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Genetic diversity and population structure in *Vallisneria spinulosa* (Hydrocharitaceae)

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Abstract

Vallisneria spinulosa is a dominant submerged macrophyte in lakes of the middle–lower reaches of the Yangtze River. Allozyme variation, clonal diversity and population genetic structure were investigated for a total of 396 individuals sampled from 10 extant populations. *V. spinulosa* maintained high levels of genetic variation both at the species ($P = 46.2$, $A = 1.69$, $H_e = 0.23$) and at the population level ($P = 46.2$, $A = 1.58$, $H_e = 0.21$). Although aquatic macrophytes commonly exhibit low genetic variation within populations, the obligately outcrossing mating system of *V. spinulosa* and pervasive gene flow likely account for the high levels of diversity maintained within populations. All *V. spinulosa* populations contained high clonal diversity with a mean proportion of distinguishable genotypes of 0.57 and a mean Simpson's diversity index of 0.95, indicating that populations were founded sexually or that successful seedling recruitment occurred after initial colonization. Partitioning of genetic diversity revealed a surprisingly low population differentiation ($G_{ST} = 0.06$) as compared to other hydrophilous angiosperms. No evidence of isolation-by-distance was found ($r = 0.056$, $P = 0.312$), suggesting that gene flow was not restricted geographically. The UPGMA cluster analysis revealed that several widely separated populations grouped together, suggesting long-distance gene flow among populations. The high vagility of *V. spinulosa* and extensive hydrologic connectivity among populations have facilitated long-distance gene flow and resulted in the pattern of population genetic structure in *V. spinulosa*.

Keywords: Clonal diversity; Genetic diversity; Genetic structure; Hydrochory; Hydrologic connectivity; *Vallisneria spinulosa*

1. Introduction

Genetic variation and population genetic structure of aquatic macrophytes are highly influenced by the environment (Barrett et al., 1993; Laushman, 1993). Most aquatic plant habitats occur as discrete islands in a terrestrial landscape. The patchy nature of isolated wetlands may influence gene flow in aquatic plants, especially if their dispersal mechanisms are relatively restricted (Barrett et al., 1993; Philbrick and Les, 1996). As a result, aquatic taxa commonly exhibit low variation within populations and high genetic differentiation among populations due to the effects of founder events and genetic drift (Les, 1991; Laushman, 1993). In contrast, genetic analyses of aquatic macrophytes that occur in hydrologically connected habitats have revealed high level of genetic diversity within populations, and relatively low population differentiation (Lokker et al., 1994; Nies and Reusch, 2005). Hydrochory (water dispersal) can result in long-distance seed or propagule dispersal events among populations, thus serving to connect discontinuous populations and effectively resulting in a larger functional population size (Waser et al., 1982; Kudoh and Whigham, 1997). Aquatic macrophytes that occur in such systems can maintain relatively high levels of genetic variation within populations and reduce levels of interpopulational differentiation as a consequence of gene flow.

Thousands of shallow lakes are located in the middle–lower reaches of the Yangtze River. Historically, most of these lakes were interconnected with the artery of Yangtze River, and formed a potamo-lacustrine system with a total lake area exceeding 20,000 km^2 (Liu, 1984). The mean depth of these lakes is less than 3 m and most areas of these shallow lakes are capable of sustaining a rich variety of submerged macrophytes (Wang and Dou, 1998; Xie and Chen, 1999). However, few studies have examined how this potamo-lacustrine habitat influences the genetic composition of aquatic macrophyte populations (Li et al., 2004). *Vallisneria spinulosa* Yan

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(Hydrocharitaceae) is one of the dominant submerged species in lakes of the middle–lower reaches of the Yangtze River (Yan and Zhu, 1984; Sun, 1992). The objective of this study was to investigate the population genetics of *V. spinulosa* in this region to gain some understanding of how genetic variation was partitioned in this species.

