Theor Appl Genet (2002) 104:1217–1228
DOI 10.1007/s00122-001-0819-7

H. W. Cai · H. Morishima

QTL clusters reflect character associations in wild and cultivated rice

Received: 31 July 2000 / Accepted: 8 June 2001 / Published online: 10 April 2002

Abstract The genetic basis of character association related to differentiation found in the primary gene pool of rice was investigated based on the genomic distribution of quantitative trait loci (QTLs). Major evolutionary trends in cultivated rice of Asiatic origin (*Oryza sativa*) and its wild progenitor (*O. rufipogon*) are: (1) differentiation from wild to domesticated types (domestication), (2) ecotype differentiation between the perennial and annual types in wild races, and (3) the Indica versus Japonica type differentiation in cultivated races. Using 125 recombinant inbred lines (RILs) derived from a cross between an Indica cultivar of *O. sativa* and a strain of *O. rufipogon* carrying some Japonica-like characteristics, we mapped 147 markers, mostly RFLPs, on 12 chromosomes. Thirty-seven morphological and physiological quantitative traits were evaluated, and QTLs for 24 traits were detected. The mapped loci showed a tendency to form clusters that are composed of QTLs of the domestication-related traits as well as Indica/Japonica diagnostic traits. QTLs for perennial/annual type differences did not cluster. This cluster phenomenon could be considered "multifactorial linkages" followed by natural selection favoring co-adapted traits. Further, it is possible that the clustering phenomenon is partly due to pleiotropy of some unknown key factor(s) controlling various traits through diverse metabolic pathways. Chromosomal regions where QTL clusters were found coincided with the regions harboring genes or gene blocks where the frequency of cultivar-derived alleles in RILs is higher than expected. This distortion may be partly due to unconscious selection favoring cultivated plant type during the establishment of RILs.

Keywords Rice · QTL analysis · Gene cluster · Domestication · Indica-Japonica differences

Communicated by J. Dvorak

H.W. Cai (✉) · H. Morishima
National Institute of Genetics, Mishima 411-8540, Japan
e-mail: hcai@jfsass.or.jp

Present addresses:
H.W. Cai, Japan Grassland Farming
and Forage Seed Association, Forage Crop Research Institute,
388-5 Higashiakada, Nishinasuno, Tochigi, 329-2742 Japan

H. Morishima, Tokyo University of Agriculture, 1737 Funako,
Atsugi, 243-0034 Japan

Introduction

Crops are genetically similar to their wild progenitors and compatible with them when hybridized. Both wild and domesticated races belong to one biological species sharing a common primary gene pool, though they usually have different species names (Harlan 1975). This holds true in rice. Cultivated rice of Asiatic origin, *Oryza sativa* L., and its wild progenitor, *O. rufipogon* Griff., belong to a single biological species (Oka 1988). Studies on inter- and intra-specific variation based on isozyme and molecular markers have clearly demonstrated that *O. sativa* and *O. rufipogon* are genetically very similar and that the genetic diversity of the former represents a part of diverse gene pool of the latter (Second 1985; Dally and Second 1990; Sano and Sano 1990; Sun et al. 2001).

Classification of *O. sativa* into Indica and Japonica types has been supported based on hybrid sterility (Kato et al. 1930), morpho-physiological traits (Oka 1958), isozymes (Second 1982; Glaszmann 1987) and various molecular markers (Wang and Tanksley 1989; Dally and Second 1990; Second and Wang 1992; Zhang et al. 1992; Ishii et al. 1995, 1996). *O. rufipogon* is widely distributed in the marshy areas in tropical Asia and Oceania. It contains a large amount of within-species genetic variability and tends to differentiate into the perennial and annual ecotypes (Sano and Morishima 1982; Morishima et al. 1984). Thus, within this *sativa/rufipogon* species complex, besides differentiation towards domestication, two other directions of differentiation have occurred, namely, differentiation into Indica and Japonica types in cultivated races, and differentiation from the perennial to annual ecotypes in wild races.

