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CLONALITY IN WILD RICE (*ORYZA RUFIPOGON*, POACEAE) AND ITS IMPLICATIONS FOR CONSERVATION MANAGEMENT[1]

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Correlations were examined between habitat characters and clonal structures determined by the RAPD (random amplified polymorphic DNA) assay in five populations of *Oryza rufipogon* in China. Nine of 175 decameric primers were used in the study because they reproducibly amplified polymorphisms. The extent of clonality together with the clonal and sexual reproductive strategies varied greatly among the five populations and correlated with the habitats where they occur. The populations under serious disturbance or seasonal drought tended to have small clones with relatively high clonal diversity caused by sexual reproduction, whereas the populations with little disturbance and sufficient supply of water were prone to have large clones with relatively low clonal variation and low sexual reproduction. Therefore, the dynamics of sexual vs. clonal reproduction of this species depended mainly on environmental factors, such as external disturbance and water supply, rather than latitudes indicated by previous study. These results have important implications for in situ conservation of *O. rufipogon.* Adequate external disturbance and water supply control are essential for maintaining high clone diversity of in situ conserved populations. According to the extent of clonality of the populations examined, we recommend that an interval of >12 m should be required for collecting samples for ex situ conservation and for population genetic studies to capture possible genetic diversity for *O. rufipogon* in China.

Key words: clonal diversity; clonal structure; conservation; *Oryza rufipogon*; Poaceae; population; wild rice.

Clonal reproduction is a common phenomenon in plants. Most perennials possess a capacity for asexual reproduction by either vegetative propagation or the formation of asexual seeds (Abrahamson, 1980; Richards, 1986). In most species, asexual reproduction is facultative and mingled with some capacity for sexual reproduction (Stebbins, 1950). Recently, the genetic and evolutionary consequences of clonal species have been addressed frequently (Ellstrand and Roose, 1987; Eckert and Barrett, 1993; Sydes and Peakall, 1998; Esselman et al., 1999), and studies have shown that reproductive mode commonly varied among species and among populations within a species (Ellstrand and Roose, 1987; Barbier, 1989; Eckert and Barrett, 1993; Widen, Cronberg, and Widen, 1994). The occurrence of clonality in rare and threatened plants has several important implications for their conservation (Sipes and Wolf, 1997; Sydes and Peakall, 1998). First, vegetative reproduction in clonal species may lead to difficulties in estimating the actual population size because a population may consist of numerous intermingled ramets belonging to only a few genets or comprise many distinct genets with each genet producing a small cluster of ramets (Ellstrand and Roose, 1987). In such cases, a census based on recognizable individuals would overestimate the number of genets. Consequently, the genetic vulnerability of particularly clonal plants may be undetected and a heavy loss of genetic variation may occur before the endangered status of species is apparent (Sydes and Peakall, 1998). Second, the size and longevity of individual are of great importance because large and/or long-lived individuals may contribute a disproportionate number of offspring, reducing the effective population size (Richards, 1986). Therefore, the genetic structure of a population may be greatly influenced by the largest genet because size and age are frequently correlated in plants (Ayres and Ryan, 1997). Finally, if the extent and patterns of clonality cannot be predicted, the general strategies for in situ conservation or ex situ collections of clonal plants would be inappropriate (Coates, 1988). Furthermore, in self-compatible plants, pollen transfers within clones may increase the level of inbreeding (Peakall and Beattie, 1991). Hence, an understanding of clonality is critical for the implementation of the most appropriate conservation management of threatened clonal plants.

Oryza rufipogon Griff. is a perennial that is widely distributed in the tropics and subtropics of monsoon Asia (Vaughan, 1994). As the progenitor of cultivated rice *O. sativa,* this species has been proven to be a valuable gene pool for rice genetic improvement and thus plays a critical role in rice breeding in the future (Chang, 1984). *Oryza rufipogon* has been found in eight provinces and autonomous regions of China (Gao, 1997). However, our recent field surveys indicated that this species was at the edge of extinction (Xie et al., unpublished data) and would be entirely extinct in China in the next 10–15 yr if no proper strategies were taken to conserve it (Hong, 1995). Therefore, it was listed as a threatened plant in China (Fu, 1992). *Oryza rufipogon* reproduces by both seeds and horizontal stems. In Thailand, it propagates mainly by asexual means (ratooning), with much investment in vegetative growth, and has a high competitive ability (Oka and Morishima, 1967; Sano and Morishima, 1982; Barbier, 1989). Based on our field observations, horizontal stems in this species extended in water or underground for up to 5 m, forming new stems and adventitious roots. In addition, physical con-

