1397 and *I* =0.2191, respectively) (Table 3). High level of genetic differentiation was detected among populations: 63.18% of the total variation occurred among populations and only 36.82% occurred within the population (Table 4), which resulted in a high Φ_{ST} value (0.6320). N_m estimated from G_{ST} was 0.4009.

Phylogenetic relationship: Neighbor-Joining cluster analysis separated 6 populations into 2 groups: the eastern group (DL population) and the western population (the other five populations) with a 74.2% bootstrap support (Fig. 2).

Isolation by distance: Nei's genetic distance and geographical distance were listed in Table 5. A significant association between Nei's genetic distance and geographical distance at the population level were revealed by Mantel analysis (r^2=0.8600, *P*=0.0470).

Table 2. Sequences of 8 primers successfully used in the ISSR analysis.

Primer	Sequence (5'- 3')	Primer	Sequence (5'- 3')
UBC 807	$(AG)_8T$	UBC 840	$(GA)_8YT$
UBC 810	$(GA)_8T$	UBC 842	$(GA)_8YG$
UBC 812	$(GA)_8A$	UBC 864	$(ATG)_6$
UBC 826	$(AC)_8C$	UBC 866	$(CTC)_6$

Table 3. Genetic diversity of *S. chinensis* at population and species level.

Population	*N*	*n*	*P*	*I*	*h*
DL	30	13	13%	0.0687 (0.1887)	0.0462 (0.1292)
DM	30	24	24%	0.1311 (0.2462)	0.0887 (0.1697)
SXW	30	18	18%	0.0891 (0.2016)	0.0584 (0.1354)
TSJ	30	18	18%	0.0923 (0.2114)	0.0616 (0.1451)
QLF	30	20	20%	0.1006 (0.2220)	0.0677 (0.1538)
LX	30	15	15%	0.0746 (0.1978)	0.0505 (0.1377)
Species[a]	180	51	51%	0.2191 (0.2528)	0.1397 (0.1722)

[a] Total genetic diversity of *S. chinensis* at species level.

N denotes sample size. *n* denotes number of polymorphic loci. *P* denotes the percentage of polymorphic loci. *I* denotes Shannon's information index. *h* denotes Nei's gene diversity. The number in the bracket indicates the standard deviation. The abbreviations of population name see Table 1.

Table 4. Analysis of molecular variance (AMOVA) for populations of *S. chinensis*.

Source of variance	d.f.	SS	MS	Variance component	Percentage Total (%)	*P*-value [b]
Among populations [a]	5	672.1889	134.438	4.3959	63.18	<0.001
Within populations [a]	174	445.6667	2.561	2.5613	36.82	<0.001

[a] all of the six populations. [b] Significance tests after 1000 permutations

d.f. is the degree of freedom, SS is the sum of squares, MS is the expected mean squares, *P*-value is the probability of null hypothesis

