Table 3. A comparison of testa variegation of F_2 plants with segregation for nodulation in the F_3 and chi-square tests for goodness-of-fit to the expected ratio of parental, crossover, and two-crossover types with the appropriate crossover percentage

Cross[a]	F_2 testa phenotype	F_3 segregation ratios (nod:nonnod)[b]			Crossover types[b]			Chi-square test		
		1:0	3:1	0:1	NC	CO	TCO	df	χ^2	*P*
UF 487A × M4–2	V	0 (TCO)	12 (CO)	112 (NC)	O 305	41	1			
	T	8 (CO)	127 (NC)	12 (CO)	E 299	46	2	2	1.16	.56
	S	66 (NC)	9 (CO)	1 (TCO)	(7.1% CO)					

[a] Also includes reciprocal of cross shown.

[b] Number of families.

V = variegated; T = trace-v; S = solid; NC = noncrossover; CO = crossover; TCO = two-crossover; O = observed; E = expected.

ed mean was 7.1% for UF 487A and M4-2. This value was then used as the best estimate of the crossover percentage when calculating the expected value to be used in the chi-square test. The results obtained from F_3 plants, which were in F_2 families that were segregating for nodulation and testa variegation, are presented in Table 2. Chi-square analyses of the frequencies of the F_2 families (F_3 data) indicated no significant differences from the expected frequencies, figuring the indicated crossover percentage. The F_3 data thus supported the F_2 findings for linkage (Table 1).

The observed frequencies in the F_1BC_1 generation were not significantly different from the expected frequencies calculated with the indicated crossover percentage ($P = .38$). F_2 plants are classified as noncrossover, crossover, or two crossover types by comparing the F_2 plant's testa variegation with the segregation for nodulation in the F_3 (Table 3). When the number of noncrossover, crossover, and two crossover F_2 plants were compared with the expected numbers, assuming the appropriate crossover percentage, no significant difference was detected.

A comparison of testa variegation of F_1BC_1 plants with segregation for nodulation in the F_2BC_1 was performed with F_1BC_1 plants being classified as noncrossover or crossover. There is no two-crossover classification for F_1BC_1 plants because a crossover can be detected only if it occurs in gametogenesis of the hybrid parent. No significant difference was detected when the number of noncrossover and crossover F_1BC_1 plants were compared with the expected values ($P = .80$).

Results from the F_2, F_3, F_1BC_1, and F_2BC_1 generations have shown that the N_I and *V* loci are linked. The recombination frequency between N_I and *V* was 7.1% for the parental combination UF 487A and M4-2. This is the strongest linkage thus far reported in peanuts.

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High Levels of Genetic Differentiation of *Oryza officinalis* Wall. ex Watt. From China

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In order to determine the population genetic structure of wild rice (*Oryza officinalis* Wall. ex Watt.), an endangered tropical and subtropical species, allozyme diversity encoded by 24 loci was analyzed electrophoretically in 145 individuals of eight natural populations from Hainan, Guangxi, and Yunnan provinces, China. A fairly high genetic differentiation (F_{ST} = 0.882 and mean I = 0.786) was found among the studied populations. Our results suggest that restricted gene flow may play a significant role in shaping such a population genetic structure. In addition, high genetic differentiation among populations within a geographically limited region may stem from a reduced population size and consequent genetic drift.

The evolutionary dynamics of a species in natural conditions are mediated by the genetic structure of their populations (Wright 1977, 1988). The nonrandom distribution of genetic variation within and among populations is often called the genetic structure of populations (Loveless and Hamrick 1984), which is primarily affected by selection, genetic drift, recolonization, and gene flow. Thus studies of population genetic structure can be crucial for assessing the actions and interactions of these evolutionary forces in natural populations (Selander and Whittman 1983). A large amount of literature on the genetic structure of plant populations has been accumulated in recent decades (Clegg and Allard 1972; Dewey and Heywood 1988; Hamrick 1989; Schaal and Smith 1980; Soltis and Soltis 1988), but further studies, particularly on tropical and subtropical plants, are needed in order to