Although these different differentiation processes must have been initiated independently in time and space, and each may have occurred more than once, different driving forces might have been superimposed on each other during the evolutionary history, resulting in the complex pattern of diversity in rice. For example, the tendency of Indica/Japonica differentiation described first in cultivated rice was also found to occur in wild rice, though not as conspicuously as in cultivars (Second 1985; Morishima and Gadrinab 1987; Sano et al. 1989; Chen et al. 1993; Cai et al. 1995; Doi et al. 2000). Wild rice populations growing in the northern fringe of its distribution tend to carry Japonica-specific genes or characteristics. Differentiation from perennial to annual type occurred not only within wild races but also can be found during the domestication process. To obtain a clearer understanding of rice evolution, we conducted research into the genetic bases underlying the three types of differentiation mentioned above.

The differences between species or ecotypes can be understood in the context of a multi-locus or multi-character system in evolution. In other words, non-random association of characters (or genes) differing among related taxa characterizes the essential nature of differentiation. Evolution from wild to cultivated rice led to "the adaptive syndrome of domestication" (Harlan 1975) common to many cereals. This adaptive syndrome includes loss of seed shedding, rapid and uniform germination, increased seed production and more determinate growth. In cultivated races, Japonica type is distinguished from Indica type by a higher resistance to potassium chlorate ($KClO_3$) solution, higher tolerance to low temperature and longer apiculus hair (Oka 1958). The perennial and annual ecotypes in wild races are characterized by a set of life-history traits: the perennial habit is associated with vigorous vegetative growth, low seed production, late flowering and allogamy, whereas the annual habit is associated with high seed production, high seed dispersal ability, early flowering and autogamy. Thus, the variation in reproductive system and mating system is associated in wild rice.

Most of the phenotypic characteristics associated with the differentiation mentioned above are quantitative traits. The objective of the study reported here was to map the quantitative trait loci (QTLs) responsible for differentiation in rice to elucidate the genetic bases of character association. A mapping population used for this study consisted of recombinant inbred lines (RILs) derived from a cross between an Indica cultivar and a Chinese wild rice strain. This wild rice strain, which is perennial and carries some Japonica-specific genes and characteristics, was selected as a parent to provide a sufficient level of polymorphism with Indica type of *O. sativa*. Almost all of the traits of interest were expected to segregate in this mapping population.

Materials and methods

Plant materials

A strain of Asian common wild rice (*Oryza rufipogon* Griff.), W1944, which is a perennial and partially outbreeding strain originally collected in China, was crossed with the Indica cultivar (*O. sativa* L.) Pei-kuh from Taiwan. F_1 plants showed partial seed sterility. The F_2 seeds used in the present study were obtained by selfing an F_1 plant.

One hundred eighty-nine F_2 plants were grown in an experimental paddy field at the National Institute of Genetics, Mishima, Japan, in 1990. The progeny of 158 F_2 plants that flowered and set seeds were used for further experiments. Using the single-seed-descent method, we raised the F_3 –F_7 generations. Two to three panicles per plant were bagged after panicle emergence, but before flowering, to avoid outcrossing and seed shedding, and seeds from bagged panicles were collected for raising the next generation. The 125 RILs thus obtained were grown in 1995 (F_6), 1996 (F_7) and 1997 (again F_7) following standard cultivation procedures in the experimental paddy field in Mishima, Japan. In 1996, the same set of RILs was planted in an automatic short-day chamber (11.5-h daylength) to assess photoperiod sensitivity. Late-flowering plants in the paddy field (natural daylength) were transferred to the greenhouse to obtain seeds.

Parental polymorphism survey and genotyping of RILs

DNA was extracted from fresh leaves of F_6 plants in 1995. DNA extraction, digestion, electrophoresis and Southern blotting were performed according to the methods described in McCouch et al. (1988). DNA hybridization was carried out using the non-radioactive ECL system (Amersham). A total of 147 markers [121 restriction fragment length polymorphisms (RFLP) markers, 17 isozymes, two protein markers, one random amplified polymorphic DNA (RAPD) marker and six qualitatively recognizable traits] that proved to be polymorphic between the parental strains were examined in all 125 RILs.