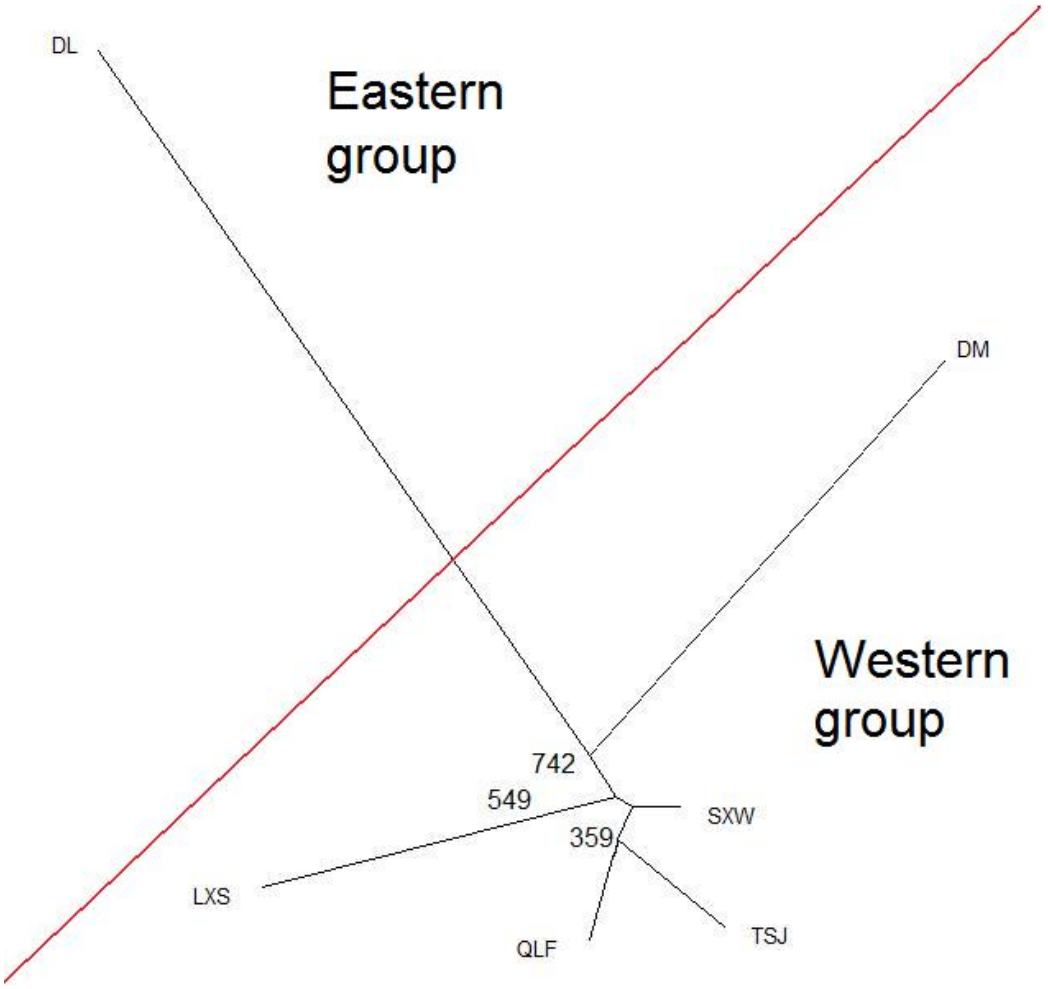

Fig. 2. Consensus dendrogram based on Nei;s genetic distance (1972) among six *S. chinensis* populations. The abbreviated names correspond to the populations as cited in Table 1. The numbers on the branches indicate the number of 1000 bootstrap replicates.

Table 5. Genetic distance and geographical distance (km) between populations of *S. Chinensis.*

Populations	DL	DM	SXW	TSJ	QLF	LX
DL	-	208.400[a]	212.400	206.700	219.900	233.400
DM	0.0729[b]	-	4.813	3.705	12.840	27.520
SXW	0.0726	0.0290	-	8.233	11.230	22.760
TSJ	0.0839	0.0288	0.0331	-	13.360	30.530
QLF	0.0652	0.0265	0.0346	0.0352	-	22.120
LX	0.0831	0.0478	0.0367	0.0415	0.0403	-

Note: geographical distance is listed above diagonal; genetic distance is listed below diagonal.

Discussion

Low genetic diversity at population level and high genetic diversity at species level were indicated in this study, which was similar with the previous reports using allozyme, RAPD and ISSR (Zhou & Ye, 2002; Li & Jin, 2006; Jin & Li, 2007b). This might be due to the biological characteristics and the long colonization history of *S. chinensis*.

The relatively high genetic diversity at species level indicated that present populations of *S. chinensis* appeared to be of multiple origins from widely distributed refugia during the last glaciations and preserved the abundant genetic diversity of its ancestors (Li & Jin, 2006). *S. chinensis* might have been widely distributed in the forest at the tertiary arctic pole (Zhang & Shen, 1999) and bear an abundant gene pool. But the biological characteristics determined that the gene flow of *S. chinensis* is low. *S. chinensis* is entomophilous and pollinated by small insects with limited winged ability or without winged ability, which mainly visited flowers in the same individual plant. *S. chinensis* is self-compatible with limited pollen flow (Zhang *et al.*, 2008). The seeds of *S. chinensis* are enwrapped are dispersed by gravity and quite limited (Li & Jin, 2006). N_m calculated from G_{ST} was only 0.4409. When N_m <1, drift dominates gene flow and strong differentiation develops between the populations of the region (Hutchison & Templeton, 1999). In addition the gene flow was further decreased by the habitat fragmentation. Recently, the number and size of wild *S. chinensis* population decreased with the increasing anthropogenic activities. The habitat was gradually fragmented and limited into small isolated areas, and eventually was divided into island-like small populations. Habitat fragmentation has many genetic consequences, such as reducing population size, increasing isolation, increasing genetic drift, elevating inbreeding, and limiting gene flow (Piotti, 2009). So, the recent genetic structure might be contributed to the biological traits of *S. chinensis* and the consequences of small fragmented populations.