Vallisneria is dioecious and relies on wind or water currents to transport the detached male flowers during pollination (Cook, 1988; Les, 1988). *V. spinulosa* is capable of both vegetative propagation and sexual reproduction. Clonal growth results from shoot production along elongating stolons during the growing season. In the fall, each shoot in turn produces several tubers which overwinter while buried in the sediments and germinate in the following spring (Yan and Zhu, 1984; Sun, 1992). Flowering and production of viable seeds are common and sexually derived seedling recruitment contributes strongly to population persistence in lakes in the middle–lower reaches of the Yangtze River (Xiong, 2000). The fruits can remain buoyant for almost one month and can be carried by water currents (or by waterfowl and other vectors) over fairly long distances (Chen and Huang, unpublished data). However, pollen movement is always limited within a water body (Les, 1988). Considering the highly connected *V. spinulosa* populations located in the hyrdologically continuous lakes of the middle–lower reaches of the Yangtze River, and the potential ability for long-distance dispersal of *V. spinulosa*, sufficient gene flow resulting in low genetic differentiation among populations would be expected. Furthermore, the combination of a dioecious reproductive system, large population sizes (at both the lake and river levels) and substantial gene flow should result in high levels of genetic variation at both the species and population level. In addition, due to the unidirectional water flow of the Yangtze River from west to east, we expect an accumulation of genetic variation in populations located in the lower reaches of the Yangtze River. We have evaluated these hypotheses using genetic data derived from allozymes. Specifically, we address the following questions: (1) Does *V. spinulosa* maintain high levels of genetic variation and clonal diversity in lakes of the middle–lower reaches of theYangtze River?; (2) Does hydrochory result in long-distance gene flow and low genetic differentiation among populations?; (3) Does hydrochory result in unidirectional gene flow in this species?

2. Materials and methods

2.1. Populations sampled

Ten lakes in the middle–lower reaches of the Yangtze River, separated by about 900 km, were selected for this study (Table 1). Ten populations and 396 individuals were surveyed during 2003–2004, with an effort to choose sites that were only minimally disturbed. More than 30 individuals spaced at intervals of at least 5 m were collected at each site. Plants were transported and grown in an experimental greenhouse for leaf sampling for electrophoretic analysis.

2.2. Electrophoresis

Enzymes were extracted from fresh leaf tissues following the procedure of Huang et al. (1994). Electrophoresis was conducted using an isoelectric focusing polyacrylamide slab gel system (Mulcahy et al., 1981). Of 11 enzyme systems pre-screened, the following nine resolved into clear and consistent banding patterns and were used for genetic analysis: acid phosphatase [ACP; EC (Enzyme Commission) 3.1.3.2], NAD(P)H-diaphorase (DIA;EC 1.6.2.2), esterase (EST; EC 3.1.1), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), malate dehydrogenase (MDH; EC 1.1.1.37), phosphogluconate dehydrogenase (PGD; EC 1.1.1.44), phosphoglucomutase (PGM; EC 2.7.5.1), peroxidase (PRX; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1). Gels were stained as described by Weeden and Wendel (1989) with minor modifications that only 1/2 of the original volume of stain solutions was used. A total of 13 loci were scored: *Acp-1*, *Acp-2*, *Dia-1*, *Dia-2*, *Est-1*, *Gpi-1*, *Gpi-2*, *Mdh-1*, *Mdh-2*, *Pgd-1*, *Pgm-1*, *Prx-1*, *Sod-1*.

Table 1
Geographic locations, regional distributions of *Vallisneria spinulosa* populations and summary of genetic statistics for *V. spinulosa* at population and species level

Site (population code)	Latitude (N)	Longitude (E)	*N*	TA	*A*	*P*	H_o	H_e	*F*	Region
Zhonghu Lake (ZH)	29°39′50.5″	112°38′03.9″	30	21	1.62	46.2	0.27	0.23	−0.22**	M
Luozhang Lake (LZH)	29°21′11.5″	112°36′19.0″	37	20	1.54	46.2	0.28	0.23	−0.21**	M
Honghu Lake (HH)	29°51′59.8″	113°25′57.8″	40	20	1.54	46.2	0.23	0.18	−0.32**	M
Huanggai Lake (HG)	29°43′21.5″	113°34′37.4″	36	21	1.62	46.2	0.26	0.21	−0.26**	M
Baoxie Lake (BX)	30°25′40.4″	114°31′26.3″	54	21	1.62	46.2	0.27	0.23	−0.20**	M
Liangzi Lake (LZ)	30°16′16.3″	114°35′54.9″	47	21	1.62	46.2	0.30	0.24	−0.28**	M
Poyang Lake (PY)	29°14′22.6″	115°53′51.6″	36	20	1.54	46.2	0.23	0.19	−0.24**	L
Shengjinzhong Lake (SJZ)	30°24′56.0″	117°04′06.3″	35	20	1.54	46.2	0.25	0.21	−0.23**	L
Shengjinshang Lake (SJS)	30°21′41.6″	117°01′42.4″	44	21	1.62	46.2	0.25	0.21	−0.19**	L
Pogang Lake (PG)	30°37′15.2″	117°10′51.8″	37	21	1.62	46.2	0.26	0.20	−0.27**	L
Mean			39.6	20.6	1.58	46.2	0.26	0.21	−0.24**	
S.D.			6.9	0.5	0.04	0.0	0.02	0.02	0.04	
Species				22	1.69	46.2	0.26	0.23		

