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Genetic structure and diversity of *Oryza sativa* L. in Guizhou, China

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Preserving many kinds of rice resources and rich variations, Guizhou Province is one of the districts with the highest genetic diversity of cultivated rice (*Oryza sativa* L.) in China. In the current research, genetic diversity and structure of 537 accessions of cultivated rice from Guizhou were studied using 36 microsatellite markers and 39 phenotypic characters. The results showed that the model-based genetic structure was the same as genetic-distance-based one using SSRs but somewhat different from the documented classification (mainly based on phenotype) of two subspecies. The accessions being classified into *indica* by phenotype but *japonica* by genetic structure were much more than that being classified into *japonica* by phenotype but *indica* by genetic structure. Like Ding Ying's taxonomic system of cultivated rice, the subspecific differentiation was the most distinct differentiation within cultivated rice. But the differentiation within *indica* or *japonica* population was different: *japonica* presented clearer differentiation between soil-watery ecotypes than *indica*, and *indica* presented clearer differentiation between seasonal ecotypes than *japonica*. Cultivated rices in Guizhou revealed high genetic diversity at both DNA and phenotypic levels. Possessing the highest genetic diversity and all the necessary conditions as a center of genetic diversity, region Southwestern of Guizhou was suggested as the center of genetic diversity of *O. sativa* L. from Guizhou.

Guizhou Province, Asian cultivated rice (*Oryza sativa* L.), phenotypic characters, microsatellites, genetic structure, genetic diversity

Rice is the most important food crop in Guizhou, with a long history of cultivation. Guizhou Province is located on the eastern slope of the Yunnan-Guizhou Plateau and is characterized by a typically arranged vertical agricultural ecosystem. Natural and artificial selection applied by this complicated environment has resulted in various ecological types exhibiting traits such as higher than natural yields, pest and disease resistance or larger seeds, attracting the attention of breeders and genetic researchers[1−8]. There were 5377 accessions of different rice varieties by 1995[9], accounting for 10% of *ex situ* germplasm collections in China. But the use of these varieties in rice breeding has been limited because of a lack of information on the genetic diversity and genetic structure of these rice resources. It is apparent that less attention has been paid to Guizhou relative to other provinces as Yunnan and Guangxi and less attention has been paid to variation at the DNA level relative to the phenotypic level.

Microsatellites, also called simple sequence repeats (SSRs), are tandem repeats of short DNA motifs (1−6

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bp in length) often exhibiting high polymorphism in the number of repeats. Because microsatellites are often highly polymorphic, codominant marker and their map positions on the rice genome are well known[10,11] they are suitable for assessing genetic diversity and genetic structure among closely-related rice cultivars and have been broadly used in genome mapping[12], population genetics and evolution[13,14], and applied in genetic diversity[15,16] and protectection of germplasm resources[17,18] and molecular breeding programs[19].

In the present study we examine genetic variation among 537 accessions of cultivated rice from Guizhou using 36 microsatellite markers and 31 phenotypic traits. Our objectives are to (1) investigate the genetic structure and diversity of cultivated rice varieties (*Oryza sativa* L.) in Guizhou, (2) examine the evolutionary relationships among intraspecific populations, and (3) determine the center of the genetic diversity in Guizhou. These data will aid rice breeding programs and the management of germplasm collections.

1 Materials and methods

1.1 Plant materials

A total of 537 accessions of cultivated rice were selected from the primary core collection of rice germplasm resources in China[20] including 249 *indica* varieties and 288 *japonica* varieties in Guizhou Province. Covering all 6 ecological zones and 76 counties in Guizhou (Figure 1), the materials used in this research are a sample representative of rice germplasm resources in Guizhou.