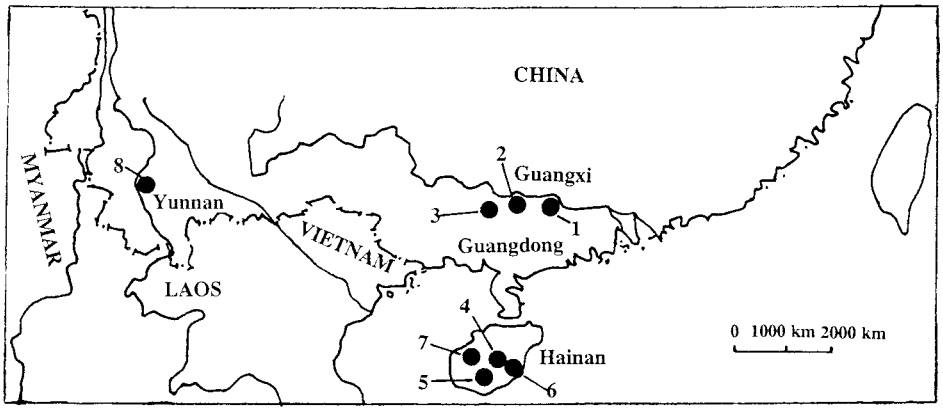

Figure 1. Geographical localities of eight populations of *O. officinalis* sampled in China.

outline a clear picture of how genetic structure is shaped.

Because *O. officinalis* Wall. ex Watt. has potentially useful genes for higher biomass production, resistance to several insects (Heinriches et al. 1985; Jena and Khush 1990), and tolerance to shade and dry land, it is one of the most important genetic resources for the improvement of cultivated rice in the world. As a wind-pollinated herbaceous species, *O. officinalis* is widely distributed in tropical and subtropical regions in the world (Vaughan 1989, 1994). In China, the species is found in southern and southwestern regions: Guangxi, Guangdong, Hainan, and Yunnan provinces (National Exploration Group of Wild Rices 1984). However, our recent field investigation suggests that a great number of populations have disappeared and the species is being seriously threatened (Gao et al. 1996; Hong 1995). As a species of one of our most precious genetic resources, but a little understood one with respect to genetic diversity (Vaughan 1989), it is of great importance to learn about its population genetic structure.

Starch-gel electrophoresis provides biologists with valuable genetic markers that are suitable for the study of population genetics and evolutionary processes (Hamrick 1989), and thus it has been successfully used to study the genetic structure of natural populations (Soltis and Soltis 1991; Soltis et al. 1992). In the present study, allozyme analyses were conducted to explore population genetic structure and explain the observed spatial patterns of genetic variability in terms of evolutionary factors.

Materials and Methods

Eight populations of *O. officinalis* were sampled from Hainan, Guangxi, and Yunnan provinces in China in October 1994 (Figure 1 and Table 1). Because *O. officinalis* has clonal ability in the populations sampled, care was taken to prevent collecting multiple samples from a single genetic individual. Individual live ratoons were randomly collected at intervals of at least 1 m in the field, numbered, transplanted to pots, and maintained at the Xishuangbanna Tropical Botanical Garden (Mengla County, Yunnan) and South China Botanical Garden (Guangzhou City). Young leaves were individually collected in March 1995, stored in plastic bags on ice, and transported to the laboratory by airplane. For each individual, 0.05 g of fresh young leaf material was crushed in 100 μl of Tris-HCl buffer (pH 7.5; see Soltis et al. 1983). The extract was absorbed into 3 mm × 8 mm paper wicks and stored at −70°C until electrophoresis was conducted.

Table 1. The sample sizes and localities of eight populations of *O. officinalis* from China

Population no.	Population locality	Sample size
1	Xiamawu, Wuzhou County, Guangxi	26
2	Yingshuan, Tengxian County, Guangxi	24
3	Fudian, Wuzhou City, Guangxi	19
4	Luokuai, Baoting County, Hainan	15
5	Gaofeng, Sanya City, Hainan	16
6	Chongpo, Ledong County, Hainan	15
7	Wuolan, Baisa County, Hainan	14
8	Langla, Simao City, Yunnan	16