TA: total number of alleles; *A*: mean number of alleles per locus; *P*: percentage polymorphic loci; H_o: observed heterozygosity; H_e: expected heterozygosity; *F*: Wright's fixation index; mean: at the population level; species: at the species level; S.D.: standard deviation; $^{**}P < 0.01$; M: locate in the middle reach of the Yangtze River; L: locate in lower reach of the Yangtze River.

2.3. Data analysis

Samples with identical multilocus genotypes were considered to be a single clone and were counted once per population to constitute the ''genet'' data set. All genetic analyses were based on the ''genet'' data set. A set of standard measures of genetic diversity was calculated using POPGENE version 1.31 (Yeh et al., 1999), including allele frequency for each locus, total number of alleles (TA), number of alleles per locus (A), percentage polymorphic loci (P, 0.99 criterion), observed heterozygosity (H_o) and expected heterozygosity (H_e). χ^2-Tests for heterogeneity of allele frequencies across populations also were performed.

Wright's fixation indices (F) for each locus and each population were estimated and tested for deviation from Hardy–Weinberg equilibrium, and genetic differentiation (G_{ST}, Nei, 1987; F_{ST}, Weir and Cockerham, 1984) was examined and tested by evaluating the significance of population subdivision using FSTAT Version 2.9.3 (Goudet, 2001). To partition total genetic diversity into geographical components, we conducted a hierarchical analysis of genetic structure. Populations were grouped into two regions (middle and lower), and the total G_{ST} was partitioned into components among regions and among populations within regions. In addition, the average level of gene flow (N_m) among sites was estimated using the formula $N_m = (1 - F_{ST})/4F_{ST}$ (Wright, 1951).

To test for isolation by distance, we performed a Mantel test (Mantel, 1967) and reduced major axis (RMA) regression on log-transformed geographic (km) distance and Rousset's (1997) genetic distance $[F_{ST}/(1 - F_{ST})]$ using IBD Version 1.52 (Bohonak, 2002). For adjacent populations, we calculated the distance between sites using their latitude and longitude coordinates. For non-adjacent populations, we calculated the distance along the Yangtze River. We evaluated pairwise genetic differentiation among populations (F_{ST}) using FSTAT (Goudet, 2001). Finally, a cluster analysis was conducted based on Nei's (1978) unbiased genetic distance/identity using unweighted pair group method using arithmetic average (UPGMA) and resulting dendrogram was tested through 2000 bootstrap replications (Felsenstein, 1985).

Clonal diversity was assessed using two measures. We first calculated genotypic diversity as the number of genets per number of ramets sampled (G/N). Second, clonal diversity was assessed as Simpson's diversity index: $D = 1 - \sum[n_i(n_i - 1)/N(N - 1)]$, where n_i is the number of ramets of the ith multilocus genotype and N is the number of samples collected for that population (Pielou, 1969). We defined widespread genotypes as those occurring in more than 75% of the populations (Ellastrand and Roose, 1987).

We tested the hypothesis that unidirectional gene flow due to hydrochory resulted in downstream populations that contained higher levels of genetic variation than upstream populations by regressing the distance of each population to the most upstream population against standard measures of genetic diversity, including mean number of alleles per locus (A), percentage polymorphic loci (P, 0.99 criterion), observed heterozygosity (H_o) and expected heterozygosity (H_e) for each population. Distances were estimated as described above. Allele frequencies of loci that showed heterogeneity among populations also were included to examine whether an obvious geographic cline existed. The significance of each regression model was tested via the F-test.

