

Association between *Pox-1* variation and seed productivity potential in wild rice

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Alleles at the *Pox-1* locus are differentially distributed in cultivated rice species and their wild relatives. Seed-propagating taxa are nearly monomorphic with allele 2A, while vegetatively propagating ones carry allele 4A at high frequency. This association between allozyme and life history was consistently found over broad areas at the species level as well as at the population level. A cross between an annual (2A2A) and a perennial (4A4A) strain of Asian wild rice *Oryza rufipogon* was extensively studied to elucidate the genetic mechanism of this association. In the F₃, 2A2A plants showed higher reproductive allocation, shorter plants, earlier flowering, and shorter anthers, which are characteristics of annual life history. When the hybrid population was selected for early flowering, allele 2A increased. When it was naturally grown under conditions favorable for vegetative propagation, allele 4A increased. In the isogenic pairs of 2A vs 4A at the *Pox-1* locus, association between allozyme and characters mostly disappeared. The above results suggest that some of the quantitative trait loci for these life-history traits are clustered on a chromosome segment marked by *Pox-1*, but their linkages are not tight. There must be certain selective interactions between those genes including *Pox-1* to maintain this gene block in nature.

Populations of the Asian common wild rice *Oryza rufipogon* Griff. tend to be differentiated into two ecotypes, annual and perennial (Barbier 1989, Morishima et al 1984, Oka and Morishima 1967). The annual type is characterized by a typical monocarpic life history, high seed productivity, high seed dispersing ability, early flowering, and high selfing rate. In contrast, the perennial type (polycarpic) is characterized by low seed productivity, high regenerating ability, late flowering, and high outcrossing rate. The annuals are found in shallow swamps that are parched in the dry season; the perennials are found in deep swamps that are relatively stable and retain soil moisture in the dry season. The contrasting characteristics and differential habitat preference of the two types represent intraspecific differentiation in adaptive strategy in *O. rufipogon*.

Fitness characters are often associated with each other in the coadapted manner in nature, characterizing the adaptive strategy of taxa. The genetic mechanism of the

development and conservation of such an adaptive syndrome has not been fully explored, although it has long attracted the interest of evolutionists. This is probably because the characters relevant to fitness or adaptation are controlled mostly by multiple genes, which cannot easily be analyzed for their location and organization in the genome. The recent availability of biochemical markers, however, has presented a new way to resolve quantitative traits into Mendelian factors (Kahler and Wehrhahn 1986, Paterson et al 1988, Weller 1987).

In the isozymic study of *O. rufipogon* and its related taxa, it was noted that, among a number of isozyme loci, *Pox-1* seemed to show a unique allele distribution associated with variation in seed vs vegetative propagation. This paper aims to elucidate the mechanism of preserving this association between allozyme and life history in this plant group. I will first present allelic variation at the *Pox-1* locus observed in cultivated rice species and their wild relatives having different propagating systems. Then I will report on the results of a series of experiments with a cross between an annual and a perennial strain of *O. rufipogon*.

Materials and methods

A number of accessions, each representing natural populations belonging to AA genome species, and several experimental populations derived in different ways from a cross, were grown to investigate isozyme and character variations.

Survey of *Pox-1* polymorphism

For wild species (*O. rufipogon*, *O. longistaminata*, *O. meridionalis*, and *O. barthii*), first-generation plants of samples collected in their natural habitats were grown in short-day plots or a greenhouse in Mishima, Japan, and scored for isozymes. For cultivated species (*O. sativa* and *O. glaberrima*), strains preserved at the National Institute of Genetics were used. Five to 30 plants per population of the wild species were examined, and 1-3 plants per accession of the cultivars.