Starch-Gel Electrophoresis

Fourteen enzymes were resolved and scored using starch-gel electrophoresis. The electrophoretic methods followed Glaszmann et al. (1988) and Soltis et al. (1983) with 12% starch gels. A modification of buffer system 1 (S1) was used to resolve malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), and phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) (the electrode buffer was diluted two times before use); aspartate aminotransferase (AAT, EC 2.6.1.1), diaphorase (DIA, EC 1.6.2.2), aminopeptidase (LAP, EC 3.4.11.1), phosphogluco isomerase (PGI, EC 5.3.1.9), and triosephosphate isomerase (TPI, EC 5.3.1.1) were resolved on buffer system 6 (S6); alcohol dehydrogenase (ADH, EC 1.1.1.1), fructose-bisphosphate aldolase (FBA, EC 4.1.2.13), glutamate dehydrogenase (G3PDH, EC 1.4.1.2), isocitrate dehydrogenase (IDH, EC 1.1.1.42), phosphoglucomutase (PGM, EC 2.7.5.1), and shikimate dehydrogenase (SKD, EC 1.1.1.25) were resolved on buffer system 1 of Glaszmann et al. (1988) (G1). Staining procedures for all enzymes followed Soltis et al. (1983). When more than one isozyme was observed for an enzyme, isozymes were numbered sequentially with the most anodally migrating enzyme designated 1. Allelic variation at a locus was coded alphabetically, with the most anodally migrating allozyme designated a.

Data Analysis

Electrophoretic data were analyzed using the computer program Biosys-1 version 1.7 for the IBM-PC (Swofford and Selander 1989). Data were entered as genotype numbers from which allele frequencies were calculated; genetic similarities between the eight populations were estimated using Nei's (1978) method and the data were used to construct an UPGMA dendrogram. The levels of genetic variability within populations were estimated using four variables: the mean number of alleles per locus (A), percentage of polymorphic loci (P), observed heterozygosity (H_o), and expected heterozygosity (H_e). The deviation from Hardy–Weinberg equilibrium (fixation indices) and F statistics were calculated. Outcrossing rates (t) were estimated using fixation indices (F), and outcrossing rate and fixation index are related by $t = (1 - F)/(1 + F)$ (Weir 1990).

Results

Enzyme electrophoresis resulted in clear staining for 14 enzymes, encoded by 24 putative loci. All the enzymes migrated anodally. *Aat-2, Fba, G3pdh, Idh, Mdh-1, Me-1, Me-2, Pgi-1,* and *Tpi-2* were monomorphic, with all individuals from the eight populations possessing a single enzyme band with identical mobility for each locus, and all the other loci were polymorphic in at least one population. *Aat-1, Aat-3, Adh, Dia-1, Dia-2, Lap-1, Lap-2, Mdh-2, 6Pgd, Pgi-2, Pgm,* and *Tpi-1* each had two alleles, *Mdh-3* and *Pgi-3* each had three alleles, and *Skd* had four alleles. Although two isozymes of PGM are typically present in diploid seed plants (Gottlieb 1982), only one PGM isozyme was observed in *O. officinalis.* Two loci of G3PDH and PGD are typically reported (Second 1982), but only one locus was observed in this study; the banding patterns of *Pgi-2* and *Pgi-3* seemed to be apparent gene duplications.

It is apparent that significant allelic differences existed between Hainan and the other two regions, and the differences came mainly from the presence of 10 specific alleles in Guangxi and Hainan (Table 2). The three populations (populations 1, 2, and 3) from Guangxi had *Aat-3a, Dia-1a, Mdh-2b, Mdh-3c,* and *Skd-c,* while the four populations (populations 4, 5, 6, and 7) from Hainan had *Aat-3b, Dia-1b, Mdh-2a, Mdh-3b,* and *Skd-b.* All populations from Guangxi had *Lap-1b* except population 2, which possessed the same allele (*Lap-1a*) as the populations from other regions. Moreover, there were two specific alleles: the three populations from Guangxi had *Pgm-a,* while the four populations from Hainan had *Pgm-b,* except populations 4 and 5 which had *Pgm-a* with low frequencies of 0.025 and 0.167, respectively. In addition, all the populations except populations 4 and 5, which possessed *Lap-2b, Pgd-a,* and *Tpi-1b* at the frequencies of 0.025 and 0.167, respectively, were monomorphic at *Lap-2, Pgd,* and *Tpi-1.* Of interest, population 8 from Yunnan shared more alleles with the populations from Guangxi (*Aat-3a, Dia-1a, Mdh-2b,* and *Mdh-3c*) than with those from Hainan (*Aat-1a*), which was responsible for the higher genetic identity between the two regions. Two specific alleles were found in population 8: one was *Skd-d* (1.00), which was also found in population 1 from Guangxi at a much lower frequency of 0.438, and the other was *Dia-2a,* which occurred at a rather low frequency of 0.031. Finally, seven alleles (*Adh-a, Adh-b, Pgi-2a, Pgi-2b, Pgi-3a, Pgi-3b,* and *Pgi-3c*) were found among all the populations studied at variable frequencies, which might also contribute to genetic differentiation among populations to a certain extent.