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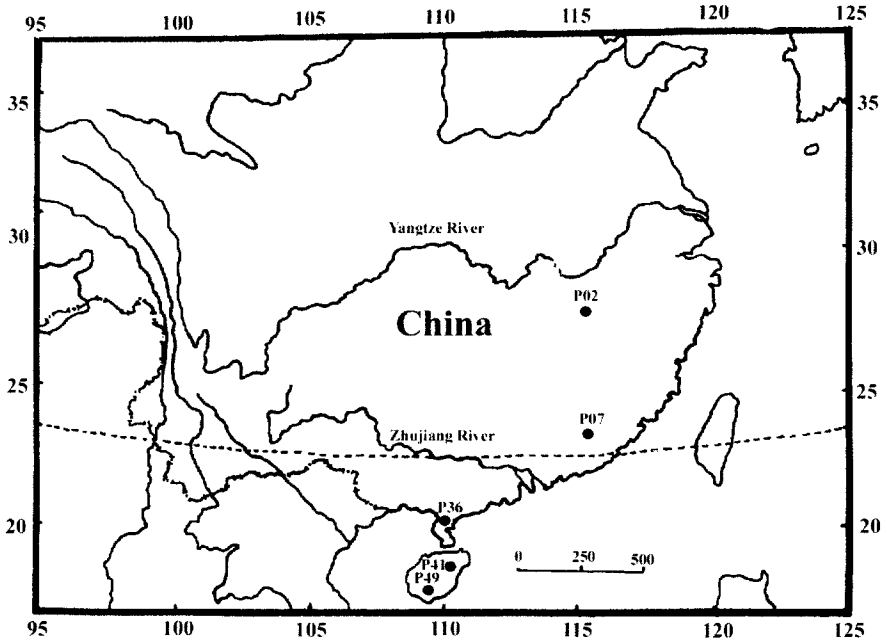

Fig. 1. Map of China showing locations of the five populations studied. Population numbers correspond to those in Table 1.

nections between parent and daughter ramets often decay, thereby obscuring the genetic relatedness of ramets within a clone and leading to intermixed dense stems from different clones. This makes it difficult to estimate clonality in the field. Until now, little has been known about clonality in *Oryza rufipogon.*

The genetic markers developed recently make it possible for more accurate determination of genetic individuals. Of them, allozyme technique is the most commonly used approach (McClintock and Waterway, 1993; Widen, Cronberg, and Widen, 1994; Sipes and Wolf, 1997; Ge, Wang, and Dong, 1999). However, a problem with the application of enzyme electrophoresis for clonal identification is the low number of polymorphic loci available in many studies (Ellstrand and Roose, 1987; Esselman et al., 1999; Wang, Ge, and Dong, 1999). The random amplified polymorphic DNA (RAPD) technique (Williams et al., 1990) has been successfully used for characterizing clones and detecting clonal diversity in plants (Wilde, Waugh, and Powell, 1992; Neuhaus et al., 1993; Hsiao and Rieseberg, 1994; Stiller and Denton, 1995; Waycott, 1995; Van de Ven and McNicol, 1995; Ayres and Ryan, 1997, 1999; Graham et al., 1997; Sydes and Peakall, 1998). In the present paper, the extent of clonality in *O. rufipogon* was investigated using the RAPD technique; the level of clonality was determined in populations subject to different levels of disturbance. We were particularly interested in whether clonal structure varies within and among populations and in the impacts of abiotic and biotic factors on the diversity and pattern of clones in *O. rufipogon.* Such information could then be used to facilitate conservation and management of the genetic resources of this crop.