IBD relationship is common in tertiary relict species with long-established populations after glaciation and positively correlated with time since colonization (Crispo & Hendry, 2005). Similar to *Arabidopsis thaliana* (Sharbel *et al*., 2000) and *Serrasalmus rhombeus* (Hubert *et al.*, 2007), *S. chinensis* has significant association between Nei's genetic distance and geographical distance at the population level were revealed by Mantel analysis (r^2=0.8600, P=0.0470). In plants, short "realized dispersal" (i.e. the successful establishment of a plant at the site), in conjunction with the limited physical distances that propagules (spores or seeds) travel, often does not allow gene flow over larger distances (Cain *et al.*, 2000). In terrestrial plants, the main obstacles for long-distance dispersal are geographical barriers such as mountain ranges, lakes, rivers, or shorelines (Bockelmann *et al.*, 2003). Geographic differentiation results from limited dispersal in many plant species and is conveniently summarized in isolation by distance models (Wright, 1946), i.e., the accumulation of genetic divergence among populations under geographically restricted dispersal (Slatkin, 1993). Phylogenetic analysis showed that the populations were clearly separated into an eastern group and a western group. Between them, the main obstacles for long-distance dispersal are geographical barriers such as Tianmu Mountain, Tiantai Mountain and Fuchun River. And even among populations in Lin'an City, there are some obstacles, such as mountains, streams and man-made constructions. This is expected to lead to increasing the genetic differentiation among populations and increasing the correlation between genetic distance and geographical distance (Kuchta & Tan, 2005).

In this study, LX population in Anhui Province had lower genetic diversity (just higher than DL population) and was clustered into the western group. According to the genetic structure and biogeographical pattern, all of the populations should be preserved to maintain the high genetic diversity. In order to increase the adaptation ability of offspring, transplantation and artificial pollination should be applied to reduce the inbreeding depression. Further study might focus on the phylogenetic relationship of *S. chinensis* populations in order to understand the complex demographic events during the recent range expansion of the species.

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References

Arif, M., S. Kousar, M.A. Bajwa, A. Arif and Y. Zafar. 2005. Genetic diversity among rice genotypes of Pakistan through random amplified polymorphic DNA (RAPD) analysis. *Pak. J. Bot.,* 37(3): 585-592.

Ashraf, M., A.S. Qureshi and A. Ghafoor. 2003. Genetic diversity in wheat under different crop-ecological zones. *Pak. J. Bot*., 35(4): 597-603.

Black, W.C. 1996. RAPDBOOT 1.0, RAPDDIST 1.0, RAPDFST 4.0, RAPDPLOT 2.4. Department of Microbiology, Colorado State University, Fort collins, Colo.

Bockelmann, A.C., T.B.H. Reusch, R. Bijlsma and J.P. Bakker. 2003. Habitat differentiation vs. isolation-by-distance: the genetic population structure of *Elymus athericus* in European salt marshes. *Mol. Ecol.,* 12(2): 505-515.

Bohonak, A.J. 2002. IBD (Isolation by distance): A program for analyses of isolation by distance. *J. Hered.,* 93(2): 153-154.

Cain, M.L., B.G. Milligan and A.E. Strand. 2000. Long-distance seed dispersal in plant populations. *Am. J. Bot.,* 87(9): 1217-1227.

Chen, X.B., L.P. Zhang, W. Wang, Q. Tiam and Q.X. Zhang. 2008. A new record of *Sinocalycanthus chinensis* in Anhui Province. *J. Trop. Subtrop. Bot.*, 16(3): 277-278.

Crispo, E. and A.P. Hendry. 2005. Does time since colonization influence isolation by distance? A meta-analysis. *Conserv. Genet.,* 6(5): 665-682.

Excoffier, L., L. Guillaume and S. Schneider. 2006. Arlequin ver 3.01: an integrated software package for population genetics and data analysis. Computational and Molecular Population Genetic Lab. University of Berne.

Felsenstein, J. 1995. PHYLIP: a phylogeny inferences package, version 3.57c, Department of Genetics, University of Washington, Seattle, Washington.

Hamrick, J.L. and M.J.W. Godt. 1996. Conservation genetics of endemic plant species. In: *Conservation Genetics*. (Eds.): J.C. Avise and J.L. Hamrick. Chapman and Hall, New York. pp. 281-304.

Hu, S.Q. 2002. The critical regions for conservation of rare and endangered plant species diversity in Zhejiang Province, *Chin. Biodiv.,* 10(1): 15-23.

Huang, J.Q. 1998. The differentiation of flower gud and development of male gametophyte in *Calycanthus chinensis*. *For. Res.,* 11(4): 439-442.

Hubert, N., F. Duponchelle, J. Nuňez, R. Rivera, F. Bonhomme and I. Renno. 2007. Isolation by distance and Pleistocene expansion of the lowland populations of the white piranha *Serrasalmus rhombeus*. *Mol. Ecol.,* 16(12): 2488-2503.

Hutchison, D.W. and A.R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evol.*, 53(6): 1898-1914.