3. Results

3.1. Genetic diversity

V. spinulosa exhibited relatively high levels of allozyme diversity both at the species and population levels (Table 1). *Acp-2*, *Gpi-1*, *Mdh-1*, *Mdh-2*, *Prx-1* and *Sod-1* were polymorphic across all populations. A total of 22 alleles were detected across loci at the species level, and the number of alleles per locus (A), observed heterozygosity (H_o) and expected heterzygosity (H_e) were 1.69, 0.26 and 0.23, respectively. Within each population, *V. spinulosa* exhibited similarly high levels of allozyme variation as at the species level. The total number of alleles per population ranged from 20 to 21 with an average value of 20.6. The average P was 46.2, the mean A was 1.58 (ranging from 1.54 to 1.62), the mean H_o was 0.26 (0.23–0.30) and the mean H_e was 0.21 (0.18–0.24). The mean observed heterozygosity (H_o) was higher than the mean expected heterozygosity (H_e) across all populations. Fixation indices (F) were significantly negative for all populations ($P < 0.01$, Table 1), and 20 of the 60 fixation indices evaluated across loci and populations were significantly negative ($P < 0.05$, Table 2), indicating a significant excess of heterozygotes. All individuals exhibited fixed heterozygosity at *Gpi-1*. χ^2-Analyses testing allele frequency heterogeneity across populations revealed significant differences at *Acp-2*, *Mdh-2* and *Sod-1* ($P < 0.001$). Further regressions of the allele frequencies at *Acp-2*, *Mdh-2* and *Sod-1* and geographic distance were not significant, indicating that no obvious geographic cline existed ($P > 0.05$) (data not shown).

Table 2
Fixation indices (F) and χ^2-tests for Hardy–Weinberg equilibrium in 10 *V. spinulosa* populations

Loci	ZH	LZH	HH	HG	BX	LZ	PY	SJZ	SJS	PG
Acp-2	-0.35^{NS}	0.22^{NS}	-0.04^{NS}	0.13^{NS}	0.17^{**}	-0.13^{NS}	-0.02^{NS}	-0.10^{NS}	-0.08^{NS}	0.07^{NS}
Gpi-1	-1.00^{**}	-1.00^{**}	-1.00^{**}	-1.00^{**}	-1.00^{**}	-1.00^{**}	-1.00^{**}	-1.00^{**}	-1.00^{**}	-1.00^{**}
Mdh-1	-1.00^{**}	-0.92^{**}	-0.86^{**}	-0.70^{**}	-0.68^{**}	-0.60^{**}	-0.56^{*}	-0.84^{**}	-0.62^{**}	-0.63^{**}
Mdh-2	0.47^{*}	0.06^{NS}	-0.08^{NS}	0.33^{NS}	-0.08^{NS}	0.11^{NS}	-0.09^{NS}	-0.39^{NS}	-0.35^{NS}	-0.08^{NS}
Prx-1	0.32^{NS}	0.23^{NS}	0.06^{NS}	-0.13^{NS}	-0.14^{NS}	-0.28^{NS}	0.26^{NS}	0.26^{NS}	0.34^{NS}	0.03^{NS}
Sod-1	0.29^{NS}	0.05^{NS}	0.41^{NS}	0.16^{NS}	0.56^{**}	0.18^{NS}	0.07^{NS}	0.56^{**}	0.42^{NS}	0.18^{NS}

$^{*}P < 0.05$, $^{**}P < 0.01$, NS: not significant.

Table 3
Hierarchical analysis of genetic structure estimated for overall populations, among populations within regions and among regions in *V. spinulosa*

	G_{ST}	F_{ST}	N_m
Overall among populations	0.06**	0.06**	3.8
Among populations within regions			
Middle region	0.05**	0.05**	4.9
Lower region	0.04**	0.05**	4.9
Among regions	0.02**		

**$P < 0.01$.

3.2. Genetic structure and geographic differentiation

Partitioning of population genetic diversity based on the six polymorphic isozyme loci revealed that a low population differentiation occurred among *V. spinulosa* populations (mean $G_{ST} = 0.06$ and $F_{ST} = 0.06$), indicating that about 6% of allozyme diversity occurred among populations, whilst 94% resided within populations (Table 3). The values of pairwise population differentiation ranged from −0.01 to 0.21 (Table 4). Twenty-one of the 45 pairwise F_{ST} comparisons were not significant at the 5% level. When the 10 populations were divided into middle and lower regions, the G_{ST} of between the two regions was 0.02. Obviously, genetic differentiation between two regions only accounted a small proportion of total genetic diversity. Indirect estimation of gene flow for the overall populations was $N_m = 3.8$, and N_m detected among populations within the middle and lower region resulted in a same value of 4.9 (Table 3). Genetic differentiation between populations was independent of geographic distance ($r = 0.056$, $P = 0.312$, Fig. 1). Further tests for geographical distribution of allozyme diversity also revealed no evidence of non-random distribution of genetic variation indicative of unidirectional gene flow ($P > 0.05$) (data not shown).