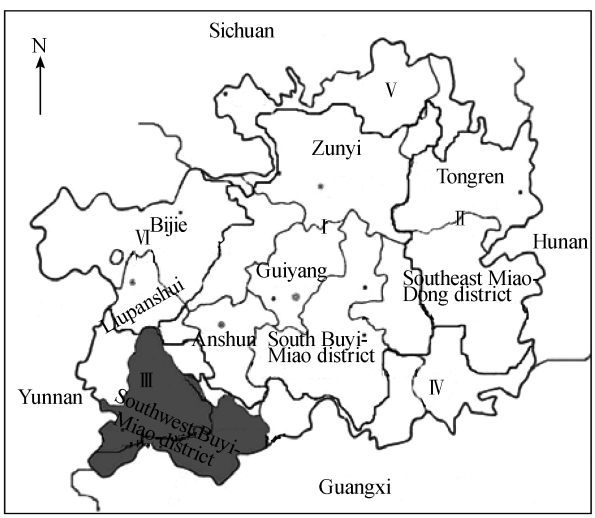

Figure 1 Map of rice ecological zones (dark line), districts (light line) in Guizhou, China. I, Zone of single-season rice in the Middle Guizhou (EZ I); II, zone of double/single-season rice in the East Guizhou (EZ II); III, zone of single-season rice in the Southwest Guizhou (EZ III); IV, zone of double/single-season rice in South Guizhou (EZ IV); V, zone of double/single-season rice in North Guizhou (EZ V); VI, zone of *Japonica* rice in Northwest Guizhou (EZ VI).

1.2 Identification of morphological traits

All the varieties in this study were planted in the China National Rice Research Institute, Hangzhou, China, in 2001 and missing data were supplemented in 2002. In a field, four rows with six plants each were planted for each variety. Data for each measured quantitative trait were averaged from ten randomly sampled individuals. Phenotypic traits including twenty-four qualitative traits and eight quantitative traits were evaluated based on *Descriptors for Rice*, *Oryza sativa L.* and *Method for characterizing the morphological traits of rice*, *Oryza sativa L.*. Measured traits included penultimate cornu, blade pubescence, sheath color, color and shape of penultimate foliage ligule, colors of collar and penultimate auricle color, diameter of culm and angle of culm, lodging resistance, color of inter-nodes, flag leaf curve and angle, panicle shape and type, exsertion, awn color, awn length, apiculus color, stigma color, lemma and palea color, lemma length and palea pubescence, seed coat color, seed shape, scent and color of rice.

1.3 SSR analysis

Fresh leaf tissues were harvested at the seedling stage from plants grown in the greenhouse. DNA was extracted following a CTAB procedure[21]. Thirty six SSR loci (3 loci per chromosome) were amplified using PCR protocols described by Panaud et al.[22]. Each 15 μL PCR reaction mix included 92.4 ng of primer, 1.5 μL 10× buffer (pH 8.3), 22.5 mmol/L $MgCl_2$, 50 ng of DNA and 1.8 mmol/L dNTP. The PCR temperature profile included the following three steps: (1) predenature at 95℃ for 5 min; (2) 30 cycles of run, each followed by denature at 95℃ for 1 min, anneal at 55℃ for 30 s and extension at 72℃ for 1 min; (3) final extension at 72℃ for 10 min. PCR products were run on 8% denaturing polyacrylamide gel at 2000 V for 120 min and marker bands were visualized using silver staining[22]. The molecular weight of each SSR allele was estimated based on a standard molecular weight marker-10 bp DNA ladder (Catalogue No. 10821-015) from Invitrogen.

1.4 Data analysis

Phenotypic diversity was measured by calculating (1) the total number of variants, and (2) Shannon-Weiner's diversity index[23] $I=-\Sigma p_i \ln p_i$, where p_i is the frequency

of the *i*th variant. Genetic diversity was measured by calculating 1) the number of alleles at an SSR locus, and 2) Nei's index[24] ($He=1-\Sigma p_i^2$) where p_i was the frequency of the *i*th allele at the locus. The population structure was inferred using a model allowing for admixture and correlated allele frequencies in STRUCTURE[25,26] with a burn-in length of 10000 and a run length of 100000. The results showed that when k = 8, the Ln$P(D)$ value and α value fluctuated distinctly. Three independent runs yielded consistent results. The neighbor-joining tree including all 537 accessions was constructed in POWERMARKER version 3.25[27] using Nei's D_A[28,29] which was proven an efficient distance measure in obtaining the correct topology. Average standardized molecular weight of PCR products in per population were calculated as in Vigouroux et al.[30]. Pairwise population differentiation among populations were estimated and tested based on the Weir and Cockerham[31] estimator of F_{st}. We also estimated regressions of genetic distance and genetic differentiation (F_{st}) on geographic distance between districts using SPSS 11.0.