Experiments with an annual/perennial cross

An annual strain (W106 from India) and a perennial strain (W1294 from the Philippines) of *O. rufipogon* were crossed. About 100 F₃ plants were examined for 6 isozymes and various characters on an individual basis. The characters included flowering date, plant height, panicle number, panicle length, awn length, anther length (correlated with outcrossing rate; see Oka and Morishima 1967, Oka and Sano 1981), seed fertility, and reproductive allocation (harvest index). This hybrid population was divided into two groups and grown in bulk during the F₃-F₆, selecting for early- and late-flowering plants, respectively. The *Pox-1* and *Acp-1* genotypes of each individual were determined for F₂, F₃, F₄, and F₆ plants. In addition, material from an experiment carried out in Taiwan, China, by H.I. Oka was also examined for isozymes: F₃ seedlings of the above-mentioned cross were transplanted at four sites in Taiwan and left for natural propagation (Oka 1988). After 3 yr, seed was collected from surviving plants for isozyme assay.

Near-isogenic lines of *Pox-1*

Ten F₂ plants heterozygous for *Pox-1* (2A/4A) were randomly chosen from the same W106/W1294 cross and repeatedly selfed until the F₆, holding this locus heterozygous. The progenies of five F₆ heterozygotes were released for allozyme and character observations. 2A2A and 4A4A plant groups derived from a heterozygous plant thus prepared can be considered as a near-isogenic pair carrying similar genetic background except for a chromosome segment surrounding the *Pox-1* locus.

Isozyme assay

Pox-1 was particularly considered in this study; other loci are briefly mentioned only for comparison. Samples for *Pox-1* assay were prepared from mature leaves at flowering, while for other isozymes mainly plumules were used. Horizontal starch gel electrophoresis (Pai et al 1973, Second 1982) was carried out.

Results

Distribution of *Pox-1* polymorphism in cultivars and their wild relatives

At the *Pox-1* locus, two major alleles—2A (slow band) and 4A (fast band)—and one rare allele—OC—are known (Pai et al 1973). The degree of polymorphism at this locus differs markedly by species. Cultivars of *O. sativa* and *O. glaberrima* are monomorphic, carrying only allele 2A (Table 1). Populations of Asian common wild rice *O. rufipogon*, which propagates both by seed and vegetatively, are highly polymorphic.

Table 1. Allelic frequency and heterozygote frequency at the *Pox-1* locus in wild and cultivated rice species. (Means of intra-population frequencies are given for wild species.)

Species	Population (no.)	Allelic frequency			Heterozygotes (%)
		OC	2A	4A	
<i>Oryza sativa</i>	452 ^a	0.01	0.99		0
<i>O. glaberrima</i>	10		1.00		0
<i>O. rufipogon</i>					
Perennial type	24	0.07	0.47	0.46	20.8
Intermediate type	11	0.17	0.72	0.11	10.2
Annual type	16	0.04	0.96		0.2
Weedy type	9		0.95	0.05	3.9
<i>O. longistaminata</i>	9		0.02	0.98	3.6
<i>O. meridionalis</i>	4	1.00			0
<i>O. barthii</i>	11		0.99	0.01	0.4

^aData of Fu and Pai (1979) are included.

Table 2. Partitioning of gene diversity calculated from 64 populations of *O. rufipogon*.

Locus	H_T^a	H_S^b	D_{ST}^c		
			a	b	c
Pox-1	.402	.152	.151	.075	.024
Acp-1	.506	.228	.173	.006	.079

^aTotal populations. ^bWithin populations. ^cBetween populations. a = between all populations, b = between ecotypes (annual, intermediate, perennial, and weedy types), c = between India and Thailand.

African perennial wild rice *O. longistaminata* preserves allele 4A at high frequency. Australian wild rice *O. meridionalis* (annual) is fixed to allele OC. African annual wild rice *O. barthii* is fixed to 2A, except for a few populations probably introgressed by *O. longistaminata*.

Populations of *O. rufipogon* were classified into perennial, intermediate, and annual ecotypes based on morphological characters, although variation among populations was continuous (Morishima et al 1961). Perennial populations were highly polymorphic, while annual and weedy (grown in ricefields) populations tended to be fixed to 2A (Table 1). To examine whether such a trend is consistently found in different localities, gene diversity between populations, D_{ST} , at the *Pox-1* locus, calculated from 64 populations, was partitioned into population, locality, and ecotype components using the method of Chakraborty et al (1982). The ecotype component was larger than the locality component at *Pox-1*, contrasting with another polymorphic locus, *Acp-1*, in which the locality component was much larger than the ecotype component (Table 2). These facts show that the frequency of 2A is associated with annual habit or seed propagating ability at the species level as well as at the ecotype level over wide areas.

