Correlation analysis of genetic diversity and population structure of *Houttuynia cordata* Thunb with regard to environment

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ABSTRACT. To study the levels of genetic diversity, and population structure, of *Houttuynia cordata* Thunb, the genetic background and relationships of populations were analyzed in terms of environmental factors. The genetic diversity and population structure of *H. cordata* were investigated using sequence-related amplified polymorphisms and correlation with environmental factors was analyzed using the SPSS software. Two thousand one hundred sixty-three sites were amplified from 41 pairs of primers, 1825 of which were polymorphic, and the percentage of polymorphic loci was 84.37%; the percentage of polymorphic sites was 72.14 and 67.77% at the species and population level, respectively. The observed number of alleles was 1.52 and 1.30 at species and population level, respectively. The effective number of alleles was 1.38 and 1.24 at species and population level, respectively. The Nei's diversity was 0.26 and 0.15 at species and population level,

respectively. The Shannon's information index was 0.87 and 0.63 at species and population level, respectively. The genetic differentiation coefficient of populations was 0.51, and 12 populations were divided into three classes based on D = 0.20; the genetic diversities of different populations are correlated at different significance levels (P < 0.05) with environmental factors. Genetic differentiation existed among populations and the populations exhibited heteroplasmy.

Key words: Correlation; Genetic diversity; Environmental factors; *Houttuynia cordata* Thunb; Population structure

INTRODUCTION

For angiosperms, asexual reproduction is widespread. Many flowering plants could reproduce asexually using rhizomes, stem buds, layers, cuttings, and grafts (Albert et al., 2003), which considerably influences their genetic diversity, population structure, and adaptation potential. The spatial distribution of genetic structures reflects the functions of adaptation in ecological evolution, environmental transition, and natural selection (O'Hanlon et al., 2000). An in-depth understanding of genetic diversity and structure are important to discern the mechanisms underlying the formation, maintenance, recession, and succession of asexual reproduction among plant populations (Waycott, 1995). For plants with both sexual and asexual reproduction, although sexual reproduction could increase their genetic diversity and decrease their genetic differentiation, their propagation depends on dispersal and the ratio of sexual reproduction to asexual reproduction. Thus, estimating genetic diversity and structure is important in understanding the dynamics and evolution of plants populations (Esselman et al., 1999).

The *Houttuynia cordata* Thunb, of the family *Saururaceae*, is a perennial herbaceous plant originating in China. It is mainly distributed in the central, southeast, and southwest regions of China, and grows in wet hillside forest understories and along the roadsides, ridges, and ditches 300-2600 meters above the sea level. *Houttuynia cordata* Thunb has been officially recognized as a developing resource with huge potential by the National Health Ministry of China. However, with increasing demand and irregular collection, wild *Houttuynia cordata* Thunb is vastly destroyed, its numbers continuously decrease, its habitats have been fragmented, its gene library is quickly diminished, and its genetic diversity is reduced (Zhong et al., 2009). The different germplasm resources of *Houttuynia cordata* Thunb vary in their morphological features, yield, quality, disease resistance, and chromosome numbers (Wu et al., 2003). Genetic diversity has been detected at the isozyme (Wu et al., 2002a) and DNA (Wu et al., 2002b) levels. However, their genetic background and associated relationships with environmental factors are still ambiguous. Evidence from population genetic studies suggests that species have a lower level of genetic diversity than progenitors (Pleasants and Wendel, 1989). Despite the economical importance and transcontinental distribution, little is about the genetic diversity and the population structure of *Houttuynia cordata* Thunb.