2 Results

2.1 Population structure of cultivated rice in Guizhou

The model-based simulation of population structure using SSRs showed that likelihood was maximized and α minimized when k=7. That is, there are seven populations, inferred from the model here denoted as POP1, POP2, …, and POP7, respectively (Figure 2(a)). The membership of the accessions among the inferred populations showed more similarity to Ding Ying's taxonomic system of cultivated rice than to a clustering pattern based on six rice ecological zones (Figure 2(b)—(d)). According to the membership pattern, we denoted POP1 as medium-late *indica* (M-L.*Ind*), POP2 as lowland *japonica* I(L.*Jap*I), POP3 as lowland *japonica* Ⅱ (L.*Jap*II), POP4 as early-medium *indica* (E-M.*Ind*), POP5 as upland *japonica* I (U.*Jap*I), POP7 as upland *japonica* II (U.*Jap*II) and POP6 as intermediate due to its unclear membership among *indica*/*japonica*, early/medium/late and lowland/upland.

The neighbor-joining tree of 537 accessions based on Nei's genetic distance[28] revealed consistent membership with the model-based structure (Figure 3). Moreover, the neighbor-joining tree showed that the cultivated rice first differentiated into two subspecies, i.e. *indica* and *japonica*, and then *indica* differentiated into two seasonal ecotypes and *japonica* differentiated into four soil-watery ecotypes. In addition, the number of accessions (84) classified into *indica* by phenotype but *japonica* by genotype was greater than that (33) classified into *japonica* by phenotype but *indica* by genotype. The overall AMOVA indicated that 17.5% of the genetic diversity was due to the differences among groups with the remaining 82.5% due to the differences within groups. The genetic difference between *indica* and *japonica* was clearer than that among subpopulations in *japonica* or *indica*.

In order to research the relationship among the seven model-based population, we selected the typical accessions ($Q\geqslant 0.95$ where Q is the probability that the inferred ancestry derived from one of the model-based populations) from every population (Table 1). The genetic distance, F_{st} and average standardized molecular weight were significantly correlated (r=0.77, P<0.001; r=0.63, P<0.01; r=0.97, P<0.000001) between primary populations and typical populations. The overall AMOVA indicated that the genetic diversity (24.6%) among typical populations was higher than that (17.5%) among primary populations. E-M.*Ind* showed the smaller genetic distance with M-L.*Ind*. L.*Jap*I showed the smaller genetic distance with L.*Jap*II. The genetic distance between *Jap*I and U.*Jap*II was also smaller. The groups of *japonica* subspecies showed greater genetic distance with the groups of *indica* subspecies than with groups of *japonica*. Within the *japonica* populations, L.*Jap*II showed smaller genetic distance with groups of *indica*. While within the *indica* populations, M-L.*Ind* showed smaller genetic distance with groups of *japonica*. The Intermediate showed smaller genetic distance with four *japonica* groups than with two *indica* groups, it indicated that the Intermediate was *japonica*-like.

We calculated the average standardized molecular weight in each model-based population to detect their directional evolution. Results were POP4 > POP1 > POP2 > POP3 > POP6 > POP5 > POP7. T-test (Table 2) showed that *japonica* is significantly smaller in molecular weight than *indica*, M-L. *indica* was significantly smaller than E-M. *indica*, Upland rice was significantly smaller than lowland rice in *japonica*. The Intermediate type was larger than *japonica*, but smaller than *indica*.