MATERIALS AND METHODS

Study populations and sampling sategies—During the 1997 field survey, 18–35 individuals were randomly chosen, and their leaves were collected from each of five populations studied according to the population sizes. These populations are typical of *O. rufipogon* in China and located at different ecological sites from the north to south of its distribution area in China (Fig. 1). The Taoshuxia population (P02) in Dongxiang Country, Jiangxi Province, is the most northern population in the entire distribution region of this species. In contrast, the Sanjia population (P49) in Dongfang Country, Hainan Province, is one of the southern marginal populations of this species in China (Gao, 1997). The Jiuqujiang population (P41) is in Qionghai Country, Hainan Province, and the Boluo (P07) and Chengxi (P36) populations are both in Guangdong Province. The Taoshuxia (P02) and Jiuqujiang (P41) populations existed in wetland and marsh habitats, respectively. For these two populations, sampling was made in a grid pattern, usually with an interval of 3 m between adjacent samples. The shape of the grids varied depending on the shape of the patches. In the Boluo (P07), Chengxi (P36), and Sanjia (P49) populations where the plants were distributed along a small river or the shoreline of a pond, leaves were sampled at 3-m intervals along one or two transects. Because the individuals were scattered irregularly in some populations, the adjacent samples were 2–5 m apart within the row and line (Fig. 2).

RAPD analysis—For RAPD analyses, harvested leaves were preserved with silica gel in the fields. The methods for leaf preservation and DNA extraction were described by Xie, Ge, and Hong (1999). In order to select a set of suitable primers, eight template DNAs from population P36, including two samples from one clone and six from different clones based on field observation, were initially screened with 175 10-mer primers (Kit B, Kit T to Kit Z, and Kit J-1-15, Operon Technologies, Alameda, California, USA). A subset of nine primers was selected for further analyses using 130 samples based on the following criteria: (1) strong and reproducible amplified bands; (2) exactly the same RAPD profiles of two samples representing a single clone; and (3) polymorphic fragments across six other samples of unknown genetic background.

The RAPD PCR was run on the Rapidcycler™ (Idaho Technology, Idaho Falls, Idaho, USA) in a reaction volume of 10 μL containing 1.2 μL mol/L primer, 10 ng DNA template, 50 mmol/L Tris-HCl (pH 8.3), 0.5 μg/μL BSA, 2 mmol/L $MgCl_2$, 0.5 units of Tag DNA polymerase, 200 μmol/L dNTPs, 0.75% Ficoll, and 1 mol/L Tartrazine. The initial two amplification cycles were carried out with 1 min at 94°C, 10 s at 35°C, and 20 sec at 72°C. The subsequent 45 cycles were run with the program: 2 s at 94°C, 10 sec at 35°C, and 1 min at 72°C, and a final 4 min extension at 72°C followed. A negative

