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Assessment of population genetic structure in common wild rice *Oryza rufipogon* Griff. using microsatellite and allozyme markers

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Abstract The genetic structure of five natural populations of common wild rice *Oryza rufipogon* Griff. from China, was investigated with 21 microsatellite loci and compared to estimates of genetic diversity and genetic differentiation detected by 22 allozyme loci. Microsatellite loci, as expected, have much higher levels of genetic diversity (mean values of A = 3.1, P = 73.3%, Ho = 0.358 and He = 0.345) than allozyme loci (mean values of A = 1.2, P = 12.7%, Ho = 0.020 and He = 0.030). Genetic differentiation detected by microsatellite loci (F_{ST} = 0.468, mean I = 0.472) was higher than that for allozyme loci (F_{ST} =0.388, mean I = 0.976). However, microsatellite markers showed less deviation from Hardy-Weinberg expectation (Wright's inbreeding coefficient F_{IS} = –0.069) than do allozymes (F_{IS} = 0.337). These results suggest that microsatellite markers are powerful high-resolution tools for the accurate assessment of important parameters in population biology and conservation genetics of *O. rufipogon*, and offer advantages over allozyme markers.

Keywords China · Allozyme variation · Microsatellite variation · *Oryza rufipogon* · Population genetic structure

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Introduction

Because rice is the most-important staple crop in the world, the scientific community has paid much attention to the study, conservation and utilization of the rice gene pool (Chang 1984; Vaughan and Chang 1992; Jackson 1994, 1997). In its categorized primary gene pool (Ge et al. 1999a; Khush 1999), the common wild rice *Oryza rufipogon* Griff. is a recognized ancestor of cultivated rice *Oryza sativa* L. It is a perennial, wind-pollinated species with mixed sexual and asexual reproductive strategies (Barbier 1989a; Gao et al. 1999a). The allocation of asexual vs sexual reproduction may vary among populations at different natural habitats (Barbier 1989a). This gene pool has greatly contributed to the development of hybrid rice in the 1970s (Yuan et al. 1989) and has led to the dramatic increases in world rice production (Khush 1999). Map-based molecular-breeding technology holds tremendous the potential for the genetic improvement of rice, with beneficial genes coming from the enormous gene pool of wild rice species like *O. rufipogon* (Xiao et al. 1996; Brar and Khush 1997; Tanksley and McCouch 1997). Our recent field survey, however, indicates that human activities have led to the extinction of many populations of *O. rufipogon* in China (Gao et al. 1996; Gao 1997; Gao et al. 1998; Gao et al., unpublished data). Successful management and conservation of the remaining threatened populations in the species, therefore, requires a more comprehensive picture of population genetic structure.

Isozyme markers have provided a valuable tool for characterizing rice germplasm resources on a large geographical scale and have greatly contributed to our understanding of the genetic structure of the genus *Oryza* (Nakagahra et al. 1975; Second 1982, 1985a, 1985b; De Kochoko 1987; Glaszmann 1987, 1988; Glazmann et al. 1988). Allozyme analysis has been used to detect the genetic variation and structure of natural populations and has yielded important information on the population genetics of *O. rufipogon* (Barbier 1989a, b; Morishima and Barbier 1990; Gao 1997; Gao et al. 1999a, b, 2000a, b,

2001a, b, c, 2002). However, limitations in the number of allozymic loci which can be resolved, and the detection of genetic changes in only coding regions of the genome which result in substitution, may have hampered an accurate assessment of population genetic structure. For instance, our recent allozyme study of 21 natural populations from seven provinces of China showed moderate allozyme variability (A = 1.33, P = 22.7%, Ho = 0.033 and He = 0.068) and genetic differentiation (F_{ST} = 0.310, I = 0.964) (Gao et al. 2000a). Of 22 loci, only 12 are polymorphic, of which nine segregate for either two or three alleles. Therefore, data on critical population genetic parameters such as genetic diversity and differentiation have been difficult to obtain because of a lack of variable genetic markers.