2.2 Genetic diversity of cultivated rice (*O. sativa* L.) in Guizhou

There were 142 phenotypic variations (4.58 on aver-

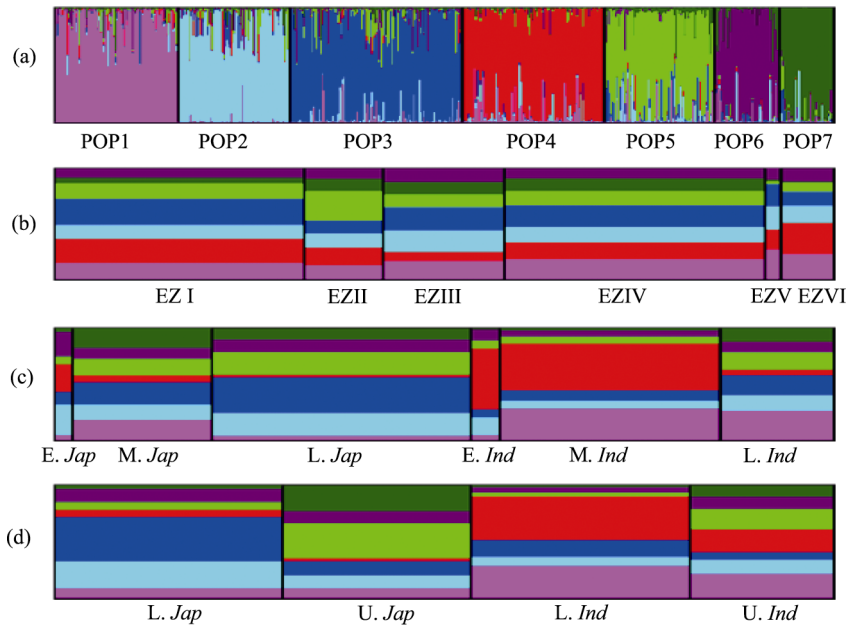

Figure 2 Model-based ancestries and their distribution in predefined populations. (a) Model-based populations; (b)—(d) distribution of model-based populations in predefined populations. L.*Ind*, Lowland *indica*; U.*Ind*, upland *indica*; L.*Jap*, lowland *japonica*; U.*Jap*, upland *japonica* (d); E.*Ind*, early *indica*; M.*Ind*, medium *indica*; L.*Ind*, late *indica*; E.*Jap*, early *japonica*; M.*Jap*, medium *japonica*; L.*Jap*, late *japonica* (c).

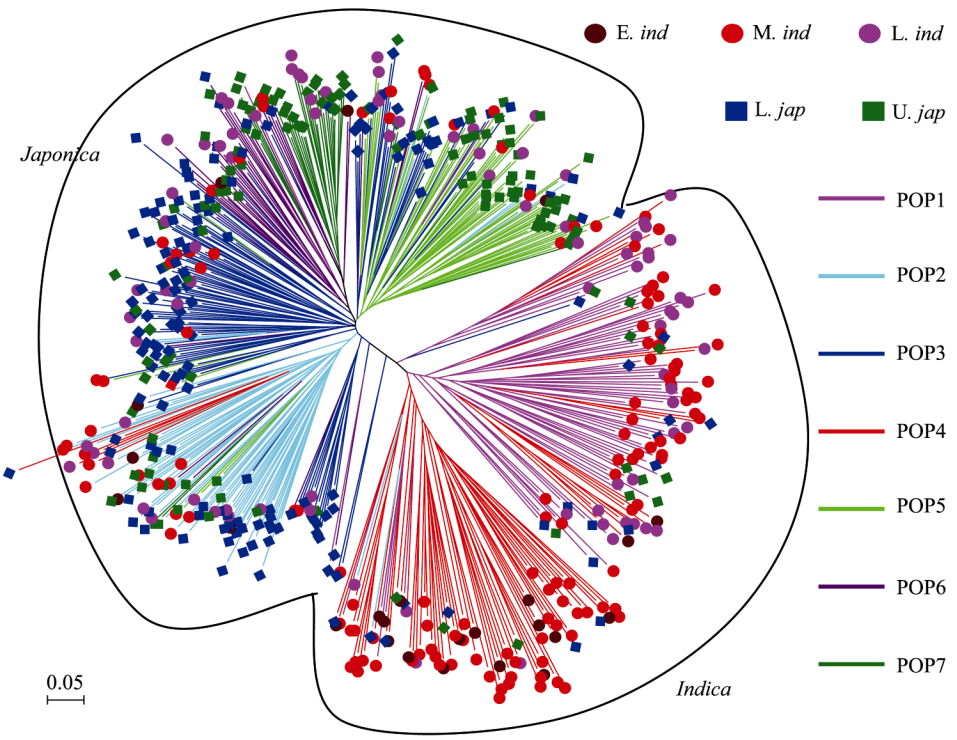

Figure 3 Unrooted neighbor-joining tree of 537 accessions based on Nei's genetic distance[27] based on 36 nuclear SSRs. Here symbols of circumambience were the predefined populations; lines of inside were model-based populations.