The sequence-related amplified polymorphism (SRAP) molecular marker is a novel molecular marker technique developed in 2001. This method has several advantages: it is simple, produces medium yield, is highly co-dominant, repeatable, and easy to isolate and sequence, and is targeted to open reading frames (ORFs). The SRAP primer design is crucial,

and is based on the amplification of two primers: the length of the upstream primer is 17 bp comprised of a non-specific filling sequence (10 bp), followed by the core sequence CCGG, three selective bases on the 3' end, and specific amplification of the exons; the length of the downstream primer is 18 bp and is composed of an 11 bp non-specific filling sequence on the 5' end, followed by the core sequence AATT, three selective bases on the 3' end, and specific amplification of the introns and promoters. The polymorphism generation is due to the gap length between introns and promoters and varies between individual and species. This is being widely used in genetic map construction (Lin et al., 2005), QTL analysis (Okazakik et al., 2007), genetic diversity analysis (Budak et al., 2004), heterosis prediction (Budak et al., 2004), species identification, and other fields.

In this study, we analyzed the genetic diversity, population structure, and spatial distribution of *Houttuynia cordata* Thunb using the SRAP technique to understand its habitation, asexual reproduction, and ecological adaptation, as well as correlating its genetic diversity with environmental factors.

MATERIAL AND METHODS

Materials

A random selection of 20-30 different plants from 12 separate *H. cordata* Thunb populations were collected from the Yunnan (YN), Guizhou (GZ), Sichuan (SC), Shanxi (SX), Jiangxi (JX), Hunan (HN), Hubei (HB), Guangxi (GX), Zhejiang (ZJ), Jiangshu (JS), Fujian (FJ), and Guangdong (GD) provinces in China (Table 1). The fresh young leaves of each plant were placed in a sealed bag with silica gel to keep them dry, and preserved for future use.

Table 1. Populations and habitation factors of *Houttuynia cordata* Thunb.

Population	pH	Altitude (m)	Latitude	Longitude	Organic (g/kg)	N (g/kg)	P (mg/kg)	K (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
YN	5.21	1200	27.88	108.71	39.4	2.34	10.74	181.25	144.25	2.23	0.41	4.84
GZ	4.85	270	25.55	108.30	27.3	1.81	13.66	217.81	161.15	1.55	0.46	5.31
SC	4.62	901	28.55	104.26	26.8	1.83	22.64	134.65	164.65	2.15	0.59	4.90
SX	4.97	653	38.09	117.04	25.1	1.75	11.28	213.25	186.65	4.72	0.50	6.95
JX	5.09	896	25.43	114.71	40.8	2.52	5.64	149.95	179.35	2.85	0.68	6.95
HN	4.84	568	27.22	109.32	24.6	1.64	9.83	236.72	180.25	2.25	0.58	6.25
HB	5.17	706	33.16	109.37	49.2	3.35	4.67	177.28	192.25	3.26	0.32	6.75
GX	5.69	112	22.24	109.17	24.1	1.46	2.56	198.85	200.16	3.82	0.43	5.22
ZJ	4.95	487	30.52	120.15	38.9	3.11	3.65	217.65	202.91	3.85	0.52	6.55
JX	5.83	391	31.47	119.58	40.4	2.64	2.98	224.35	166.83	6.34	0.38	8.15
FJ	5.81	846	27.32	117.24	18.2	0.86	5.43	129.42	128.25	1.05	0.25	6.43
GD	5.51	497	23.53	120.03	20.1	1.11	11.34	127.75	142.56	2.33	0.14	5.21

Methods

DNA extraction and polymerase chain reaction (PCR) amplification were performed as previously reported (Zhong et al., 2010). Sequences of forward and reverse primers are shown in Table 2.

The PCR amplification mixture consisted of 60 ng DNA template, 30 ng each primer, 200 µM dNTP, 1X PCR buffer, and 1 U TAKARA Taq DNA polymerase for a total volume of 20 µL. The thermal cycling profile was as follows: 94°C for 2 min, followed by 4 cycles of 1 min at 94°C, 1 min at 35°C, and 1 min at 72°C, then 34 cycles of 1 min at 94°C, 1 min at 50°C

and 1 min at 72°C, and a final extension step for 7 min at 72°C. Amplified fragments were separated on a 6% denaturing gel and silver stained.

Table 2. SRAP primers used in the experiment.