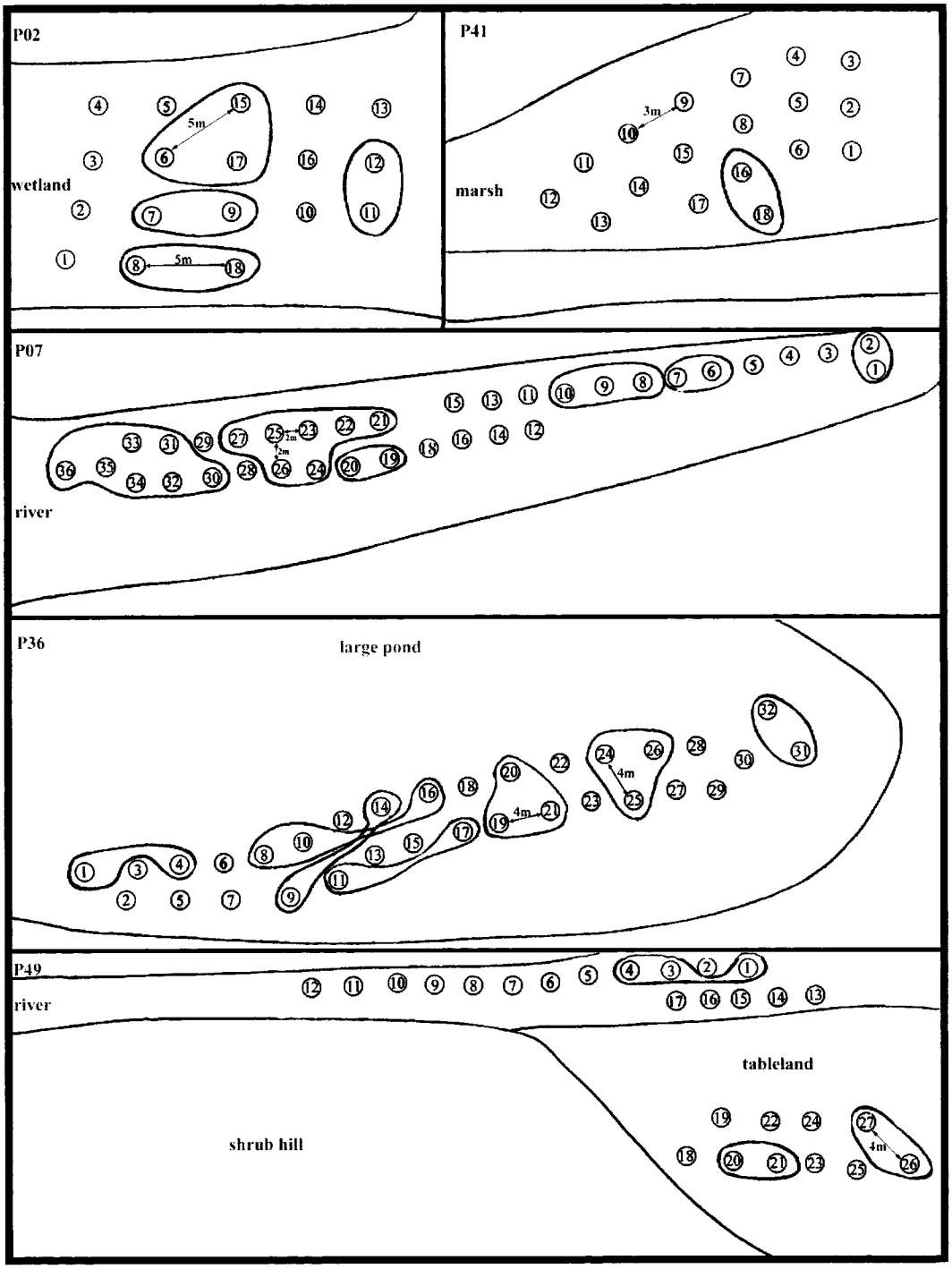

Fig. 2. Maps of clonal structure at the sampling populations. Each circled number represents a sampled individual, and larger circles uniting individuals indicate putative clones. Population numbers correspond to those in Table 1.

control, in which template DNA was omitted, was included with every run in order to verify the absence of contamination. DNA samples were run with each primer at least twice to check the reproducibility of the bands scored for the analysis.

The amplification products were electrophoresed through 1.4% agarose gel (Promega Corporation, Madison, Wisconsin, USA) containing 0.5 μL/mL ethidium bromide and were visualized and photographed on an UV transilluminator. The molecular mass of RAPD fragments was estimated by using a 100 bp DNA Ladder (Pharmacia Biotech, SE-751 84, Uppsala, Sweden).

We defined a clone to be a repeatable combination of polymorphic amplified bands.

Clonal diversity analysis—The mean clone size (N/G) was calculated for each population by dividing sample size (N) by the number of genotypes (G) detected (McClintock and Waterway, 1993). A modified version of the Simpson's diversity index (D) (Pielou, 1969) was calculated for each population as $D = 1 - \Sigma [N_j(N_j - 1)/N(N - 1)]$, where N_j is the number of samples of the jth genotype, and N is the sample size. This index was originally devel-

TABLE 1. Clonal diversity and distributional uniformity in five populations of *Oryza rufipogon.*

Population	N[a]	G[b]	N/G[c]	D[d]	E[e]
P02	18	13	1.38	0.96	0.78
P07	35	17	2.06	0.92	0.80
P36	32	19	1.68	0.96	0.90
P41	18	17	1.06	0.99	0
P49	27	23	1.17	0.99	0.66
Mean		18	1.47 (0.36)[f]	0.96 (0.029)[f]	0.63 (0.36)[f]
Species	130	69	1.88	—	—

[a] Sample size.
[b] The number of genotypes detected.
[c] The mean clone size.
[d,e] $D = 1 - \Sigma [N_J (N_J - 1)/N(N - 1)]$ and $E = (D - D_{min})/(D_{max} - D_{min})$, where $D_{min} = (G - 1)(2N - G)/N(N - 1)$, $D_{max} = (G - 1)N/G(N - 1)$, N_J is the number of samples of the jth genotype, and N is the sample size, G is the number of genotypes.
[f] Standard errors in parentheses.

oped as a measure of species diversity and evenness and has also been employed to measure the clonal diversity within a population (Parker, 1979; Ellstrand and Roose, 1987; Eckert and Barrett, 1993; McClintock and Waterway, 1993; Ge, Wang, and Dong, 1999). Because the D value depends in part on the total number of clones identified in a population, Fager's (1972) E value was also calculated as $E = (D - D_{min})/(D_{max} - D_{min})$, where $D_{min} = (G - 1)(2N - G)/N(N - 1)$, $D_{max} = (G - 1)N/G(N - 1)$. This statistic describes uniformity of distribution of genotypes within a population. The D and E values vary between 0 and 1.