The UPGMA cluster analysis revealed two distinct groups (Fig. 2). However, the dendrogram did not reflect clear separation between middle and lower regions. One population (PG) in the lower reach of Yangtze River and another population (ZH) in the middle reach were the most widely separated populations, but clustered together into the Middle reach region with 51% of bootstrap support; while population HH and population BX which occurred in the middle reach of the Yangtze River were grouped into the lower reach region with 52% of bootstrap support.

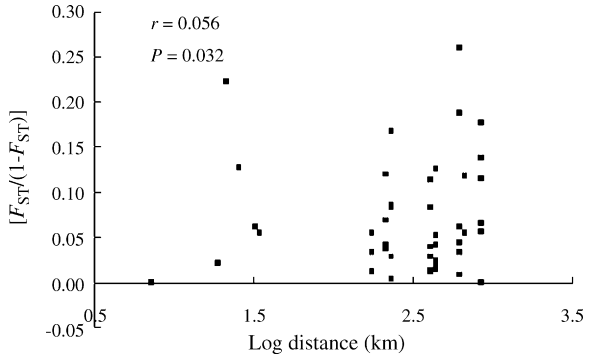

Fig. 1. Relationship between pairwise genetic $[F_{ST}/(1 - F_{ST})]$ and geographic distances between populations along the Yangtze River.

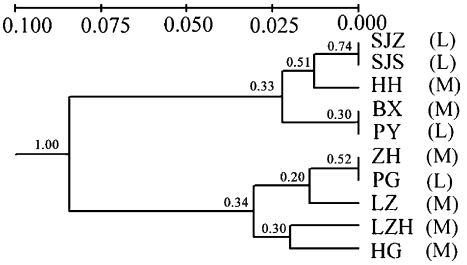

Fig. 2. UPGMA dendrogram showing genetic relationships of 10 populations of *Vallisneria spinulosa*, based on Nei's unbiased genetic distance (Nei, 1978) and statistical support based on 2000 bootstraps (numbers above branches). The two regions in the hierarchical analysis are indicated by the letters in parentheses.

3.3. Clonal diversity

A total of 225 unique multilocus genotypes were identified from the 396 individuals, and only one genotype was widespread. The mean number of multilocus genotypes per population was 22.5 and ranged from 13 to 32. The average number of genets per ramet was 0.57 (0.33–0.70), and the mean Simpson's clonal diversity index (D) was 0.95 (0.84–0.98), together indicating a high probability that a randomly sampled individual has a unique genotype, which suggested that all populations contained high clonal diversity.

Table 4
Pairwise genetic differentiation (F_{ST}) among 10 *V. spinulosa* populations

	LZH	HH	HG	BX	LZ	PY	SJZ	SJS	PG
ZH	0.05*	0.08*	0.03NS	0.02NS	0.01NS	0.05*	0.10*	0.06NS	0.00NS
LZH		0.14*	0.03*	0.04NS	0.02*	0.11*	0.15*	0.12*	0.05*
HH			0.18*	0.04NS	0.11*	0.05NS	0.04NS	0.01NS	0.06NS
HG				0.07*	0.04*	0.11*	0.21*	0.16*	0.03NS
BX					0.02NS	0.01NS	0.04*	0.01NS	0.01NS
LZ						0.08*	0.10*	0.08*	0.03NS
PY							0.05*	0.01NS	0.03NS
SJZ								−0.01NS	0.11*
SJS									0.06NS

*$P < 0.05$, NS: not significant.

4. Discussion

4.1. Genetic diversity

Consistent with our predictions, *V. spinulosa* maintained high levels of allozyme diversity in lakes of the middle–lower reaches of the Yangtze River. The value of H_e (0.23) at the species level was substantially higher than the mean value of plant species with both asexual and sexual reproduction ($H_e = 0.138$), and even higher than the mean value of plant species with an outcrossing mating system in general (Hamrick and Godt, 1989). The values of P and A were comparable to the mean levels of species with both asexual and sexual reproduction (Hamrick and Godt, 1989). Many investigations have provided examples of submerged macrophytes that have low levels of allozyme diversity at the population level (Les, 1991; Laushman, 1993). Obviously, this is not the case for *V. spinulosa*. This species exhibited similarly high levels of genetic variation at the population as well as the species level. The values of P (46.2) and A (1.58) both were high compared to the mean values within populations ($P = 34.2$, $A = 1.53$), and the expected heterozygosity ($H_e = 0.211$) was almost twice the average obtained for a sample of 468 plant taxa ($H_e = 0.113$) (Hamrick and Godt, 1989).