Cosegregation between allozyme and quantitative traits

In the F_3 of a W106 (annual)/W1294 (perennial) cross, 10 quantitative traits and 6 isozymes were examined. Character measurements were compared between two homozygous genotypes of each isozyme locus. As shown in Table 3, the genotype effect of *Pox-1* was significant on a spectrum of characters: 2A homozygotes had higher reproductive allocation, shorter plants, earlier flowering, and shorter anthers—all characteristic of an annual life history—than 4A homozygotes. This suggests that quantitative trait loci for these characters are linked with the *Pox-1* locus. The directions of all these effects were as expected from parental or ecotypic differences. Four other isozyme loci respectively showed linkage with one of the characters, with the effects of expected or unexpected directions from parental means.

Characters examined here are known to be correlated with each other among natural populations of *O. rufipogon*, resulting in ecotypic differentiation within the species (Sano and Morishima 1982). Among the F_3 lines of the W106/W1294 cross, however, most correlations disappeared, suggesting that the character correlations were gener-

Table 3. Association between characters and allozymes in F₃ plants of W106 (annual)/W1294 (perennial).^a

Character	Locus (homozygous allele)					
	<i>Pox-1</i> (2A:4A)	<i>Sdh-1</i> (1:3)	<i>Acp-1</i> (+9:-4)	<i>Amp-2</i> (1:2)	<i>Est-2</i> (1:2)	<i>Pox-2</i> (0:4C)
Flowering time	<	ns	ns	>>	>>	ns
Culm length	<<	ns	ns	ns	ns	ns
Panicle length	ns	ns	ns	ns	ns	ns
Panicle number	ns	ns	ns	ns	ns	ns
Awn length	ns	< ^b	ns	ns	ns	ns
Anther length	<	ns	ns	ns	ns	ns
Regeneration index	ns	ns	< ^b	ns	ns	ns
Self-fertility	ns	ns	ns	ns	ns	ns
Grain weight	ns	ns	ns	ns	ns	ns
Harvest index	>	ns	ns	ns	ns	ns

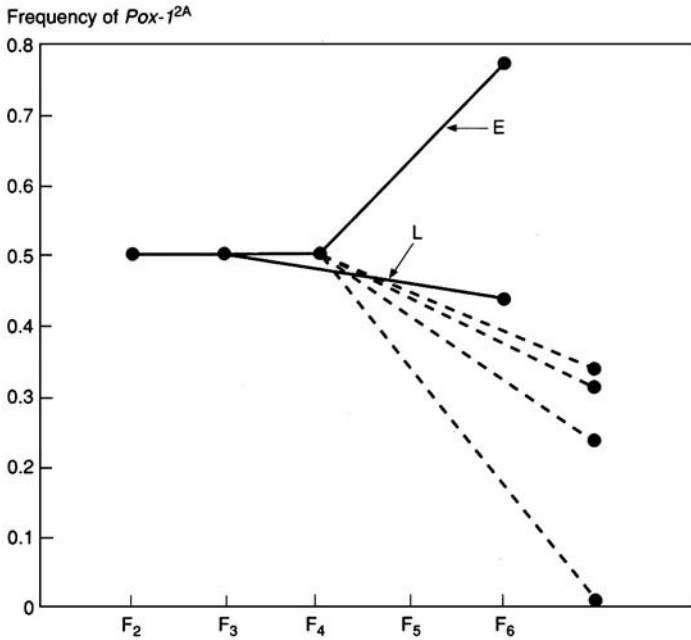
^aSignificant at the 1% (<<,>>) and 5% (<,>) levels. ns = nonsignificant. Direction of caret indicates relationship. For example, flowering time of 4A4A plants is significantly longer than that of 2A2A plants at the 5% level.
^bDirection of association is opposite to parental type.