RESULTS AND DISCUSSION

Clonal diversity and the extent of clonality—The mean clone size (N/G) and values for Simpson's diversity index (D) and the uniformity of clonal distribution within the populations (E) are listed in Table 1. Over the five populations, the mean clone sizes ranged from 1.06 to 1.94, and the means of clonal diversity and evenness were 0.96 and 0.62, respectively. A total of 70 genotypes were identified for all the populations using nine selected primers.

The spatial pattern of clones varied greatly among the five populations studied. Population P02 occupied a small and isolated wetland with an area of 300 m^2 and <150 individuals in total. Based on 14 polymorphic loci, a total of 13 clones were revealed in this population. The largest clone consisted of three adjacent samples (6, 15, and 17), and three other multisample clones were composed of two adjacent samples. Each of these multisample clones spread at maximum ~5 m long (Fig. 2: P02). The remaining samples were all distinct clones. According to our field observations, it seemed that horizontal stems of *O. rufipogon* stretched from dry to wet habitats in the dry season, and new ramets might be produced in the areas of deep water. As a result, all the multisample clones were located between shallow and deep water of small puddles. Handel (1985) and Schmid and Harper (1985) indicated that clonal plants could extend by rhizomes to more favorable environments or for water or mineral resources in response to environmental heterogeneity. We infer that there might be ~100 actual genets in the P02 population.

Population P07 was distributed along the river ~100 m long and grew vigorously. Twenty-five polymorphic bands were generated (Table 2). These identified 18 clones among the 35 samples shown in Fig. 3. Two large clones, each consisting of seven samples, were identified along the river. One (samples 20–26) covered ~11 × 4 m^2 area, and the other (samples 29–35) extended an area of ~12 × 5 m^2 along the river. In addition, samples 1 and 2, samples 6 and 7, samples 8, 9, and 10, and samples 18 and 19 belonged to four distinct clones, respectively. Each of these multisample clones comprised two or three neighboring samples, suggesting that the direction of clonal stretch was consistent with that of water flow. Accordingly, water flow may be an important factor that affects clone formation and distribution (Fig. 2: see clone distribution of P07). Twenty-four polymorphic loci were produced to identify clones in population P36. Of 32 samples, 19 clones were detected. Interestingly, mosaic clonal distribution was found in this population. For example, the clone of samples 1 and 4 interspersed with clone 3, while the clone of samples 8, 10, and 16 occurred between clones 12 and 14 (Fig. 2: P36). Furthermore, these mosaic clones extended from 6 m to >12 m in distance. Population P36 existed in a large pond with high density and horizontal stems woven closely. The water level in the pond fluctuated seasonally 1–2 m in different years. In the dry season, the crawling stems or stolons sprawled across other individuals when the water level decreased severely. We observed in the field that buds and roots were generated from the nodal parts of crawling stems or stolons, which further could develop new ramets. Therefore, mosaic pattern of clone distribution observed is not unexpected in this population.

Population P41 was distributed in a marsh adjacent to a river and was frequently disturbed by grazing, mowing, and digging. Therefore, most of the plants were short with few scrambling stems. Sixteen polymorphic bands were amplified and used for recognizing 17 clones from 18 samples (Fig. 2: P41). Samples 16 and 18 belonged to one clone, which was the only one multisample clone in this population. This population was the most disturbed one compared with the other four populations examined. The severe disturbance may result in its high clone diversity. In population P49, plants were scattered in a river and the neighboring tableland. Of 27 samples, 23 clones were identified by 30 polymorphic fragments (Fig. 2: P49). In the river, *O. rufipogon* plants exhibited lush growth with long and scrambling stems. Therefore, one mosaic clone consisting of samples 1, 3, and 4 was identified along the river. In contrast, in the tableland, plants were short and small with slender crawling stems because the water supply was not sufficient from October to April of the next year. That we identified only two small multisample clones (samples 20 and 21 and samples 26 and 27) is reasonable in these disturbed habitats.