Forward primer sequence	Forward primer sequence	Reverse primer sequence	Reverse primer sequence
F1: TGAGTCCAAACCGGACC	F7: TGAGTCCAAACCGGAAT	R1: GACTGCGI'ACGAATTGAC	R7: GACTGCGTACGAATTCTG
F2: TGAGTCCAAACCGGTAA	F8: TGAGTCCAAACCGGAAG	R2: GACTGCGTACGAATTTGA	R8: GACTGCGI'ACGAATTAAT
F3: TGAGTCCAAACCGGTCC	F9: TGAGTCCAAACCGGACA	R3: GACTGCGTACGAATTGAT	R9: GACTGCGTACGAATTTGC
F4: TGAGTCCAAACCGGATA	F10: TGAGTCCAAACCGGTGC	R4: GACTGCGTACGAATTCAG	R10: GACTGCGTACGAATTAAC
F5: TGAGTCCAAACCGGAGC	F11: TGAGTCCAAACCGGAGA	R5: GACTGCGTACGAATTCTC	R11: GACTGCGTACGAATTGCA
F6: TGAGTCCAAACCGGACC	F12: TGAGTCCAAACCGGACG	R6: GACTGCGTACGAATTCAA	R12: GACTGCGTACGAATTGTC

The genetic parameters of all populations were analyzed with Popgene Version 1.31 to calculate allele number (N_A), effective number of alleles (N_E), Nei's genetic diversity index (h), Shannon phenotype index (I), and percentage of polymorphic site (P). The genetic structure was evaluated using the migration number per population generation (N_m) and the genetic differentiation coefficient (G_{ST}). The computational formulas are as follows (Yeh et al., 1997): $h = 1 - å p_i^2$, p_i is the i allele frequency of population; $I = 1 - \sum F_i$, F_i is the length of amplicons; $P = k/n \times 100\%$, k and n are the number of polymorphic sites and total sites, respectively; $G_{ST} = (H_T - Hs)/H_T$, H_T and H_S is the genetic diversity among and within populations; $N_m = (1 - G_{ST})/2G_{ST}$.

Nei' genetic distance (D) = $1 - 2N_{ab}/(N_A + N_b)$; N_{ab} was the number of common bands at two populations, N_A, N_b was the number of peculiar bands for each population, respectively.

Each band was manually scored as 1 (present) or 0 (absent). The NTSYS-pc2.10e software was used to calculate simple matching coefficients, perform cluster analysis, and identify the goodness of fit of the cluster analysis.

The soil factors among populations were determined using the Analytic Service Center of the Hunan Agricultural University. The correlations between environmental factors and genetic diversity indexes were analyzed using SPSS 12.0 software.

RESULTS

Amplified polymorphism among populations using SRAP primers on *H. cordata* Thunb

A total of 41 pairs of repeatable and highly polymorphic primers were selected from 144 pairs of sequence-related amplified polymorphism primers, and 2163 sites were amplified from 41 pairs of primers, 1825 of which were polymorphic. The percentage of polymorphic loci was 84.37% (Table 3). The above data showed that populations are genetically diverse.

Genetic diversity of *H. cordata* Thunb populations

At the species level of *H. cordata* Thunb, the percentage of polymorphic sites was 72.14%, the observed number of alleles (N_A) was 1.52, the effective number of alleles (N_E) was 1.38, the Nei's diversity (h) was 0.26, and the Shannon's information index (I) was 0.87. At population level of *H. cordata* Thunb, the percentage of polymorphic sites was 67.77%, the observed number of alleles (N_A) was 1.30, the effective number of alleles (N_E) was 1.24, the Nei's diversity (h) was 0.15, and the Shannon's information index (I) was 0.63 (Table 4).

Table 3. Polymorphism level of SRAP analysis on *Houttuynia cordata* Thunb populations.