V. spinulosa exhibits a dioecious sexual system and therefore all offspring are the result of obligate outcrossing (Richards, 1986). Generally, outcrossing species tend to maintain the highest level of genetic variation within populations (Hamrick and Godt, 1996). For instance, similarly high levels of genetic diversity were also found in the related (also dioecious) species *V. americana* (Lokker et al., 1994). Certainly, the outbreeding system of *Vallisneria* accounts at least partially for the relatively high level of genetic variation revealed in our study.

However, we believe that the high level of interconnectivity that characterizes waterbodies in this region also has a significant influence on the pattern of genetic diversity observed. Because aquatic macrophytes occur often in isolated and ephemeral habitats, their life histories are likely to be characterized by repeated population bottlenecks and cycles of colonization and extirpation, features that may lead to a substantially reduced genetic variation within populations (Barrett et al., 1993). As a result, aquatic plant populations may exhibit low levels of genetic variation as they become fixed for different alleles at several loci due to the effects of founding events, genetic drift and vegetative reproduction (Les, 1991; Barrett et al., 1993). All of our studied populations were connected historically through the Yangtze River and it is well known that hydrologic connectivity facilitates the exchange of genes among geographically isolated populations of aquatic plants (Kudoh and Whigham, 1997). Accordingly, these hydrologically connected populations can function as a single genetic metapopulation that acts as a reserve of genetic variation and reduces the effect of random genetic drift (Kudoh and Whigham, 2001). It is conceivable that all *V. spinulosa* populations located in this potamo-lacustrine habitat are linked together to form a very large population at the river level; thus it is reasonable to expect that they might share many allozyme alleles as well as maintain a high amount of genetic variation within populations. The observation that each *V. spinulosa* population examined contained 20–21 of the 22 total alleles (>90%) detected in this ecosystem indicates that the exchange of allozyme alleles among these hydrologically connected lakes indeed has occurred.

In addition, all lakes surveyed are relatively large in size (>20 km^2), with most of their areas suitable for the growth of macrophyte populations. Large population size at the river and lake level should contribute to the maintenance of genetic diversity in *V. spinulosa* by reducing the effect of genetic drift (Barrett et al., 1993). Moreover, as all lakes where *V. spinulosa* populations were sampled are at least several hundred years or even thousands of years old (Liu, 1984; Wang and Dou, 1998), populations have had sufficient time to accumulate genetic variation. Certainly, the combination of an outbreeding system in *Vallisneria spinulosa* together with the interconnected character of these particular lakes has served to maintain high levels of genetic diversity in this species.

4.2. Clonal diversity

All 10 *V. spinulosa* populations were multiclonal. We characterized an average of 22.5 multilocus genotypes per population in size ranging from 30 to 54 randomly sampled individuals, although allozymes typically underestimate the extent of clonal variation. Clonal diversity in *V. spinulosa* was remarkably high ($G/N = 0.57$, Simpson's $D = 0.95$), compared to the mean values observed for clonal terrestrial species ($G/N = 0.17$, Simpson's $D = 0.62$) (Ellastrand and Roose, 1987). In *V. spinulosa*, fruit dispersal is probably the major means of long-distance population colonization because stolons disperse plants only locally. Due to prevalent seed dispersal in *V. spinulosa*, populations are likely to be founded or colonized sexually, as opportunities for seedling recruitment are particularly good during periods of colonization (Piquot et al., 1998). Widespread dispersal of sexually derived propagules would explain why only one widespread multilocus genotype was found along with an average of 22.5 distinct genotypes per population. Following the initial phase of sexual seedling recruitment, *V. spinulosa* populations would contain a large number of genets at the time of population establishment. Therefore, even if repeated seedling recruitment became rare, the genetically rich pool of founders would be sufficient to maintain a pattern of high clonal diversity, as these genets are virtually immortal due to their strong asexual growth (Eriksson, 1993).