Microsatellites have become powerful new genetic markers for measuring intraspecific variability and differentiation due to their high polymorphism, codominance, abundance throughout the genome and relative ease of scoring (Bowcock et al. 1994; Estoup et al. 1995). Since Condit and Hubbell (1991) first noted that microsatellite analysis has great potential for understanding plant population genetics in natural populations, it is now widely used in population and conservation genetic studies (e.g. Bruford and Wayne 1993; Saghai-Maroof et al. 1994; Terauchi and Konuma 1994; Chase et al. 1996a, b; Inna et al. 1997; van Treuren et al. 1997; Aldrich et al. 1998; Provan et al. 1999; Olsen and Schaal 2001). Recently, hundreds of microsatellites from rice have been developed, mapped and published (Wu and Tanksley 1993; Akagi et al. 1996; Panaud et al. 1996; Chen et al. 1997; McCouch et al. 1997; Cho et al. 2000; Temnykh et al. 2000). Our recent studies show that the microsatellites from *O. sativa* can be amplified in *O. rufipogon* and exhibit numerous alleles (Gao et al., unpublished). Therefore, rice microsatellites are untapped resources of highly polymorphic genetic markers for studies on genetic diversity in natural populations of *O. rufipogon*.

To-date, no studies on the application and comparison of microsatellites and allozyme loci for detecting the genetic structure of natural populations have been reported in wild rice. Such work will help to verify the relative merits of these two kinds of codominant markers in population biology and provide an access to rice microsatellites for studies on molecular population and conservation genetics in the *Oryza* species. The purposes of this study are: (1) to evaluate the levels and distribution of genetic variability in *O. rufipogon*, at both the protein and DNA levels, to provide a deep insight into the genetic diversity of the species; (2) to compare the levels and distribution of genetic variability, within and among the same populations of *O. rufipogon*, at microsatellite and allozyme loci in order to determine the relative levels of resolution for measuring population genetic structure; and (3) to assess the relative values of both techniques in population and conservation genetic studies, and to access the potential application of rice microsatellites for further studies on the evolution and conservation of *O. rufipogon*.

Materials and methods

Study sites and plant samples

Five populations of *O. rufipogon* were sampled in Southern China (see Table 2), including the three surviving populations from Yunnan Province, and one each from the Guangxi and Hainan provinces. These populations represent the major distribution of *O. rufipogon* in China. The sampling methods are described in detail elsewhere (Gao et al. 2000a).

Starch-gel electrophoresis and microsatellite assay

Fourteen enzymes were resolved and scored using starch-gel electrophoresis (Gao et al. 2000a). The electrophoresis methods followed Soltis et al. (1983) and Glaszmann et al. (1988a) with 12% starch gels. Staining procedures and the nomenclature for the designation of loci and alleles is identical to the previous studies (Gao et al. 2000a, 2001a, b, 2002).

DNA was isolated from freeze-dried leaf tissues according to the method of Edwards et al. (1991). A total of 21 rice microsatellite primer pairs were used in this study (Table 1). Microsatellite polymorphism was analyzed by specific PCR conditions as described in Panaud et al. (1996). PCR products were resolved by 4% polyacrylamide denaturing gels. The gels were stained with the silver staining as described by Panaud et al. (1996).

Statistical analysis

Statistical analysis was performed using Biosys-1 (Swofford and Selander 1989) version 1.7 for the IBM-PC. Data were entered as genotype numbers from which allele frequencies were calculated. The amount of genetic variation in each locus and population was indicated by the mean number of alleles per locus (A), the percent of polymorphic loci (P), and observed and expected heterozygosities (Ho and He). Departures from Hardy-Weinberg expectations and divergences among populations were measured using the *F-statistics* of Wright (1978) and their significance was evaluated with a chi-square test following the method of Workman and Niswander (1970). Overall genetic differences between populations were evaluated by the Cavalli-Sforza chord distance (Dc) (Cavalli-Sforza and Edwards 1967). Using NEIGHBOR in the PHYLIP computer package (version 3.5c; Felsenstein 1995), two UPGMA phenograms were generated. The robustness of Nei's unbiased genetic distance trees was assessed by creating 100 bootstrap replicates of the data set with the SEQBOOT algorithm in PHYLIP, and then generating a majority rule consensus tree in the CONSENSE program. All distance trees were viewed in TREEVIEW (Page 1996).