Table 2. Allele frequencies for polymorphic loci in the eight populations of *O. officinalis*

	Population							
Locus	1	2	3	4	5	6	7	8
Aat-1								
a	.000	1.000	.000	1.000	1.000	1.000	1.000	1.000
b	1.000	.000	1.000	.000	.000	.000	.000	.000
Aat-3								
a	1.000	1.000	1.000	.000	.000	.000	.000	1.000
b	.000	.000	.000	1.000	1.000	1.000	1.000	1.000
Adh								
a	.875	.000	.000	.000	.500	1.000	1.000	1.000
b	.125	1.000	1.000	1.000	.500	.000	.000	.000
Dia-1								
a	1.000	1.000	1.000	.125	.000	.000	.000	1.000
b	.000	.000	.000	.875	1.000	1.000	1.000	.000
Dia-2								
a	.000	.000	.000	.000	.000	.000	.000	.031
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.969
Lap-1								
a	.000	1.000	.000	1.000	1.000	1.000	1.000	1.000
b	1.000	.000	1.000	.000	.000	.000	.000	.000
Lap-2								
a	1.000	1.000	1.000	.975	.833	1.000	1.000	1.000
b	.000	.000	.000	.025	.167	.000	.000	.000
Mdh-2								
a	.000	.000	.000	1.000	1.000	1.000	1.000	.000
b	1.000	1.000	1.000	.000	.000	.000	.000	1.000
Mdh-3								
a	.000	.000	.000	.025	.167	.000	.000	.000
b	.000	.000	.000	.975	.833	1.000	1.000	.000
c	1.000	1.000	1.000	.000	.000	.000	.000	1.000
Pgd								
a	.000	.000	.000	.025	.167	.000	.000	.000
b	1.000	1.000	1.000	.975	.833	1.000	1.000	1.000
Pgi-2								
a	1.000	.958	1.000	.050	1.000	.250	.000	1.000
b	.000	.042	.000	.950	.000	.750	1.000	.000
Pgi-3								
a	.000	.042	.000	.125	.833	.250	.000	.000
b	.000	.125	1.000	.875	.167	.000	.000	.000
c	1.000	.833	.000	.000	.000	.750	1.000	1.000
Pgm								
a	1.000	1.000	1.000	.025	.167	.000	.000	1.000
b	.000	.000	.000	.975	.833	1.000	1.000	.000
Skd								
a	.000	.000	.000	.025	.167	.000	.000	.000
b	.000	.000	.000	.975	.833	1.000	1.000	.000
c	.563	1.000	1.000	.000	.000	.000	.000	.000
d	.438	.000	.000	.000	.000	.000	.000	1.000
Tpi-1								
a	1.000	1.000	1.000	.975	.833	1.000	1.000	1.000
b	.000	.000	.000	.025	.167	.000	.000	.000

The values of genetic diversity varied among populations (Table 3). It is clear that populations 4 and 5 from Hainan showed relatively higher levels than others, while population 3 from Guangxi and population 7 from Hainan showed the lowest levels of genetic diversity. Moreover, the mean levels of genetic diversity for Hainan were relatively higher than those for Guangxi as well as those for Yunnan. At the species level, genetic diversity was higher than that at the population level.

The above results were further demonstrated by Wright's *F* statistics (Table 4). The high, statistically significant F_{ST} values observed at most of the polymorphic loci indicate a considerable genetic differentiation among populations of *O. officinalis.* The statistically significant average F_{ST} value of 0.882 suggests that 88.20% of the total genetic variation existed among populations. On a regional level, the statistically significant F_{ST} average value from Guangxi (F_{ST} = 0.615) was higher than that from Hainan (F_{ST} = 0.415) (not shown), indicating

Table 3. Genetic variability at 24 loci in eight populations of *O. officinalis*

Population	*A*	*P*[a]	H_o	H_e[b]	*F*
1. Wuzhou Xiamawu	1.1	8.3	.000	.031	1.000
2. Tengxian Yingshu	1.1	8.3	.007	.016	0.334
3. Wuzhou Fudian	1.0	.0	.000	.000	—
Mean for Guangxi	1.07	5.53	.0023	.0157	0.445
4. Baoting Luokuai	1.4	37.5	.013	.038	0.854
5. Sanya Gaofeng	1.3	33.3	.000	.111	1.000
6. Ledong Chongpo	1.1	8.3	.000	.033	1.000
7. Baisa Wuolan	1.0	.0	.000	.000	—
Mean for Hainan	1.20	19.78	.0033	.0455	0.714
8. Simao Langla	1.0	4.2	.003	.003	−.032
Mean for all populations	1.13	12.49	.0029	.0290	0.520
Species level	1.83	62.50		.0266	

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.