4.3. Population structure and gene flow

Vallisneria spinulosa exhibited lower genetic differentiation ($G_{ST} = 0.06$) than expected for a mixed breeding system. The proportion of genetic diversity partitioned among populations was surprisingly low compared with other hydrophilous angiosperms (Les, 1991; Laushman, 1993), and even obviously lower than the mean value observed for outcrossing species in general (Hamrick and Godt, 1989). Low genetic differentiation

also was revealed by pairwise genetic differentiation analysis, which suggested that nearly half of the pairwise F_{ST} values were negligible. Isolation-by-distance tests revealed that gene flow was not geographically limited (data not shown). In addition, populations that clustered genetically by UPGMA did not mirror their geographic patterns, with several populations falling into clusters that contained sites from opposite regions. This result also indicates that long-distance gene flow was effective among populations.

Habitat features are expected to have a major influence on population genetic structure in aquatic macrophytes (Barrett et al., 1993; Laushman, 1993; Nies and Reusch, 2005). Hydrochory is a common means of long-distance seed dispersal in wetland species (Nies and Reusch, 2005) and hydrologic connectivity will facilitate long-distance dispersal among geographically isolated populations. In investigating the population structure of *Potamogeton pectinatus*, Nies and Reusch (2005) found that genetic differentiation among marine populations was less than half of the differentiation detected among isolated freshwater lake populations, and attributed the result to the higher population connectivity in the sea relative to freshwater populations. Therefore, the historical hydrologic connectivity among lakes of the middle–lower reaches of the Yangtze River provides a reasonable explanation for the low degree of subdivision observed among *V. spinulosa* populations in our study.

Several studies of aquatic plants have found evidences for unidirectional gene flow, as indicated by higher levels of genetic variation in downstream populations (Kudoh and Whigham, 1997; Gornall et al., 1998). However, we found no evidence that Lower region populations contained higher genetic variation than did the middle region populations, although allozymes may not be sufficiently polymorphic to test this hypothesis effectively in *V. spinulosa* (Barrett et al., 1993). Additional DNA markers such as amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) are needed in order to accurately to test the gene flow model of *V. spinulosa* in this potamo-lacustrine habitat.

To our knowledge, this study represents the first population genetic survey to be made of any aquatic macrophyte that covers the entire basin of the middle–lower reaches of the Yangtze River. We found that *V. spinulosa* maintained high levels of genetic variation within populations and low subdivision among populations in these lakes. Historical hydrologic connectivity of these shallow lakes contributes to the high degree of genetic variation observed within populations and low genetic differentiation among populations in *V. spinulosa*. Further population genetic studies of aquatic plants in these lakes are needed to demonstrate whether the pattern provided by *V. spinulosa* is typical or anomalous for macrophytes in this region. Because recent human activities have increasingly modified this freshwater ecosystem, and have disturbed the stability and hydrologic connectivity of these shallow lakes (Liu, 1984; Xie and Chen, 1999), additional studies of submerged macrophytes in this area may help to determine the impact of these processes on the aquatic macrophyte populations, which help to maintain ecological stability in the system (Alberte et al., 1994; Xie and Chen, 1999). Such information should provide additional insight and help to develop strategies for the conservation and management of aquatic plant communities in this important freshwater ecosystem.

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References

Alberte, R.S., Suba, G.K., Procaccini, G., Zimmerman, R.C., Fain, S.R., 1994. Assessment of genetic diversity of seagrass populations using DNA fingerprinting: implications for population stability and management. Proc. Natl. Acad. Sci. USA 91, 1049–1053.

Barrett, S.C.H., Eckert, C.G., Husband, B.C., 1993. Evolutionary processes in aquatic plant populations. Aquat. Bot. 44, 105–145.

Bohonak, A.J., 2002. IBD (isolation by distance): a program for analyses of isolation by distance. J. Hered. 93, 153–154.

Cook, C.D.K., 1988. Wind pollination in aquatic angisosperms. Ann. Missouri Bot. Gard. 75, 768–777.

Ellastrand, N.C., Roose, M.L., 1987. Patterns of genotypic diversity in clonal plant species. Am. J. Bot. 74, 123–131.

Eriksson, O., 1993. Dynamics of genets in clonal plants. Trends Ecol. Evol. 8, 313–316.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gornall, R.J., Hollingsworth, P.M., Preston, C.D., 1998. Evidence for spatial structure and directional gene flow in a population of an aquatic plant, *Potamogeton coloratus*. Heredity 80, 414–421.

Goudet, J., 2001. FSTAT, A Program to Estimate and Test Gene Diversities and Fixation Indices (Version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hamrick, J.L., Godt, M.J.W., 1989. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), Plant Population Genetics, Breeding, and Genetic Resources. Sinauer Associates, Sunderland and Massachusetts, pp. 43–63.