Results

Allele frequencies and polymorphisms

Allele frequencies and genotype data for both markers are available from Lizhi Gao on request. In allozyme analysis, a total of 33 alleles at 22 loci were identified in 216 individuals of the five populations. *Aat-1*, *Aat-3*, *Dia-2*, *Fba*, *Gdh*, *Lap-1*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Me*, *Pgd-1*, *Pgd-2*, *Pgi-1*, *Tpi-1* and *Tpi-2* were monomorphic, and other loci were polymorphic in at least one population. *Adh*, *Idh* and *Pgi-2* each had two alleles, *Dia-1*, *Pgi-3* and *Pgm* each had three, and *Skd* had five. All 21 microsatellite loci were polymorphic in this study. A total of 163 alleles were detected in 108 individuals

Table 1 Microsatellite pair sequences, expected product size, and chromosomal location in the present study (*Not Available)

Name	Chromal location	References	Primer sequences	Annealing temperature (°C)	Product size (bp) in IR36
RM225	6	Chen et al. (1997)	TGCCCATATGGTCTGGATG GAAAGTGGATCAGGAAGGC	55	140
RM234	7	Chen et al. (1997)	ACAGTATCCAAGGCCCTGG CACGTGAGACAAAGACGGAG	55	156
OSR20	12	Akagi et al. (1996)	TGGTCAAGTGACTTAGGTGG AGAGCTCCAACTCTTTACAAG	55	NA*
RM233A	2	Chen et al. (1997)	CCAAATGAACCTACATGTTG GCATTGCAGACAGCTATTGA	55	162
RM244	10	Chen et al. (1997)	CCGACTGTTCGTCCTTATCA CTGCTCTCGGGTGAACGT	55	163
RM164	5	Wu and Tanksley (1993)	TCTTGCCCGTCACTGCAGATATCC GCAGCCCTAATGCTACAATTCTTC	55	246
RM242	9	Chen et al. (1997)	GGCCAACGTGTGTATGTCTC TATATGCCAAGACGGATGGG	55	225
RM252	4	Chen et al. (1997)	TTCGCTGACGTGATAGGTTG ATGACTTGATCCCGAGAACG	55	216
RM206	11	Chen et al. (1997)	CCCATGCGTTTAACTATTCT CGTTCCATCGATCCGTATGG	55	147
RM26	5	Chen et al. (1997)	GAGTCGACGAGCGGCAGA CTGCGAGCGACGGTAACA	55	112
RM224	11	Chen et al. (1997)	ATCGATCGATCTTCACGAGG TGCTATAAAAGGCATTCGGG	55	157
OSR22	7	Akagi et al. (1996)	CTGAGTCTCCTGTCCTCATC CTTGAATCTCTGCACTGCAC	55	NA*
OSR27	1	Akagi et al. (1996)	GGGTGATTTCTTGGAAGCGA TCTCGGAGAGCTTCTCCATC	55	NA*
OSR28	9	Akagi et al. (1996)	AGCAGCTATAGCTTAGCTGG ACTGCACATGAGCAGAGACA	55	NA*
OSR32	12	Akagi et al. (1996)	CTCCAGCTTCGGCAACGTCA CTTCTTGATGCCCTCAATCGT	55	NA*
OSR35	8	Akagi et al. (1996)	GCCTCGAGCATCATCATCAG ATCAACCTGCACTTGCCTGG	55	NA*
RM212	1	Chen et al. (1997)	CCACTTTCAGCTACTACCAG CACCCATTTGTCTCTCATTATG	55	136
RM261	4	Chen et al. (1997)	CTACTTCTCCCCTTGTGTCG TGTACCATCGCCAAATCTCC	55	125
RM60	3	Chen et al. (1997)	AGTCCCATGTTCCACTTCCG ATGGCTACTGCCTGTACTAC	55	165
RM55	3	Chen et al. (1997)	CCGTCGCCGTAGTAGAGAAG TCCCGGTTATTTTAAGGCG	55	226
RM210	8	Chen et al. (1997)	TCACATTCGGTGGCATTG CGAGGATGGTTGTTCACTTG	55	140

from five populations. The locus OSR22 was the most polymorphic with 14 alleles, while the least polymorphic loci were RM242 and RM244, each with four alleles.

Genetic variability

The number of alleles per locus per population (n), the observed heterozygosity (*Ho*) and the expected heterozygosity (*He*) at seven polymorphic allozyme and 21 microsatellite loci suggested that most of the microsatellite loci showed larger numbers of alleles, as well as higher levels of heterozygosities, than the allozyme loci (data not shown). *A*, *P*, *Ho* and *He* at the microsatellite loci for each population were much higher than those at the allozyme loci (Table 2), and the mean values for the 21 microsatellite loci (*A* = 3.1, *P* = 73.3%, *Ho* = 0.358 and *He* = 0.345) were much higher than those for the 22 allozyme loci (*A* = 1.2, *P* = 12.7%, *Ho* = 0.020 and *He* = 0.030). Although the values varied among populations, the patterns of genetic diversity within these populations at microsatellite loci are consistent with those at allozyme loci.