Clonal diversity and structure in populations of clonal species vary greatly. Some endangered species, especially those with little or no sexual reproduction, such as *Taraxacum obliquum* (Van Oostrum, Sterk, and Wusman, 1985; Ellstrand and Roose, 1987) and *Haloragodendron lucasii* (Sydes and Peakall, 1998), have only one or a few genets. For several species, the number of genets varies greatly among populations with some populations consisting of only one genet but others of many (Aspinwall and Christian, 1992; Eckert and Barrett, 1993; Ayres and Ryan, 1997). For those species with both asexual and sexual reproductions, populations usually consisted of a number of genets (Ellstrand and Roose, 1987; Eckert and Barrett, 1993; Eriksson and Bremer, 1993; Ayres and Ryan, 1999; Ge, Wang, and Dong, 1999). In the present study, all five populations of *O. rufipogon* sampled comprised multiple genets. This is consistent with the reproductive strategy of *O. rufipogon* (Oka and Morishima, 1967; Gao, 1997). Moreover, clonal structures of four of the five populations

TABLE 2. Polymorphic nonoverlapping fragments useful for distinguishing different clones in the P07 population.

Sample/ Size bp	Primer																										
	B20		V18	Y02				Y20							X18	Z04	Z09			Z10					Z11		
	580	750	440	420	480	580	620	300	320	380	400	510	620	740	350	630	380	700	790	320	360	390	510	740	480		
1	0	1	1	0	1	0	1	0	0	0	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0		
2	0	1	1	0	1	0	1	0	0	0	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0		
3	0	1	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0		
4	1	0	0	1	0	0	1	1	0	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1	0		
5	0	0	0	1	0	0	1	1	0	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1	0		
6	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	1	0	1	1	1	1		
7	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	1	0	1	1	1	1		
8	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0		
9	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0		
10	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0		
11	0	1	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	1	0		
12	0	1	1	0	1	1	1	0	0	0	1	1	0	1	1	0	0	1	0	0	0	1	1	1	0		
13	0	1	1	0	1	1	1	1	0	0	1	1	0	1	1	0	1	1	0	1	0	1	1	1	0		
14	0	0	1	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0		
15	0	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	0	0	0	1	0	1	1	1	1		
16	0	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	0	1	1	1	0		
17	0	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0		
18	0	0	0	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0		
19	0	0	0	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0		
20	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0		
21	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0		
22	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0		
23	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0		
24	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0		
25	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0		
26	0	1	0	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0		
27	0	1	0	1	1	0	0	1	0	1	0	1	0	1	1	0	0	0	1	1	1	1	0	1	0		
28	0	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	0	0	0	1	0	1	1	1	1		
29	0	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0		
30	0	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0		
31	0	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0		
32	0	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0		
33	0	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0		
34	0	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0		
35	0	0	0	1	0	0	1	1	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0		

[a] Presence and absence of fragments are represented as 1 and 0 in the samples.

(with the exception of population P41) were similar to those of the majority of 21 clonal plants summarized in Ellstrand and Roose (1987). That is they were neither dominated by a single genotype nor consisted of numerous genotypes in roughly equivalent frequencies. However, the proportion of distinguishable genotypes (i.e., the number of clones identified divided by the sample size) ranged from 0.51 to 0.94 and was 0.54 across all the five populations in the present study. This value is much higher than the average value (0.17) for clonal populations (Ellstrand and Roose, 1987). The following reasons may apply. First, *O. rufipogon* has a certain degree of sexual reproduction (Ge et al., 1999) and thereby maintains high genetic diversity in wild populations. In contrast, approximately half of the clonal plants described by Ellstrand and Roose (1987) were dominated by asexual reproduction through apogamety or agamospermy or by some mechanism of permanent translocation heterozygotes. Second, RAPD assays can yield abundant polymorphic loci and thus allow more precise identification of clones (Stewart and Porter, 1995; Esselman et al., 1999). In the present study, the numbers of polymorphic loci used for discriminating clones varied from 14 (P02) to 30 (P49), which were higher than the average number of polymorphic loci used for allozymes (Ellstrand and Roose, 1987). Thus, many more clones could be detected for *O. rufipogon* populations by RAPD markers. On the other hand, genotypic diversity (as measured by *D* and *E*) may have been overestimated in natural populations of *O. rufipogon* because of the coarse spatial scale of sampling (~3-m intervals).