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

Huang, H., Dane, F., Norton, J.D., 1994. Allozyme diversity in Chinese, Seguin and American chestnut (*Castanea* spp.). Theor. Appl. Genet. 88, 981–985.

Kudoh, H., Whigham, D.F., 1997. Microgeographic genetic structure and gene flow in *Hibiscus moscheutos* (Malvaceae) populations. Am. J. Bot. 84, 1285–1293.

Kudoh, H., Whigham, D.F., 2001. A genetic analysis of hydrologically dispersed seeds of *Hibiscus moscheutos* (Malvaceae). Am. J. Bot. 88, 588–593.

Laushman, R.H., 1993. Population genetics of hydrophilous angiosperms. Aquat. Bot. 44, 147–158.

Les, D.H., 1988. Breeding systems, population structure, and evolution in hydrophilous angiosperms. Ann. Missouri Bot. Gard. 75, 819–835.

Les, D.H., 1991. Genetic diversity in the monoecious hydrophile *Ceratophyllum* (Ceratophyllaceae). Am. J. Bot. 78, 1070–1082.

Li, W., Xia, L.Q., Li, J.Q., Wang, G.X., 2004. Genetic diversity of *Potamogeton maackianus* in the Yangtze River. Aquat. Bot. 80, 227–240.

Liu, J.K., 1984. Lakes of the middle and lower basins of the Chang Jiang (China). In: Tabu, F.B. (Ed.), Lakes and Reservoirs. Elsevier, Amsterdam, pp. 331–355.

Lokker, C., Susko, D., Lovett-Doust, L., Lovett-Doust, J., 1994. Population genetic structure of *Vallisneria americana*, a dieoecious clonal macrophyte. Am. J. Bot. 81, 1004–1012.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.

Mulcahy, D.L., Robinson, R.W., Ihara, M., Kesseil, R., 1981. Gametophytic transcription for acid phosphatase in pollen of *Cucurbita* species hybrids. J. Hered. 72, 353–354.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nies, G., Reusch, T.B.H., 2005. Evolutionary divergence and possible incipient speciation in post-glacial populations of a cosmopolitan aquatic plant. J. Evol. Biol. 18, 19–26.

Philbrick, C.T., Les, D.H., 1996. Evolution of aquatic angiosperm reproductive systems. Bioscience 46, 813–826.

Pielou, E.C., 1969. An Introduction to Mathematical Ecology. Wiley Interscience, New York.

Piquot, Y., Petit, D., Valero, M., de Cuguen, J., Laguerie, P., Vernet, P., 1998. Variation in sexual and asexual reproduction among young and old populations of the perennial macrophyte *Sparganium erectum*. Oikos 82, 139–148.

Richards, A.J., 1986. Plant Breeding Systems. Allen and Unwin, London.

Rousset, F., 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145, 1219–1228.

Sun, X.Z., 1992. Flora Reipublicae Popularis Sinicae, Tomus, vol. 8. Science Press, Beijing (in Chinese).

Wang, S.M., Dou, H.S., 1998. ZhongGuo HuBo Zhi. Science Press, Beijing (In Chinese).

Waser, N.M., Vickery Jr., P.K., Price, M.V., 1982. Patterns of seed dispersal and population differentiation in *Mimulus guttatus*. Evolution 36, 753–761.

Weeden, N.F., Wendel, J.F., 1989. Visualization and interpretation of plant isozymes. In: Soltis, D.S., Soltis, P.S. (Eds.), Isozymes in Plant Biology. Dioscorides, Porland and Oregon, pp. 46–72.

Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38, 1358–1370.

Wright, S., 1951. The genetical structure of populations. Ann. Eugenet. 15, 323–354.

Xie, P., Chen, Y.Y., 1999. Threats to biodiversity in Chinese inland waters. Ambio 26, 674–681.

Xiong, B.H., 2000. The Ecological Studies on *Vallisneria* L. in Poyang Lake, Jiang Xi. Masters Thesis. Wuhan Institute of Botany, the Chinese Academy of Sciences (in Chinese, with English abstract).

Yan, S.Z., Zhu, W.J., 1984. Comparative morphological studies on three species of *Vallisneria* from China. Guihaia 4, 309–315 (in Chinese, with English abstract).

Yeh, F.C., Yang, R.C., Boyle, T.B., 1999. POPGENE Version 1.31, Microsoft Window-based Freeware for Population Genetic Analysis. Available at http://www.ualberta.ca/~fyeh.