Primers code	Total number of amplified sites	Number of polymorphic sites	Percentage of polymorphic (%)	Primers code	Total number of amplified sites	Number of polymorphic sites	Percentage of polymorphic (%)
F2-R5	72	47	62.7	F6-R6	72	72	100
F2-R6	70	44	61.1	F6-R7	79	54	65.8
F2-R7	70	70	100	F6-R10	34	34	100
F3-R5	30	30	100	F7-R9	26	26	100
F3-R6	84	59	67.8	F7-R8	73	45	61.6
F3-R7	78	52	65.0	F7-R8	40	40	100
F3-R9	24	24	100	F8-R8	26	26	100
F3-R10	69	43	60.6	F9-R10	77	49	63.6
F3-R11	45	45	100	F10-R3	50	50	100
F3-R12	70	45	61.6	F10-R8	48	48	100
F4-R1	27	27	100	F10-R10	50	50	100
F4-R3	73	47	62.7	F10-R11	53	53	100
F4-R9	29	29	100	F11-R6	66	40	58.8
F4-R10	49	49	100	F11-R7	63	37	56.9
F4-R11	20	20	100	F11-R10	61	61	100
F4-R12	45	45	100	F11-R11	71	45	61.6
F5-R4	48	48	100	F12-R2	44	44	100
F5-R5	41	41	100	F12-R3	35	35	100
F5-R8	51	51	100	F12-R5	43	43	100
F6-R3	45	45	100	F12-R9	50	50	100
F6-R5	62	62	100	Total	2163	1825	84.37

Table 4. Genetic diversity of *Houttuynia cordata* Thunb populations.

Population	Observed number of alleles (N_A)	Effective number of alleles (N_E)	Nei's gene diversity (h)	Shannon's Information index (I)	Percentage of polymorphic (%)
YN	1.23	1.18	0.14	0.75	61.04
GZ	1.16	1.14	0.10	0.66	55.69
SC	1.22	1.20	0.12	0.71	59.83
SX	1.36	1.32	0.10	0.61	53.57
JX	1.39	1.34	0.23	0.65	85.51
HN	1.46	1.33	0.28	0.56	74.28
HB	1.12	1.09	0.16	0.61	85.71
GX	1.60	1.53	0.14	0.55	78.18
ZJ	1.18	1.13	0.19	0.60	66.18
JX	1.49	1.41	0.13	0.53	71.43
FJ	1.19	1.13	0.11	0.65	78.57
GD	1.21	1.15	0.15	0.74	67.96
Mean	1.30	1.24	0.15	0.63	67.77
Species level	1.52	1.38	0.26	0.87	72.14

The genetic differentiation and gene flow

The genetic diversity among and within populations was 0.5331 and 0.2612, respectively. The genetic differentiation coefficient (G_{ST}) of *H. cordata* Thunb populations was 0.51, which the genetic differentiation among population was slightly higher than of within population (Table 5).

Genetic differentiation existed among populations, and the percentage was 51% ($P < 0.001$) according to the AMOVA analysis. The gene flow (N_m) for *H. cordata* Thunb populations was 0.48, which indicates that the gene flow of 12 populations was blocked. The Nei's diversity index and gene flow analysis were consistent among populations (Table 6).

Table 5. Nei's analysis of genetic diversity of *Houttuynia cordata* Thunb populations.

	Total gene diversity (Ht)	Gene diversity in population (Hs)	Index of genetic differentiation (G_{ST})	Gene flow (Nm)
Mean	0.5331	0.2612	0.51	0.48

Table 6. Analysis of molecular variance (AMOVA) analysis of *Houttuynia cordata* Thunb. populations.

Source of diversity	Sums of square	Degree of freedom	Mean squares	Variance components	Percentage of variance components (%)	P
Diversity among populations	1856.92	11	168.81	15.784	51	<0.001
Diversity within population	973.21	312	3.12	15.165	49	
Total	2830.13	323	171.93	30.949		

Genetic identity (I) and genetic distance (D) among *H. cordata* Thunb populations

The range of genetic identity (I) two populations of the 12 *H. cordata* Thunb. populations was 0.6881-0.9664, and that of genetic distance (D) was 0.0336-0.3119. The genetic identity (I) was largest between the HN and JX populations (0.9664) and was smallest between the ZJ and FJ population (0.6881) (Table 7).