[b] Unbiased estimate (see Nei, 1978).

that there existed more genetic differentiation among Guangxi populations than among Hainan populations. F_{IS} was 0.899, suggesting that most of the populations deviated from Hardy–Weinberg expectation within populations and that there was a deficiency of heterozygotes. Table 3 also gives the fixation indices for all the populations studied, suggesting most of the populations deviated from Hardy–Weinberg expectation. In general, the populations studied showed a deficiency of heterozygotes, with a mean fixation index of 0.520. The outcrossing rate (*t*) was also estimated to obtain preliminary insights into the mating system of the species; the populations of *O. officinalis* possessed an average estimated outcrossing rate of 31.6%.

Nei's (1978) unbiased genetic identity values varied between the pairs of populations (data not shown). The mean of all pairwise comparisons of 0.786 suggests a fairly low genetic similarity and corresponds to the high level of F_{ST} observed.

Table 4. Summary of *F* statistics at all polymorphic loci

Locus	F_{IS}	F_{IT}	F_{ST}
Aat-1	—	1.000	1.000***
Aat-3	—	1.000	1.000***
Adh	1.000	1.000	.819***
Dia-1	−.143	.937	.945***
Dia-2	−.032	−.004	.027*
Lap-1	—	1.000	1.000***
Lap-2	1.000	1.000	.127***
Mdh-2	—	1.000	1.000***
Mdh-3	1.000	1.000	.922***
Pgd	1.000	1.000	.127***
Pgi-2	.848	.977	.847***
Pgi-3	.892	.970	.723***
Pgm	1.000	1.000	.918***
Skd	1.000	1.000	.840***
Tpi-1	1.000	1.000	.127***
Mean	.899	.988	.882***

* $P < .5$; *** $P < .001$.

The mean genetic identity values estimated for the regions (Table 5) were somewhat lower than those among populations from both Hainan and Guangxi, indicating that high genetic differentiation in *O. officinalis* occurred between Hainan and the other two regions. The only population from Yunnan had a close genetic relationship with Guangxi despite the great geographic isolation and agreed with the difference of allelic frequencies observed. These results are shown in Figure 2, which not only shows the above relationships, but also indicates that pairs of populations that were geographically closer to each other had higher genetic identities than those separated by greater distances.

Discussion

Levels of genetic variation are highly variable among plant populations (Hamrick 1989; Hamrick and Godt 1989), as is the case in *O. officinalis.* Compared to the mean estimate of allozyme diversity in the herbaceous perennial and wind-pollinated plant species reviewed by Hamrick and Godt (1989), *O. officinalis* possesses low levels of allozyme variation. The levels of genetic diversity in the species are much lower than those reported in *O. rufipogon* Griff. (Barbier 1989b; Second 1985a), and also lower than those in Chinese *O. rufipogon* ($A = 1.3$, $P = 22.7\%$, and $H = 0.068$) according to our recent allozyme survey (Gao et al. 2000a). However, the species possesses a higher amount of genetic diversity than that of *O. glumaepatula* Steud. (Akimoto et al. 1995) and Chinese *O. granulata* Nees et Arn. ex Watt. ($A = 1.09$, $P = 6.33\%$, and $H = 0.016$) (Gao et al. 2000b). The results therefore suggest that *O. officinalis* probably maintains a moderate level of genetic diversity in the genus *Oryza.* It is noteworthy that there exists a fairly high level of genetic differentiation, with the F_{ST} value (0.882) being much higher than the average for short-lived herbaceous perennials and wind-pollinated plants (Hamrick and Godt 1989) as well as gravity-dispersed plants (Loveless and Hamrick 1984). As compared to the other wild rice species studied, the genetic differentiation among populations of *O. officinalis* is not only much higher than that in *O. rufipogon* from Thailand (Barbier 1989b) and China ($F_{ST} = 0.310$) (Gao et al. 2000a), but it also seems somewhat higher than that in *O. granulata* from China ($F_{ST} = 0.859$) (Gao et al. 2000b). The level of mean genetic identity further supports the findings in the present study ($I = 0.786$), which is obviously lower than the mean $I = 0.900$ among populations within a species (Gottlieb 1981) and lower than those for other *Oryza* species studied, such as *O. rufipogon* from China (Gao et al. 2000a; Second 1985b) and Thailand (Barbier 1989a), *O. granulata* from China ($I = 0.901$) (Gao et al. 2000b), and *O. glumaepatula* from the Amazon basin (Akimoto et al. 1995).