Population genetic structure

In the five populations of *O. rufipogon* detected by allozyme loci, F_{IS} was 0.337 (Table 3), indicating that most populations deviated from Hardy-Weinberg expectation within populations with a deficiency of heterozygotes; F_{ST} was 0.388, indicating that 38.8% of the total genetic variation existed among populations. As detected by microsatellite loci (Table 3), however, F_{IS} was –0.069, suggesting that most of the populations deviated slightly from Hardy-Weinberg expectation within populations

Table 2 The sample sizes, geographical origin and the comparison of genetic variability at microsatellite and allozyme loci of five populations of *O. rufipogon*. *N*: mean sample size per locus; *A*: mean number of alleles per locus; *P*: percentage of polymorphic loci (a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99); *Ho*: observed heterozygosity based on a direct count of heterozygotes; *He*: unbiased heterozygosity (Nei 1978); M: microsatellite analysis; A: allozyme analysis

Populations	Geographical origin	*N* (M)	*A* (M)	*P* (M)	*Ho* (M)	*He* (M)	*N* (A)	*A* (A)	*P* (A)	*Ho* (A)	*He* (A)
Ledong	Ledong County Hainan Province	14	2.8 (0.0)	90.5 (0.2)	0.480 (0.105)	0.335 (0.054)	40	1.2 (0.0)	18.2 (0.1)	0.052 (0.038)	0.053 (0.032)
Beise	Beise City, Guangxi Province	18	6.7 (0.1)	100.0 (0.5)	0.276 (0.055)	0.696 (0.036)	28	1.3 (0.0)	22.7 (0.1)	0.028 (0.019)	0.065 (0.035)
Meiting	Meiting, Jinghong City, Yunnan Province	27	1.5 (0.0)	42.9 (0.2)	0.425 (0.110)	0.223 (0.058)	32	1.0 (0.0)	0.0 (0.0)	0.000 (0.000)	0.000 (0.000)
Yuanjiang	Yuanjiang County, Yunnan Province	29	2.7 (0.1)	85.7 (0.3)	0.172 (0.072)	0.218 (0.045)	87	1.2 (0.0)	13.6 (0.1)	0.017 (0.015)	0.031 (0.023)
Gasa	Gasa, Jinghong City, Yunnan Province	10	1.6 (0.0)	47.6 (0.1)	0.435 (0.107)	0.253 (0.061)	29	1.1 (0.0)	9.1 (0.1)	0.003 (0.002)	0.003 (0.002)
Mean		20	3.1	73.3	0.358	0.345	43	1.2	12.7	0.020	0.030

Table 3 Summary of *F-statistics* at all the polymorphic allozyme and microsatellite loci

Allozyme					Microsatellite				
Locus	F_{IS}	F_{IT}	F_{ST}	*P*	Locus	F_{IS}	F_{IT}	F_{ST}	*P*
Adh	0.321	0.544	0.329	0.00000	RM224	0.453	0.728	0.502	0.00000
Dia-1	0.974	0.992	0.690	0.00000	RM261	0.893	0.969	0.706	0.00000
Idh	1.000	1.000	0.029	0.00911	RM225	–0.391	0.065	0.328	0.00000
Pgi-2	1.000	1.000	0.029	0.00911	RM212	–0.414	0.121	0.378	0.00000
Pgi-3	–0.423	–0.092	0.232	0.00000	RM164	0.842	0.951	0.688	0.00000
Pgm	–0.015	–0.005	0.011	0.20924	RM233A	0.671	0.907	0.718	0.00000
Skd	0.521	0.671	0.312	0.00000	RM206	0.757	0.889	0.544	0.00000
					RM60	0.122	0.615	0.562	0.00000
					RM252	–0.135	0.223	0.316	0.00000
					RM244	1.000	1.000	0.114	0.00000
					RM26	–0.605	–0.299	0.191	0.00000
					RM242	0.706	0.953	0.840	0.00000
					RM55	0.924	0.980	0.732	0.00000
					OSR22	–0.338	0.138	0.356	0.00000
					OSR20	1.000	1.000	0.600	0.00000
					OSR35	–0.482	0.074	0.376	0.00000
					OSR27	0.612	0.864	0.649	0.00000
					OSR28	–0.519	0.010	0.349	0.00000
					OSR32	–0.456	–0.156	0.206	0.00000
					RM210	–0.535	–0.048	0.318	0.00000
					RM234	0.256	0.678	0.568	0.00000
Mean	0.337	0.594	0.388	0.00000	Mean	–0.069	0.431	0.468	0.00000