Table 7. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among *Houttuynia cordata* Thunb. populations.

	YN	GZ	SC	SX	JX	HN	HB	GX	ZJ	JX	FJ	GD
YN		0.8248	0.8094	0.7653	0.8537	0.8376	0.8443	0.8366	0.7276	0.7753	0.6972	0.8079
GZ	0.1752		0.8179	0.7540	0.8938	0.8883	0.8757	0.8158	0.7044	0.8053	0.6986	0.8177
SC	0.1906	0.1821		0.8344	0.8258	0.8247	0.8299	0.7946	0.7463	0.7744	0.7063	0.8144
SX	0.2347	0.2460	0.1656		0.7796	0.7639	0.7743	0.7576	0.7663	0.7092	0.6576	0.8169
JX	0.1463	0.1062	0.1742	0.2204		0.9664	0.9581	0.8522	0.7453	0.8679	0.7643	0.8384
HN	0.1624	0.1117	0.1753	0.2361	0.0336		0.9444	0.8476	0.7279	0.8591	0.7673	0.8146
HB	0.1557	0.1243	0.1701	0.2257	0.0419	0.0556		0.8438	0.7459	0.8473	0.7580	0.8257
GX	0.1634	0.1842	0.2054	0.2424	0.1478	0.1524	0.1562		0.7118	0.7547	0.7276	0.8861
ZJ	0.2724	0.2956	0.2537	0.2337	0.2547	0.2721	0.2541	0.2882		0.7737	0.6881	0.7672
JX	0.2247	0.1947	0.2256	0.2908	0.1321	0.1409	0.1527	0.2453	0.2263		0.7637	0.7673
FJ	0.3028	0.3014	0.2937	0.3424	0.2357	0.2327	0.2420	0.2724	0.3119	0.2363		0.7333
GD	0.1921	0.1823	0.1856	0.1831	0.1616	0.1854	0.1743	0.1139	0.2328	0.2327	0.2667	

According to the Nei's genetic distance, a genetic relationships dendrogram among populations was established using the UPGMA method, which was based on SRAP data, where 12 populations were divided into three classes on D = 0.20. One class was composed of nine populations of YN, GZ, SC, SX, JX, HN, HB, GX, GD; the second class comprised the ZJ and JX populations; the third class was the FJ population (Figure 1).

Correlation between the genetic diversity and environmental factors of *H. cordata* Thunb populations

H. cordata Thunb. exhibits spatial scalability. Compared with other plants, it has a stronger adaptability and competitiveness. To further understand the ecological adaptation of *H. cordata* Thunb, we analyzed the correlation of genetic diversity and structure with environmental factors. The results showed that NC had an extremely significant negative correlation with soil organic matter (P = 0.01, r = -0.632) and N content (P = 0.01, r = -0.563), while PD and D had an extremely significant positive correlation with soil organic matter (P = 0.01, r = 0.635 and P = 0.05, r = 0.469, respectively) and N content (P = 0.01, r = 0.561 and P =