Jin, Z.X. and J.M. Li. 2007a. Genetic differentiation in endangered *Heptacodium miconioides* Rehd. based on ISSR polymorphism and implications for its conservation. *For. Ecol. Manag.*, 245(1-3): 130-136.

Jin, Z.X. and J.M. Li. 2007b. ISSR analysis on genetic diversity of endangered relic shrub *Sinocalycanthus chinensis*. *Yingyong Shengtai Xuebao*, 18(2): 247-253.

Keller, L.F. and D.M. Waller. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.,* 17(5): 230-241.

Kuchta, S.R. and A.M. Tan. 2005. Isolation by distance and post-glacial range expansion in the rough-skinned newt, *Taricha granulose*. *Mol. Ecol.,* 14(1): 225-244.

Lewontin, R.C. 1972. The apportionment of human diversity. *Evol. Biol.*, 6(38): 381-398.

Li, J.M. and Z.X. Jin. 2006. High genetic differentiation revealed by RAPD analysis of narrowly endemic *Sinocalycanthus chinensis* Cheng et S.Y. Chang, an endangered species of china. *Biochem. Syst. Ecol.*, 34(10): 725-735.

Li, J.M. and Z.X. Jin. 2007. Genetic variation and differentiation in *Torreya jackii* Chun, an endangered plant endemic to China. *Plant Sci.*, 172(5): 1048-1053.

Li, J.M. and Z.X. Jin. 2008. Genetic structure of endangered *Emmenopterys henryi* Oliv. based on ISSR polymorphism and implications for its conservation. *Genetica,* 133(3): 227-234.

Li, L.C. 1990. Study on the morphological anatomy of pollen of *Sinocalycanthus chinensis*. *Bull. Bot. Res*., 10(1): 93-97.

Michalski, S.G. and W. Durka. 2007. High selfing and high inbreeding depression in peripheral populations of *Juncus atratus*. *Mol. Ecol.,* 16(22): 4715-4727.

Nei, M. 1972. Genetic distance between populations. *Am. Nat.*, 106(949): 283-292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl Acad. Sci.,* 70(12): 3321-3323.

Ni, S.F., Y.J. Pan, C.X. Fu, P. Wu and Y.C. Chen. 2003. Study on naphtha of *Sinocalycanthus chinensis* by Gas Chromatography/Mass Spectrometry. *Chi. J. Anal. Chem*., 31(11): 1405.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.,* 12(4): 357-358.

Piotti, A. 2009. The genetic consequences of habitat fragmentation: the case of forests. *iForest-Biogeosci. For.,* 2: 75-76.

Schaal, B.A., D.A. Hayroth, K.M. Olsen, J.T. Rauscher and W.A. Smith. 1998. Phylogeographic studies in plants: problem and prospects. *Mol. Ecol.*, 7(4): 465-474.

Sharbel, T.F., B. Haubold and T. Mitchell-Olds. 2000. Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. *Mol. Ecol.,* 9(12): 2109-2118.

Slatkin, M. 1993. Isolation by distance in equillibrium and non-equillibium populations. *Evol.,* 47(1): 264-279.

Wrigh, S. 1943. Isolation by distance. *Genetics,* 28(2): 114-138.

Wright, S. 1946. Isolation by distance under diverse systems of mating. *Genetics*, 31(1): 39-59.

Yeh, F.C. and T.J.B. Boyle. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian J. Bot.,* 129: 157.

Yu, H.H., Z.L. Yang, B. Sun and R.N. Liu. 2011. Genetic diversity and relationship of endangered plant *Magnolia officinalist* (Magnoliaceae) assessed with ISSR polymorphism. *Biochem. System. Ecol.,* 39(2): 71-78.

Zhang, F.G., Z.H. Chen, Y.D. Qiu, G.Y. Li and S.H. Jin. 2001. Distribution population number and main types of community of *Calycanthus chinensis*. *Bull. Bot. Res*., 21(4): 620-623.

Zhang, H.W., D.M. Weng and R.Z. Xu. 1997. Study on ecological biological properties of *Sinocalycanthus chinensis*. *J. Zhejiang For. Sci. Tech.*, 17(1): 15-17.

Zhang, R.H. and X.L. Shen. 1999. Taxonomy Geographical Distribution and Evolution of Calycanthaceae. *J. Beijing For. Uni.,* 21(2): 8-11.

Zhang, W.B. and Z.X. Jin. 2008. Flowering phenology and pollination success of an endangered plant *Sinocalycanthus chinensis*. *Acta Ecol. Sinica,* 28(8): 4037-4046.

Zhou, S.L. and W.G. Ye. 2002. The genetic diversity and conservation of *Sinocalycanthus chinensis*. *Chi. Biodiv.,* 10(1): 1-6.

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