The genetic differentiation of *O. officinalis* in this work exists not only between Hainan and the other two regions, but also within a geographically limited region. A possible explanation for the highly regional differentiation between Hainan and the other regions is that large populations might have been on Hainan Island before being isolated, and consequently geographical isolation between them has led to restricted gene flow. For immigrants that possibly arrived on the island before it was totally separated from the mainland, the founder effect may play a small role in its levels of genetic diversity. In addition, the tropical climate of the island might have advantages in maintaining the lower frequencies of genetic bottlenecks

Table 5. Matrix of Nei's (1978) unbiased identity values estimated by regions

Region	Populations	Guangxi	Hainan	Yunnan
Guangxi	3	.892 (.874–.918)		
Hainan	4	.635 (.542–.721)	.938 (.906–.996)	
Yunnan	1	.869 (.791–.915)	.698 (.648–.720)	—[a]

[a] No comparison.

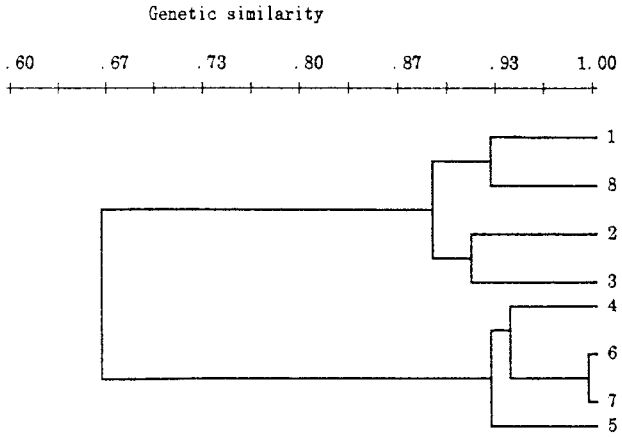

Figure 2. Cluster analysis of eight populations of *O. officinalis* using unweighted pair group method and Nei's (1978) unbiased genetic identity values.

after establishment, and the influence of genetic bottleneck effects on outcrossing species with a large effective population size will be less than in smaller populations. These explanations are supported by the high level of genetic diversity observed within the populations of Hainan in the present study. Great geographical isolation and the channel barrier may have led to fairly restricted gene movement, and thus a great deal of specific alleles were fixed in the populations of different geographical regions and a high genetic differentiation was observed.

High genetic differentiation among populations of *O. officinalis* within a geographically limited region may stem from limited gene flow and genetic drift due to the smaller populations. Taking Guangxi as an example, relatively isolated natural habitats may play an important role in genetic differentiation. Most of the populations of *O. officinalis* grow in humid habitats beside streams in mountain valleys, and the water flow may act as a seed dispersal agent as well as live ratoons. It is possible that gene flow occurs along the same stream, but the populations along different streams are relatively isolated. In addition, although *O. officinalis* was widely distributed in Guangxi and once had a large population (Wu 1981), our recent field survey suggests that many populations of Guangxi have fallen into extinction, and the surviving ones have become fairly small in size and greatly isolated (Gao et al. 1996). The reduction in population size may lead to changes in allelic frequencies due to genetic drift (Ayala 1982), and very small, isolated populations in the respective streams may also reduce the chances of migration among populations. A high genetic identity between Yunnan and Guangxi, despite their distant geographical isolation, may be due to the same gene pool source that was shared by the populations in the two regions, as well as historical migration events.