0.05, r = 0.429, respectively), respectively. By contrast, NC, PD, and D were not significantly correlated with other habitation factors. PPB had a significant positive correlation with soil organic and N content, and an extremely significant positive correlation with soil Cu content, but not with other environmental factors. The value for h had a significant positive correlation with soil Cu content, but not for other environmental factors. The value of I had an extremely significant positive correlation with altitude and soil P content, but an extremely significant negative correlation with K, Fe, Mn, and Zn contents. G_{ST} had a significant positive correlation with latitude, N, and Zn and an extremely significant positive correlation with K, Fe, and Cu. N_m had a significant positive correlation with latitude, N, K, and Zn contents, and an extremely significant positive correlation with Fe and Cu contents. In addition, all genetic parameters were not significantly correlated with soil pH and longitude (Table 8).
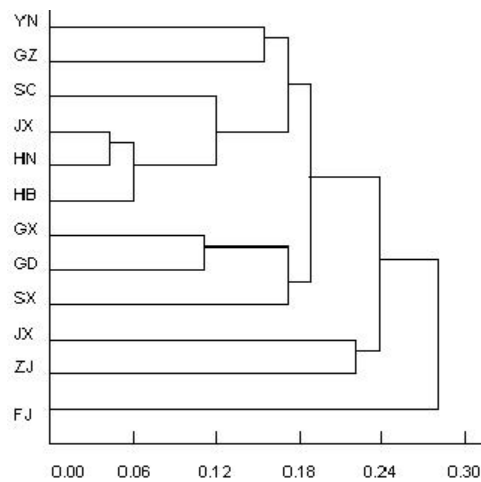
Figure 1. Dendrogram of UPGMA method cluster analysis based on Nei's genetic distance of SRAP data generated by the 41 primer combinations.

Table 8. The correlation between genetic diversity and habitation factors of *Houttuynia cordata* Thunb populations.

	pH	Altitude (m)	Latitude	Longitude	Organic (g/kg)	N (g/kg)	P (mg/kg)	K (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
NC	-0.125	-0.010	0.235	-0.108	-0.632**	-0.563**	0.269	-0.054	0.045	-0.076	0.251	0.057
PD	0.129	0.054	-0.218	0.115	0.635**	0.561**	-0.271	0.046	-0.064	0.067	-0.259	-0.059
D	-0.226	0.301	-0.053	-0.049	0.469*	0.429*	0.303	-0.246	-0.280	-0.142	-0.162	-0.274
PPB	-0.269	0.169	-0.169	-0.151	0.466*	0.425*	-0.075	0.269	0.054	-0.039	0.612**	0.251
h	-0.254	0.069	-0.211	-0.007	0.226	0.226	-0.233	0.207	0.352	-0.059	0.438*	0.179
I	-0.238	0.606**	-0.237	-0.178	-0.145	-0.234	0.617**	-0.680**	-0.638**	-0.626**	-0.224	-0.655**
G_{ST}	-0.312	-0.013	0.519*	0.226	0.377	0.475*	-0.271	0.515*	0.705**	0.316	0.589**	0.479*
Nm	0.285	-0.028	-0.474*	0.282	-0.376	-0.456*	0.243	-0.505*	-0.625**	-0.286	-0.639**	-0.443*

*P < 0.05; **P < 0.01.

DISCUSSION

Genetic diversity and structure of *H. cordata* Thunb populations

The Simpson diversity index (D) reflects the relative frequency of a special genotype

of a certain population. A high D value means that nearly every plant of the sampling population has a different heredity, D values equal to 1 indicate that each plant has a unique multi-locus genotype, a low D value means that certain genotypes are sampled repeatedly within populations, and D values equal to 0 indicate that all plants have a common multi-locus genotype (Waycott et al., 1997). Many studies have shown that the average ratio of different genotypes (PD) and Simpson diversity index (D) of asexually reproduced plants are 0.62 (0.1-1.00) and 0.75 (0.13-1.00), respectively (Widén et al., 1994). Our results showed that the average values of PD and D were 0.91 and 0.97, respectively. Although these values are higher than those mentioned in the literature, they are within the range of previously reported values.

Earlier studies have considered that the genetic diversity of the asexually reproducing plants was lower than that of the sexually reproducing plants (Hamrick and Golt, 1990), but more and more research has found that the genetic diversity of the asexually reproducing plants is not low (Eckert and Barrett, 1993), and sometimes can be very high. For example, the genetic diversity was as high as 0.983 for *Leymus chinensis* (Zu and Cui, 2002), 0.988 for the *Cyclobalanopsis glauca* population (Chen and Song, 1997), and 0.992 was measured using the ISSR molecular marker technique for *Potamogeton malaianus population* (Chen et al., 2006). These phenomena could be attributed to the existing population and was established with plants of different origins and populations (Pappert, 2002).