with a small excess of heterozygotes; F_{ST} was 0.468, suggesting that 46.8% of the total genetic variation existed among populations.

Genetic relationships

Nei's (1978) unbiased genetic identities among the pairs of the five populations (data not shown) indicated that the mean genetic identity of 0.976 among populations detected by allozyme loci is much higher than that of 0.472 for microsatellite loci. Cluster analysis (UPGMA) was further used to produce two phenograms and show the genetic relationships of the populations for both allozyme and microsatellite loci, respectively (Fig. 1). Genetic distances based on allozymes and microsatellites are not completely correlated. The three populations from Yunnan Province clustered together before forming a cluster with any other populations, with which the Beise population from Guangxi Province showed a closer genetic relationship than the Ledong population from Hainan Province based on allozyme loci. However, the Yuanjiang population from Yunnan Province formed a cluster with the Beise population, and the Ledong population showed the highest genetic identity with the Meiting population at microsatellite loci with high bootstrap support (100 and 100%, respectively; Fig. 1).

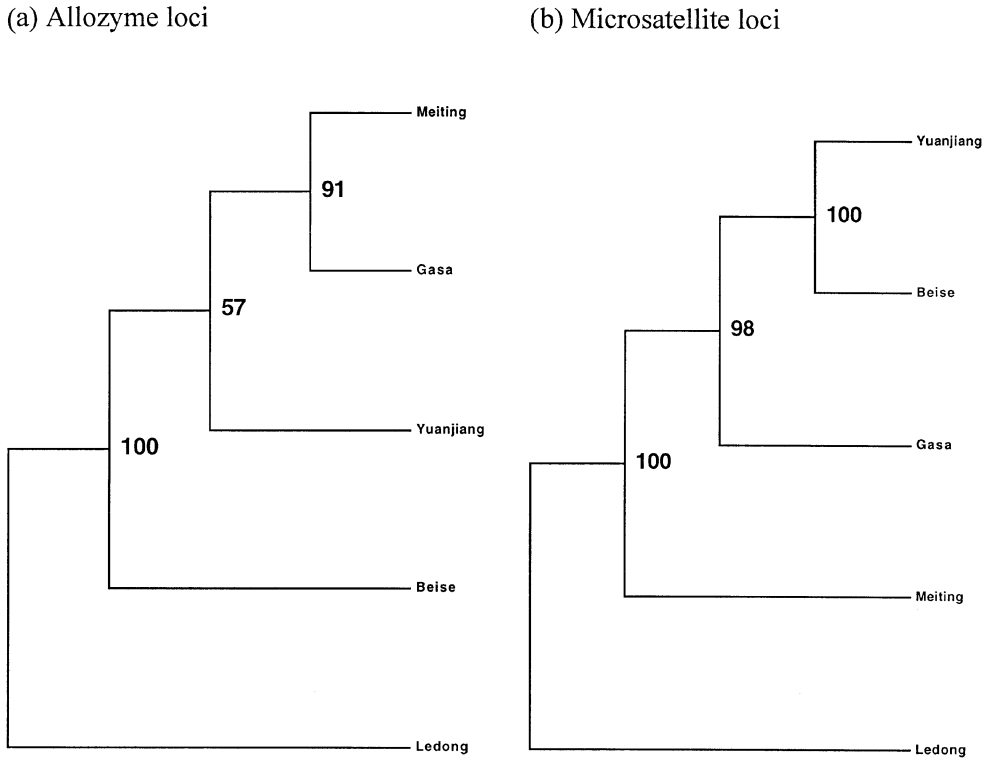

Fig. 1 UPGMA dendrogram of five populations of *O. rufipogon* using the Cavalli-Sforza chord distance (Dc) detected by allozyme (*a*) and microsatellite (*b*) loci. Numbers on branches indicate bootstrap values of >50% (100 replications)