age), 129 and 133 of which were found in *indica* and *japonica* , respectively (Table 3). 435 alleles were detected from 36 SSR markers in the population of 537 varieties. The number of alleles per locus varied widely among these markers, ranging from 3 to 22 with an average of 12.1 (Table 3). It was apparent that SSR markers

Table 1 Pairwise F_{st} and Nei's genetic distance among typical groups[a)]

	M-L. *Ind*	E-M. *Ind*	Intermediate	L. *Jap*I	L.*Jap*II	U. *Jap*I	U. *Jap*II
M-L. *Ind*		0.2390	0.4708	0.5352	0.3752	0.4553	0.4900
E-M. *Ind*	0.1168		0.5935	0.5403	0.3823	0.5937	0.6374
Intermediate	0.3106	0.3774		0.3687	0.2521	0.3853	0.2995
L. *Jap*I	0.2975	0.3985	0.2137		0.2436	0.3980	0.4342
L.*Jap*II	0.2446	0.2491	0.1679	0.1209		0.2911	0.3497
U. *Jap*I	0.3255	0.3979	0.2133	0.2457	0.2010		0.2495
U. *Jap*II	0.3498	0.4203	0.1886	0.3023	0.2725	0.1736	

a) Nei' genetic distance estimates appear above the diagonal and Pairwise F_{st} appear below the diagonal.

Table 2 *T*-test of the average standardized molecular weight among typical groups[a)]

	M-L. *Ind*	E-M. *Ind*	Intermediate	L. *Jap*I	L.*Jap*II	U. *Jap*I	U. *Jap*II
M-L. *Ind*							
E-M. *Ind*	4.88**						
Intermediate	3.86**	7.25**					
L. *Jap*I	1.10^{NS}	5.11**	2.39*				
L.*Jap*II	3.26**	7.30**	0.39^{NS}	1.81^{NS}			
U. *Jap*I	4.80**	7.66**	1.58^{NS}	3.32**	1.67^{NS}		
U. *Jap*II	6.03**	9.63**	1.96^{NS}	4.17**	2.13*	0.02^{NS}	

a) NS, Not significant ($P > 0.05$). *, $P< 0.05$. **, $P< 0.01$.

Table 3 Genetic diversity of the cultivated rice at phenotypic traits and SSRs[a)]

Populations	Sample size	Phenotypic traits		SSR		
		N_v	Shannon-Weiner index	N_a	N_{sa}	Nei's index
Japonica	288	133	0.6912	407	42	0.6167
Indica	249	129	0.6891	391	29	0.6960
Total	537	156	0.7178	435		0.6960

a) N_v, Number of phenotypic variations; N_a, number of alleles; N_{sa}, number of specific alleles in predefined population.

showed much more polymorphism than phenotypic markers. The genetic diversity of *japonica* was higher than that of *indica* at the phenotypic level but lower at SSR level.

The genetic differentiation coefficients between the two subspecies for phenotypic traits varied from 0.0001 to 0.2297, with the average G_{st}=0.0320, higher than that (0.0258, 0.0013－0.0868) estimated from SSR markers. The G_{st} of only 6 loci, RM71, RM267, RM25, RM296, RM262 and RM270, were higher than 0.05. This indicates that the differentiation between *indica* and *japonica* subspecies was low at both phenotypic and DNA levels.

The differentiation between *indica* and *japonica* subspecies at the DNA level was similar to that at the phenotypic level within all zones except the South and Northwest zones (IV and VI); within these zones the inter-subspecific differentiation at the DNA level was distinctly lower than that at the phenotypic level (Figure 4). The two zones (I and III) along with the zone upstream of the Wujiang River revealed lower differentiation at both the DNA and phenotypic levels. In general, the differentiation in south Guizhou was lower than that in north Guizhou at the DNA level; the differentiation at the phenotypic level, however, was lower within the zones along with the Wujiang River.