Implications for conservation management—*Oryza rufipogon* is a perennial with mixed sexual and asexual reproductive strategies (Barbier, 1989; Gao, 1997). The allocation of asexual vs. sexual reproduction may vary among populations in different habitats (Barbier, 1989). In the present study, population P41 was frequently disturbed, while population P49 grew under harsh conditions such as drought. Thus, it is difficult for them to produce large dominant clones. The high clone diversity and small clone sizes indicated that sexual reproduction might be more important than clonal reproduction for these two populations. Oka and Morishima (1967) and Barbier (1989) also demonstrated that wild rice (*O. rufipogon*) populations growing in disturbed habitats produced many seeds and promoted seed propagation. In contrast, populations P07 and P36 existed in pond or river with sufficient water supply and little disturbance. They grew luxuriantly and formed large clones through stretching of sprawling stems enhanced by water flow or seasonal fluctuation of water level. Consequently, these two populations have relatively low clone diversities. Investment for clonal reproduction might be high in both populations. Gao (1997) studied variation in the breed-

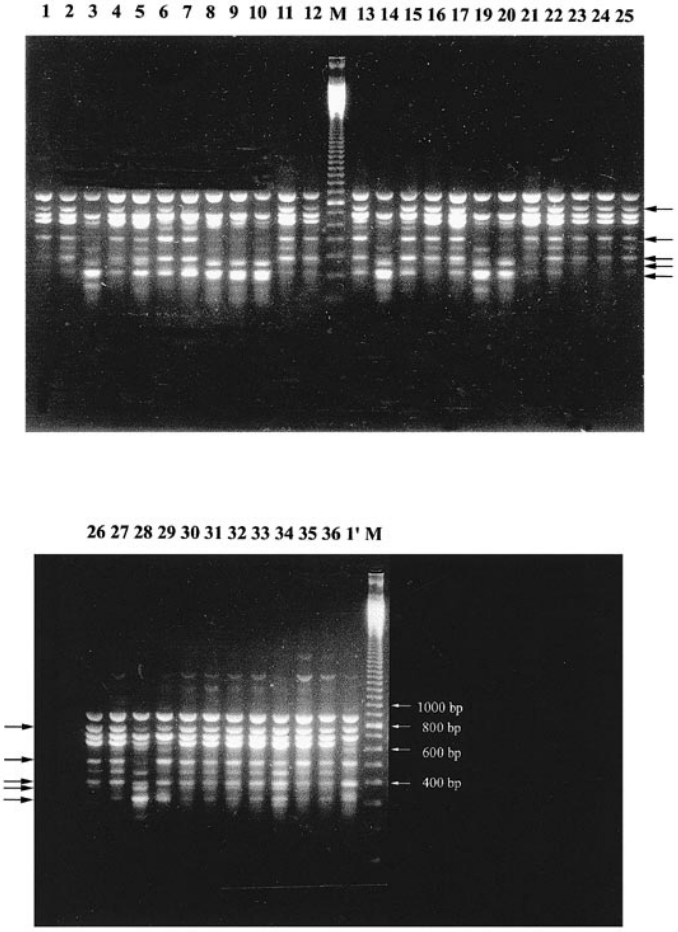

Fig. 3. RAPD profile using the template DNAs of P07 population with the primers of OPZ10. Arrows show the polymorphic bands scored. The numbers 1 to 36 are sampled individuals of P07, while 1′ belongs to another population.

ing system of *O. rufipogon* and found an increased allotment to sexual reproduction over clonal reproduction when going from lower to higher latitudes in China. According to our results, the recruitment of sexual vs. clonal progeny depends mainly on environmental factors, such as external disturbance and water condition, rather than on the latitude. These results have important implication for conservation of *O. rufipogon* in situ. Moderate disturbance and reduction of the water supply, gradually from sufficient to wet and finally to dry, at the stage of vegetative growth (from April to September of a year in China) could be helpful for maintenance of genetic diversity of natural populations of *O. rufipogon.* As indicated by the present study, the extent of clonality varies from 3 to 12 m in the five populations of *O. rufipogon* examined, suggesting that an interval of 12 m or more in sampling would probably result in collection of completely distinct genotypes from a population in most cases. Therefore, we recommend that an interval of ~12 m be used for comparative studies on population genetics in *O. rufipogon* as well as for sampling for ex situ conservation of this species in the future.

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