Discussion

Comparison of levels of genetic diversity assessed using microsatellite and allozyme markers

The principal aim of this study was to compare levels of microsatellite and allozyme variation in five natural populations of *O. rufipogon* to assess the relative potential of the markers for assaying genetic diversity in the species. Microsatellite loci are a demonstrated rich source of highly polymorphic genetic markers in an ever-increasing number of organisms, and are promising markers for the detection of genetic variation in species, while allozyme analysis has revealed only low levels of variation (Saghai-Maroof et al. 1994; Terauchi and Konuma 1994; Lehmann et al. 1996; Sanchez et al. 1996; Estoup et al. 1998; Meglecz et al. 1998; Streiff et al. 1998; Dje et al. 1999; Sun et al. 1999; Lemaire et al. 2000). For example, in the yam (*Dioscorea tokoro*), Terauchi and Konuma (1994) found that microsatellites show higher levels of genetic variability than allozymes with respect to heterozygosity. In the current study, all the microsatellite loci surveyed were polymorphic with four to 14 alleles per locus, and an average of 3.1 alleles per locus. Protein-coding allozyme loci from the same populations show only between one and four alleles per locus, with an average of 1.2. Most of the allozyme loci were monomorphic and those loci that were polymorphic usually displayed one allele in high frequency with several rare alleles (data not shown). Therefore, many high levels of genetic variation at 21 microsatellite loci, which were 2.58-, 5.77-, 17.90- and 11.50-times higher than those at 22 allozyme loci, were observed in the same five natural populations of *O. rufipogon* in this study (Table 2).

The comparison of levels of genetic diversity in the five populations shows a similar pattern between allozyme and microsatellite loci. The three populations from Yunnan Province, which have low levels of genetic variation at allozyme loci, also displayed the lowest genetic diversity at microsatellite loci. The results support the previous allozyme studies in which the three surviving populations of *O. rufipogon* from Yunnan possess low levels of genetic variability, compared to other populations analyzed from south China (Gao et al. 2000a, 2002). As for the two populations from Xishuangbanna, the Meiting population possessed lower levels of genetic polymorphisms than the Gasa population for allozyme and microsatellite loci to a varying extent. Genetic diversity within the Meiting population (A = 1.0, P = 0.00%, Ho = 0.000 and He = 0.000) is much lower than those of the Gasa population (A = 1.1, P = 9.10%, Ho = 0.003 and He = 0.003) for allozyme loci. However, at the microsatellite loci, the Meiting population (A = 1.5, P = 42.9%, Ho = 0.425 and He = 0.223) was nearly as variable as the Gasa population (A = 1.6, P = 47.6%, Ho = 0.435 and He = 0.253). These results further suggest that microsatellite loci are more effective in detecting small genetic differences among populations than are allozyme loci. These differences in the nature of allozyme vs microsatellite loci would affect variation at different levels of genetic variation between these two kinds of markers (Clegg 1989; Smith et al. 1992; Sun et al. 1998, 1999).

Population genetic structure based on microsatellite and allozyme data analysis

The second aim of this study was to compare the distribution of microsatellite and allozyme variation within and among the five natural populations of *O. rufipogon*. In this study, both the microsatellite and allozyme data indicate that more genetic variation exists within populations than among populations. This observation corresponds well to what has previously been reported for allozyme and RAPD markers in *O. rufipogon* (Morishima 1985; Morishima and Barbier 1990; Gao 1997; Barbier 1989b; Ge et al. 1999b; Gao et al. 2000a, 2001a, 2001b, 2002). However, genetic differentiation detected by microsatellite loci (F_{ST} = 0.468) seems higher than using allozyme loci (F_{ST} = 0.388). The degrees of differentiation are further indicated by different values of genetic identities using microsatellite and allozyme markers. The pattern of genetic differentiation in *O. rufipogon* is consistent with data from natural populations of other organisms (Hughes and Queller 1993; Lanzaro et al. 1995). Microsatellites evolve more rapidly than allozyme loci, and thus recent genetic differentiation is more easily detected between populations by microsatellite loci. In contrast, Estoup et al. (1998), Meglecz et al. (1998) and Dje et al. (1999) observed that higher levels of polymorphism at microsatellite loci resulted in a higher power for statistical tests of population differentiation but does not significantly increase F_{ST} values. Strieff et al. (1998) detected a greater genetic structure in oaks inferred from microsatellite data than from allozymes, but pointed out that the main difference between the two markers resides in the interlocus variation in F_{ST}, which is much greater for allozymes. However, the F_{ST} estimate for the microsatellite loci in *Anopheles gambiae* (F_{ST} = 0.016) was lower than the F_{ST} for the allozyme loci (F_{ST} = 0.036) (Lehmann et al. 1996) in spite of the SSRs hypervariability. Similar results were observed in *Elymus fibrosus* (Sun et al. 1998). The failure of microsatellite loci to detect greater divergence than allozyme loci in these cases suggests the existence of constraints on their evolution, such as biased mutation rates (Garza et al. 1995) and/or selection for certain allele sizes (Epplen et al. 1994). This is probably an explanation that there is actually no divergence or else just a failure to detect it. The population genetic structure of the species can be better outlined if extensive populations are sampled in the future.