Among the different causes proposed by Brown (1979), two may be used for the case of *O. officinalis*: selfing and isolation by distance. To explain the similar result that was observed in perennial populations of *O. rufipogon* from Thailand, Morishima and Barbier (1990) proposed that some inbreeding may occur in outcrossing asexual populations because of intraclonal outcrossing events. It is probably the case in *O. officinalis* in this study. In addition to regional geographic isolation, most of the populations studied are greatly isolated due to discrete natural habitats and human destruction (Gao et al. 1996), which has resulted in a deficiency of heterozygotes. Another possible explanation for the high differentiation observed in the present study may be that those lower sample sizes could tend to increase the F_{ST} estimates, since small sample sizes cause more deviant estimates of allele frequencies.

In conclusion, restricted gene flow as well as genetic drift may account for the high genetic differentiation of *O. officinalis* observed in the present study. However, the spatial distribution of genetic variation within plant populations results from the joint action of mutation, migration, selection, and genetic drift (Hamrick 1989). It should be noted that populations from other regions, such as Guangdong Province, as well as other worldwide regions, are not included in the present study. Therefore a full picture of population genetic structure for the species, as well as possible evolutionary causes, can be better outlined if extensive studies on these other populations are completed in the future. Further detailed information on the variation of reproductive and mating systems should also be helpful to explain the population genetic structure of *O. officinalis*. It is certainly a critical issue for the conservation of these wild genetic resources.

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A Radiation Hybrid Mapping Panel for the Rhesus Macaque

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The genomes of nonhuman primates have recently become highly visible candidates for full genome analysis, as they provide powerful models of human disease and a better understanding of the evolution of the human genome. We describe the creation of a 5000 rad radiation hybrid (RH) mapping panel for the rhesus macaque. Duplicate genotypes of 84 microsatellite and coding gene sequence tagged sites from six macaque chromosomes produced an estimated whole genome retention frequency of 0.33. To test the mapping ability of the panel, we constructed RH maps for macaque chromosomes 7 and 9 and compared them to orthologous locus orders in existing human and baboon maps derived from different methodologies. Concordant marker order between all three species maps suggests that the current panel represents a powerful mapping resource for generating high-density comparative maps of the rhesus macaque and other species genomes.

Radiation hybrid (RH) mapping is becoming a powerful tool for whole genome characterization in model organisms. RH maps provide a useful resource for high-resolution comparative mapping, comparative candidate positional cloning, and ultimately a guide to whole genome sequence assembly (Band et al. 2000; Kwitek et al. 2001; Murphy et al. 2000; O'Brien et al. 2001; van de Sluis et al. 2000). There is increasing interest in charting the genomes of nonhuman primates for both their close evolutionary kinship with mankind and the potential that nonhuman primates, especially the rhesus macaque, possess for effectively and accurately modeling human biological phenomena (Dawes 2001; McConkey and Varki 2000; VandeBerg et al. 2000). Here we describe the construction and initial characterization of a rhesus macaque RH panel as a resource for comparative evolutionary inference with other genomes and effective utilization of this species as an animal model. We demonstrate its potential resolving power by producing comparative chromosome maps for two macaque chromosomes and comparison of these to human and baboon maps of homologous chromosomes. These comparative mapping data are useful for elucidating the evolutionary history of human chromosome 14 and 15.

Materials and Methods

The 5000 rad RH panel was constructed by fusing approximately 2×10^7 irradiated cells from a male rhesus macaque fibroblast donor cell line with an equivalent number of cells from the A23 thymidine kinase (TK)-deficient hamster cell line. The origin of the donor fibroblasts was a male rhesus macaque (Mm123–87) housed at the New England Regional Primate Center. The radiation dosage was 5000 rads, similar to what has been used for other domestic species (Band et al. 2000; Murphy et al. 2000). Fusions were plated onto alpha-MEM+20% fetal bovine serum, penicillin, streptomycin, oubain, and HAT supplement, and grown at 37°C. Appropriate controls for the TK selection and irradiation procedures showed no growth 10 days after fusion. Seven to 14 days after fusion, colonies were isolated and grown separately in the wells of 24-well microtiter plates. Ninety-three clones were selected and expanded based on retention of macaque-specific DNA, determined using previously optimized markers (Rogers et al. 2000) and an Inter-Alu PCR assay. DNA harvests yielded an average of 1.9 mg of DNA per hybrid.

Genotyping was performed with 25 ng of hybrid DNA from the 93 cell lines as well as 3 control samples (macaque genomic DNA, hamster [A23] genomic DNA, and a water blank) in a 10 μl reaction volume using Perkin-Elmer dual 384-well 9700 thermal cyclers under the following conditions: 10 min denaturation at 95°C, fol-