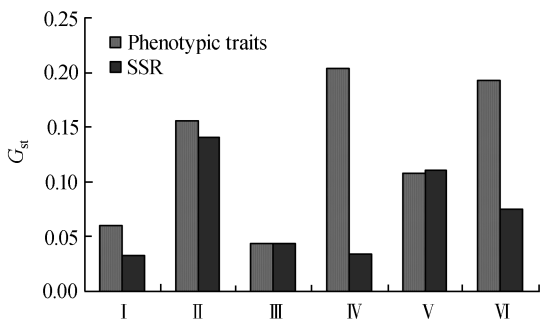

Figure 4 Genetic differentiations between *indica* and *japonica* within each rice ecological zone. Code of ecological zones is the same with Figure 1.

2.3 Geographic differentiation of cultivated rice in Guizhou

In Guizhou, rice-growing areas were divided into six ecological zones based on altitude, temperature, rainfall, sunlight and soil (Figure 1)[32]. Table 4 shows the geographic distribution of the alleles and genetic diversity at phenotypic traits and SSR markers.

The correlation between the number of variations at the phenotypic level and that at the SSR level was significant (r=0.98, P<0.001) in each ecological zone, but the correlation between the genetic diversity at the phenotypic level and that at the SSR level was not significant (r=0.22, P>0.05). For phenotypic traits the genetic diversity in the Middle (I) and the East (IV) of Guizhou was significantly higher than that in others regions. For SSR markers, the genetic diversity in the East, the Center and South-west of Guizhou was significantly higher than that in others.

We analyzed the genetic diversity of 9 districts at the phenotypic level and the SSR level (Table 5). The correlation of number of variations between at phenotypic level and at SSR level and the correlation of genetic diversity between two levels were significant (r=0.95, P<0.001; r=0.77, P<0.05). This indicated that the much better consistence between DNA and phenotypic levels could be reached when accessions were grouped according to districts. The southwest Buyi-Miao district preserved the most number of accessions and the largest variation at both levels, accounting for 85% and 86% respectively, and had the highest genetic diversity. The south Buyi-Miao district, east Miao-Dong district and Guiyang were the next three districts with higher genetic diversity. The other districts, northern and northwestern Guizhou, had lower genetic diversity. That is, the genetic diversity of cultivated rice in Guizhou showed a geographical decrease from southwest to north. Figure 5 shows that genetic distance between districts were sig-

Table 4 Genetic diversity of cultivars in six rice ecologic zones of Guizhou[a)]

Code of ecological zones	Sample zize	Phenotypic traits		SSR		
		N_v	Shannon-Weiner index	N_a	N_{sa}	Nei's index
I	166	145	0.7157	376	22	0.6754
II	50	110	0.5773	290	6	0.6790
III	93	127	0.6472	310	10	0.6681
IV	182	144	0.6888	360	20	0.6350
V	11	81	0.4400	175	0	0.6394
VI	35	108	0.6314	240	3	0.6314

a) N_v, Number of phenotypic variations; N_a, number of alleles; N_{sa}, number of specific alleles in predefined population.

Table 5 Genetic diversity of cultivars in nine districts of Guizhou[a)]

District	Number of accessions		Phenotypic traits		SSR	
	Total	Sample	N_v	Shannon-Weiner index	N_a	Nei' s index
Anshun	265	41	100	0.5991	252	0.6191
Bijie	362	25	100	0.6405	211	0.6130
Guiyang	273	32	108	0.6603	259	0.6660
Liupanshui	121	16	83	0.5596	191	0.6119
Southeast Miao-Dong district	794	81	120	0.6680	320	0.6621
South Buyi-Miao district	712	71	123	0.6744	298	0.6634
Southwest Buyi-Miao district	1215	222	133	0.7074	374	0.6733
Tongren	364	15	79	0.5019	189	0.6221
Zunyi	528	34	97	0.5700	259	0.6362

a) N_v, Number of phenotypic variations; N_a, number of alleles.