Table 4. Character correlations among 33 F₃ lines derived from an annual (W106)/perennial (W1294) cross (above diagonal), compared with those found among 57 strains of common wild rice from different Asian countries (below diagonal).^a

Character	DH	CL	PN	AW	SF	GW	RG	AN	RA
Days to heading (DH)		.09	-.29	.21	-.41*	-.10	.09	.55**	-.42*
Culm length (CL)	.20		.13	.10	-.31	.11	.11	.28	-.21
Panicle no./plant (PN)	-.48**	-.28*		.07	.19	.31	.27	-.02	.49**
Awn length (AW)	-.12	-.17	.30*		-.15	.56**	.32	.25	.02
Seed fertility (SF)	-.47**	-.38**	.42**	.51**		-.05	-.23	-.58**	.65**
Single grain weight (GW)	-.35**	.17	.06	.16	.20		.17	.11	.27
Regenerating ability (RG)	.65**	.36**	-.45**	-.12	-.49**	-.14		.26	-.16
Anther length (AN)	.56**	.47**	-.41**	-.16	-.43**	.01	.62**		-.43*
Reproductive allocation (RA)	-.53**	-.32*	.39**	.17	.70**	.18	-.52**	-.61**	

^aSignificant at the 1% (**) and 5% (*) levels.

ated mostly by natural selection during differentiation (Table 4). Yet the following correlations remained in the F₃: high reproductive allocation associated with early flowering, many panicles, high seed fertility, and short anther; short anther associated with early flowering and high seed fertility; and early flowering associated with high seed fertility. This also indicated that a number of quantitative trait loci representing perennial-annual life history are partly linked.



1. Changes in frequencies of allele $Pox-1^{2A}$ observed in differently selected hybrid populations derived from W 106/W1294. Solid line = selected for early (E) and late (L) flowering, dashed line = naturally grown at 4 sites in Taiwan, China.

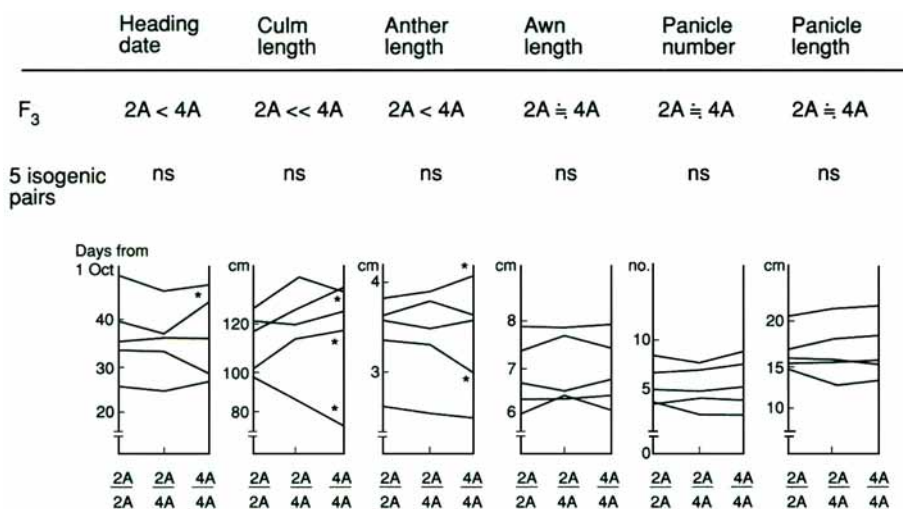
Changes in allelic frequency in bulk populations

Allelic frequency at $Pox-1$ and $Acp-1$ was monitored in two bulk populations that were originally derived from the same cross but selected for early and late flowering, respectively. The frequency of allele 2A increased in the early-selected group during the F_2 - F_6 ; no such trend was observed in the late-selected group (Fig. 1). The allelic frequency at $Acp-1$, which was also segregating in this hybrid population, did not show any significant shift.

On the other hand, in naturally propagated populations in Taiwan, China, a decrease in 2A frequency was consistently observed at 4 sites during 3 yr. These plants were supposed to propagate mainly by vegetative means, seed propagation being unsuccessful under conditions in Taiwan (H.I. Oka, National Institute of Genetics, Japan, pers. comm.). The results of the preceding artificial and natural selection experiments indicated that 2A was selected in seed-propagated populations, particularly when selected for early flowering, while 4A was selected in vegetatively propagated populations.