Our study shows that allozyme and microsatellite data did not fit Hardy-Weinberg expectation. Allozymes, which have been used most frequently in rice (Gao 1997; Gao et al. 2000a, b, d), indicate that most populations deviate from Hardy-Weinberg expectation with a deficiency of heterozygotes (F_{IS} = 0.337). In contrast, although 12 out of 21 loci exhibited a deficiency of heterozygotes, the five populations studied here by microsatellites (Table 3) showed a mean F_{IS} of –0.069, indicating that most populations deviated slightly from Hardy-Weinberg expectation by a small excess of heterozygotes. A possible cause for such a contradiction is the different technical resolution between two classes of markers so that many heterozygotes may not have been detected by allozyme analysis.

Potential application to studies on the population biology and conservation genetics of wild rice

Our final question is whether microsatellite markers have the potential for studying the evolution and conservation genetics of *O. rufipogon*? The value of genetic markers to detect population differences depends strongly on the number and frequencies of the alleles (Nei 1987). Our results indicate that microsatellite markers detected higher intra-population polymorphisms than do allozymes. Microsatellite analysis, therefore, provides a highly polymorphic set of markers that will be useful for assaying genetic variation within species, particularly where any other methods have weakened or failed. For example, microsatellite analysis is more effective in detecting small genetic differences within those seriously fragmented small populations or marginal populations than is allozyme analysis in this study, and thus is able to provide useful evolutionary and conservation application. Although some new allozyme loci have recently been adapted to examine genetic diversity and the population genetic structure of wild rice species (Gao 1997; Gao et al. 2000a, b, 2001a, b, c, 2002), it is apparent that few new allozyme loci will be available in the short-term. In contrast, over 300 microsatellites have been published and mapped in rice (Wu and Tanksley 1993; Akagi et al. 1996; Panaud et al. 1996; Chen et al. 1997; McCouch et al. 1997; Cho et al. 2000; Temnykh et al. 2000), which provide a large reservoir of genetic markers for the rice research community. Thus microsatellite analysis is a useful tool for population-biology and conservation-genetics studies in wild rice *O. rufipogon*. For instance, allozyme analysis in studying the mating system and gene flow of *O. rufipogon* usually confronted difficulties in the availability of allelic variation (Gao, unpublished data). However, lots of microsatellite loci with high polymorphisms have changed the situation and offer us the opportunities to further address these important questions. Nevertheless, allozymes are often well suited for population genetic structure because there is a large body of available literature for comparison with other plant species (Hamrick and Godt 1989, 1996). In particular, allozymes may be less biased in their measures since microsatellite markers are chosen based on their polymorphisms. Hence, for a plant species with a poor knowledge of population genetic structure, one practical strategy could be to initially use a limited number of individuals with the cheapest and fastest method, like allozyme analysis, to approximately estimate the levels of genetic diversity and determine whether microsatellite analysis will necessarily be applied. We believe that the most suitable method to be used will depend on the amount of genetic variation, and the molecular evolutionary and conservation genetic questions addressed in *O. rufipogon*. Microsatellite analysis has great potential for exploring some specific ques-

tions in the species, such as: (1) the variability and evolution of mating systems, (2) gene flow among natural populations as well as between wild populations and cultivated varieties, (3) the clonal structure of natural populations, and (4) genetic erosion of natural populations under fragmented habitats. However, allozyme analysis also seems informative in investigating the population genetic structure on a large geographical scale as compared to previous allozyme literature in the species.

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