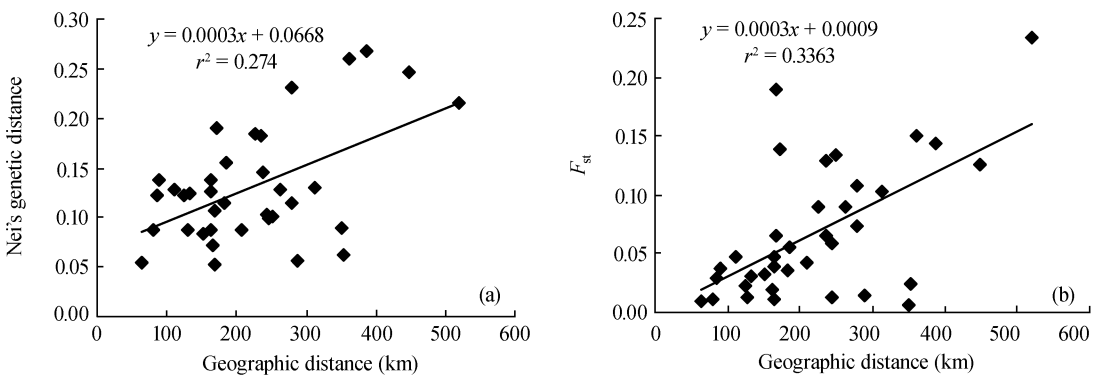

Figure 5 Regression between genetic estimators and geographic estimators. (a) Regression between Nei's genetic distance and geographic distance; (b) regression between F_{st} and geographic distance.

nificantly correlated with geographic distance. This suggests that the environmental differences has driven genetic differentiation among districts.

3 Discussion

3.1 Comparing the genetic diversity revealed by SSRs and phenotypic traits

The southwest of China is one of the largest centers of genetic diversity of cultivated rice in China and the world and is the center of origin of Asian *O. sativa* L.[33,34]. Tang et al.[35] analyzed the genetic diversity of cultivated rice in China using isozymes and their results suggested that the Southwest of China harbored the highest genetic diversity. We analyzed 4310 accessions of *O. sativa* L. using the same 36 SSR loci as those in this study and the results showed that the genetic diversity in Guizhou and Yunnan were high and the difference of genetic diversity between two provinces was not statistical significant (unpublished). Guizhou possesses a very large gene pool of rice germplasm resources with 5548 accessions including 4238 accessions of landrace/local rice varieties, 743 accessions of upland rice, 396 accessions of HE resources and 171 accessions of cultivars. Many accessions were characterized by elite variations such as faster growth, higher yields, pest and disease resistance, larger seeds and better quality, but the percentage of utilization in whole rice resources was low. This study of genetic structure and diversity provides valuable information for rice breeding, germplasm collection and management and gene exploitation of cultivated rice.

Our study revealed that SSRs were more suitable for assessing genetic diversity than phenotypic traits due to their ability to detect significantly higher polymorphism and codominance. SSRs estimate variation at the DNA level whereas phenotypic traits are controlled by the interaction between DNA and the environment. In this study the correlation coefficients of genetic diversity between the DNA and phenotypic levels were not statistically significant when the accessions were grouped into rice subspecies or ecological zones. However, the correlation of genetic diversity between two levels was showed statistically significant when the accessions were grouped into districts. These showed that the consistence between genetic diversity of phenotypic traits and genetic diversity of SSR markers was possibly influenced by groups. In a word, the genetic diversity of DNA level could not be always truly reflected by that of phenotypic level.

3.2 Genetic structure and intraspecific differentiation of cultivated rice in Guizhou

Classification of cultivated rice has been previously documented[36,37]. But these taxonomic systems were deduced based on phenotypic traits and experiences of breeder and thus were somewhat influenced by the environment or subjectivity. Recently, the analysis of genetic structure[38−40] using DNA markers and new statistical methods has become a popular topic[25,26]. Because the southwest of China is one of the largest centers of genetic diversity of cultivated rice in China and the world, an analysis of the genetic structure of cultivated rice in Guizhou is important. In the present study, *O. sativa* L. showed significant differentiation between *indica* and *japonica*. Within *indica* or *japonica* populations, however, the sub-structure was different from Ding Ying's taxonomic system[36]. The differentiation between soil-watery ecotypes in *japonica* was clearer than that in *indica*, but differentiation among seasonal ecotypes in *indica* was clearer than that in *japonica*. These results indicate that different selection pressures influenced the formation of sub-structure in the two subspecies. The complicated cropping system and ample photothermal resources in a tropical environment caused distinct seasonal ecotypes in *indica*. In contrast, the simple cropping system and restricted soil-watery condition in a temperate environment caused distinct soil-watery ecotypes and less clear differentiation of seasonal ecotypes in *japonica*. The results were consistent with our other results (Genome, in press) using rice landraces in Yunnan, another Chinese province adjacent Guizhou. Of course, further research using materials from other geographic areas and including wild rice should be done to further test our conclusions. The smaller average standardized allele size in *japonica* than that in *indica* indicates that *japonica* may be more primitive than *indica*. Similar to our other results (Genome, in press) using rice landraces in Yunnan, the upland rice was more primitive than the lowland rice in *japonica* and the late rice was more primitive than early rice in *indica*.