Near-isogenic lines for $Pox-1$

In five sets of isogenic lines for $Pox-1$, various characters were compared among the 2A2A, 2A4A, and 4A4A plant groups (Fig. 2). No single character showed a consistent



2. Comparison of character measurements among 3 *Pox-1* genotypes (2A2A, 2A4A, and 4A4A) observed in 5 sets of near-isogenic lines. * = significant difference between 2A2A and 4A4A plant groups, ns = nonsignificant, \approx means the character measurements do not differ significantly (or are nearly equal) between 2A homozygotes and 4A homozygotes.

difference between two homozygote groups over all five pairs. This implies that the *Pox-1* locus itself has no direct effect; nor are closely linked genes responsible in so far as the characters examined are concerned. In some pairs, however, allozyme-character associations were preserved: between the 2A2A and 4A4A groups, flowering date was significantly different in one pair, culm length in three pairs, and anther length in two pairs. Irrespective of *Pox-1* genotype, anther length and plant height were correlated in one pair, and flowering date and panicle number were correlated in three pairs. But no correlations were found between members of the former (anther length, plant height) and those of the latter (flowering date, panicle number) groups. Judging from the rate of breakup of initial linkage blocks, the following arrangement could be presumed: anther length - plant height - *Pox-1* - flowering date - panicle number.

Discussion

Evolutionary forces influencing the conservation of multilocus associations or gametic-phase disequilibrium include selection, linkage, inversion, selfing, migration, and drift in a small population (Hedrick et al 1978). In the present study, a cluster of quantitative trait loci for flowering date, reproductive allocation, panicle number, anther length, and plant height was located on a chromosomal segment marked by the *Pox-1* locus. The chromosomal location of the *Pox-1* locus has not been determined. Other isozyme markers used in the F_3 study covered only two linkage groups (6 and 12). Therefore, the gene block detected in the present study might explain only part of natural variation. Yet, the preceding characters proved to be associated in a coadapted

manner in the detected chromosome block, as expected from ecotypic differentiation. The selectional process of clustering of coadapted genes is little known in eukaryotes. Studying a cross between two ecotypes of *Plantago lanceolata*, Wolff (1987) reported that ecologically relevant morphological genes were linked with effects expected and unexpected from parental combinations.

In the near-isogenic lines examined in the present study, the allozyme-character linkages observed in early generations largely disappeared. Selfing for six generations holding a particular locus heterozygous is expected to conserve a heterozygous segment of about 10% of the length of the chromosome on both sides of the selected locus (Hanson 1959). This means that the linkage between *Pox-1* and genes for those characters should not be strong enough to counteract the randomization force by outcrossing and recombination. There should be factors other than linkage to conserve this gene block.

In nature, an association between *Pox-1* and the perennial-annual syndrome was widely found at the species as well as the ecotype level, as mentioned. Even within a population, it was found that a seed-propagating subpopulation was fixed to 2A, while an adjacent subpopulation that propagated vegetatively was polymorphic (Morishima et al 1984). Furthermore, a similar trend of linkage between allozyme and life history traits as found in this study was also observed in other crosses of annual/perennial strains (Barbier 1990). Coadaptive interaction between genes including *Pox-1* seems to be only one plausible explanation for such an association, although whether it is because of the effect of *Pox-1* itself or some other tightly linked genes could not be detected in the present study. In the differentiation process from perennials to annuals, high seed productivity associated with earliness and selfing must have been selected for adaptation to a drier and more disturbed habitat, while, in the domestication process, rice plants must have been selected by nature and by people for high seed productivity as well as for low degree of seed shedding and seed dormancy. Such adaptive differentiation must be a gradual process to accumulate a number of coadapted genes with minor effects all over the genome. The selection of the gene block dealt with in the present study, however, could have played a key role with an appreciable effect, not only in the differentiation of annual types under natural conditions, but also in domestication. In this sense, the two processes are conceptually independent of each other, but partly interrelated in the real world.

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Notes

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