Our study showed that the N_m among *H. cordata* Thunb. populations was lower than 1, and the G_{ST} was higher than 0.51 (reaching a 1% significance level), indicating that the gene exchange among populations was fewer and genotypes were strongly, regionally distributed. These features were possibly due to the nature of asexual reproduction, and the important function of sexual reproduction, in the life history of *H. cordata* Thunb. Moreover, the existence of many genotypes among populations may contribute to regional ecological adaptation and subsequently lead to differentiation among populations. The number of plants per clone was 1.58 on average, but varied significantly among different clones, indicating that different clones may have different growth and adaptation abilities, and therefore different competitiveness.

Correlation between the genetic diversity and habitation factors

The effects of environmental factors on genetic diversity are debatable among scholars. Some believe that adversities such as disease, pests, cold, drought, and others are limiting factors on plants growth. After long-term exposure to the adverse conditions, plants growing in this environment develop adaptive mechanisms, such as genetic resistance, thereby increasing the genetic sub-differentiation and diversity within populations (Ricardo and O'Connell, 2005). In contrast, some scholars believe that environmental factors are not correlated with genetic diversity (Dorken and Husband, 1999; Ruggiero et al., 2005a). Our results showed that the genetic variation of different *H. cordata* Thunb. populations is significantly (P < 0.05) orrelated at different levels with environmental factors, except soil pH and longitude, indicating that *H. cordata* Thunb. populations exhibit heteroplasmy. Moreover, there are few gene flows (0.47) among *H. cordata* Thunb. populations, which influence the spatial pattern of their genetic diversity and their mating patterns.

Causes of differentiation among *H. cordata* Thunb populations

The breeding system is an important factor affecting genetic diversity and structure.

Generally, distant mating and terminally successional species have a higher genetic diversity. The genetic diversity of self-mating and mixed-mating species is lower than that of outbreeding species (Parker and Hamrick, 1992; McClintock and Waterway, 1993; Li and Ge, 2001; Hayashi et al., 2001; Ferriol et al., 2003). The difference in the genetic diversity of *H. cordata* Thunb. populations is related to their life history, especially the renewal and addition to the population, which includes sexual reproduction and sexual reproduction patterns (Hamrick and Godt, 1996). The function of sexual reproduction lies in creating new gene combinations and accumulating a large number of mutations, thus accelerating environmental adaption and new species formation (Aspinwall and Christian, 1992). When seedling renewal becomes difficult, asexual reproduction is thought to ensure the stabilization of partial populations (Ruggiero et al., 2005b). If there were no mutations, migration, selection, or seedling renewal through sexual reproduction, genetic diversity would disappear over time until only one genotype exists within the population. However, if there were few seedlings renewed in a short time, the genetic diversity could be maintained or raised (Watkinson and Powell, 1993). In this study, the average number of plants per clone among *H. cordata* Thunb. populations was 1.58. Moreover, the ratio of different genotypes was higher, suggesting that in the early stages of population establishment, *H. cordata* Thunb populations consisted of many plants with different genotypes, rather than being formed by fragmentation or the spread of existing clones through asexual reproduction; that is, *H. cordata* Thunb populations can have multiple origins, which creates rich genetic diversities.

Genetic obstacles, establisher effects, and gene flow have important functions that affect the genetic diversity of asexually reproducing plants. In addition, statistics and the phenological conditions of different regions also affect the level of genetic diversity (Li et al., 1996; Schnabel and Hamrick, 1997; Persson and Gustavsson, 2001; Hangelbroek et al., 2002). It is possible that only a few plants established the population at an early stage, which, together with plants of different genotypes, affected the genetic structure to a large extent. Therefore, the genetic diversity and structure of *H. cordata* Thunb. populations were the systematic result of many factors closely correlated to spread, spread pattern, and sexual reproduction.

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