The accession membership based on the model-based genetic structure was consistent with that based on the reconstructed neighbor-joining tree, but different from that based on phenotypic traits. Whether all 537 accessions were used or typical accessions were used, the

number of accessions classified into *indica* by phenotype but *japonica* by genetic structure was more than those classified into *japonica* by phenotype but *indica* by genetic structure. This phenomenon is partly due to the low differentiation as well as the multifarious phenotypes ecological types (such as Nuda and upland rice).derived from the complicated environment in Guizhou. In addition, the grain length and grain shape were generally used to distinguish the two subspecies. *Japonica* with long grain was easily classified as *indica*. In this study, upland rice accounted for 67% of accessions misclassified between the two subspecies using different types of data. This further suggested that upland rice is more primitive. The inconsistence in classification of subspecies between the phenotypic level and SSR level indicated that the differentiation or evolution was asynchronous between phenotype and DNA.

The deep genetic structure in rice may also be an effect of the autogamous breeding system. In self-pollinated species, one would predict a greater partitioning of diversity among rather than within populations in the absence of human-mediated gene flow between populations via breeding. In our current research, the genetic diversity attributable to differences between groups accounted for only 17.5% of the variation, much less than results obtained by Garris et al. (37.5%)[38]. And the high ratio (64%) of accessions with multiple ancestries also revealed a lower genetic differentiation among groups. It could be explained as followed. On one side, the materials of this research are geographically distant, so gene flow was more frequently occurred among districts or groups due to the variety introduction among districts or groups. On another side, the introduced materials may suffer the selection of the local environment, and thus formed the similar genetic structure. These phenomena are supported by the results in the current research, for example, the genetic structure was not consistent with the geographic distribution. In summary, several factors including the autogamous property of cultivated rice, human activity and the local environment resulted in the lower differentiation and the lower genetic diversity among model-based populations in *O. sativa* L. of Guizhou.

3.3 Center of genetic diversity of cultivated rice in Guizhou

Guizhou Province is located at the east slope of the Yunnan-Guizhou Plateau. It covers a subtropical mountainous area between the Sichuan Basin and the Guangxi Hill, north latitude 24°37′—29°13′ and east longitude 103°36′—109°35′. Due to the complicated environment and numerous folk-customs, multifarious phenotypes and ecological types were accumulated in Guizhou cultivated rice. Though no wild rice was found in Guizhou, analysis of *O. sativa* L. from the whole of China using the same primers showed that Guizhou had the smallest genetic distance from Yunnan (unpublished) where large area of wild rice ever found. How does the genetic diversity appear among different ecological zones or districts?

The correlation between genetic diversity estimated by DNA and that by phenotype was not significant among ecological zones but significant among districts. This indicated that the rice in each district shared a more similar environment and genetic structure than that in each ecological zone, and thus should be treated as a deme. Compared with other districts, the southwest Buyi-Miao district adjacent to Yunnan had the highest genetic diversity, the lowest differentiation between two subspecies and the most number and types of rice resources. Thus, this district appears to be the center of genetic diversity of Guizhou *O. sativa* L. Similar to the geographic distribution of genetic diversity in Yunnan Province (Genome, accepted), the genetic diversity of cultivated rice in Guizhou showed a geographical decline from the center of genetic diversity to north. It is of interest to study the geographic distribution of genetic diversity of the rice from both provinces.

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