The six qualitative traits observed were panicle shape, new tiller emergence after heading, awning, gold furrows on hull, hull color and pericarp color (Table 1). The cultivar parent has a compact panicle, weak tiller emergence after heading, no awn, straw-colored hull and white pericarp. In contrast, the wild parent has an open panicle, vigorous tiller emergence after heading, long awn, black hull and red pericarp. Isozyme assays were performed using the starch and polyacrylamide gel systems described in Ishikawa et al. (1989) and Cai et al. (1995). The method for analysis for the protein marker (salt-soluble storage protein; APAGE1 and APAGE2) is given in Cai and Morishima (1997).

Trait measurements

A total of 34 morphological and physiological traits listed in Table 1 (37 including four different tests of dormancy) were evaluated in the F_7 generation. Five traits (seed shedding, seed dormancy, seed fertility, heading date and $KClO_3$ resistance) were evaluated twice, in 1996 and 1997 (both F_7). The traits were grouped into four categories: (1) life-history traits, (2) heading behavior, (3) Indica-Japonica diagnostic traits and (4) plant morphology (Table 1). Measurements were taken on an individual basis, and line means were used for the statistical analysis. The evaluation methods for the respective traits are briefly described in Table 1.

Domestication-related traits differing between wild and cultivated rice are essentially life-history traits affecting the propagation and mating systems. Wild rice has a natural propagation ability, either by seeds or by ratoons, conferred by a high degree of seed shedding, strong seed dormancy, long awn, pronounced panicle exertion and regenerating ability (in the perennial type), while cultivated rice has acquired a high and efficient seed pro-

Table 1 Description of qualitative and quantitative traits examined

Trait	Evaluation method[a]
(A) Qualitative traits	
Panicle shape (OP)	Open or compact panicle
New tiller after heading (NT)	Presence or absence of new tiller emergence after heading
Awn (AW)	Presence or absence of awn
Gold furrow on spikelet (*gf*)	Presence or absence of gold furrows of hull
Hull color (*Bh*)	Black or yellow hull
Pericarp color (*Rc*)	Red or white pericarp
(B) Quantitative traits	
Life-history traits	
Seed shedding[b]	Scored as 0-1-2 according to the degree of shedding (1)
Seed dormancy	Germination % evaluated under the four different test conditions (1)
Awn length	Measured for five spikelets
Anther length	Average of ten anthers taken from three flowers
Panicle exertion	Distance from the uppermost leaf neck to the panicle base
Pollen fertility	Percentage of good pollens
Seed fertility[b]	Percentage of filled grains
Ratooning ability	Degree of ratooning after harvest in the field
Root regeneration	Degree of root regeneration from the stem-cut in the wet sand (2)
Shoot regeneration	Degree of shoot regeneration from the stem-cut in the wet sand
Panicle number	Number of panicles per plant
Tiller number	Number of tillers per plant
Panicle/Tiller ratio	Ratio of panicle number to tiller number
Leaf and stem weight	Dry matter weight of leaf and stem per plant
Heading behavior	
Heading date[b]	Number of days from seeding to heading
Basic vegetative phase	Number of days from seeding to heading under short-day length
Photoperiod sensitive phase	Difference between heading date '97 (DTH) and basic vegetative phase (BVP)
Temperature sensitive phase	Difference in DTH between 2 years
Indica/Japonica-related traits	
$KClO_3$ resistance[b]	Degree of resistance of the seedlings to $KClO_3$ solution (3)
Apiculus hair length	Hair length on the apiculus of spikelet (3)
Germination speed	Germination % at 2 days after soaking (3)
Low temperature resistance	Degree of injury of seedlings treated by low temperature (3)
Panicle base to lowest branch	Distance from panicle neck to the lowest branch base
Plant morphology	
Mesocotyl length	Mesocotyle length germinated in the dark for 10 days
Culm length	Highest culm
Panicle length	Average of three normal panicles
Primary branch number	Number of primary branches per panicle
Secondary branch number	Number of secondary branches per panicle
Spikelet length	Average of ten spikelets
Spikelet width	Width of the widest part of the spikelet
L/W ratio of spikelet	Ratio of length to width of the spikelet
Leaf length	Mean of the first and second top leaves
Leaf width	Width of the widest part of three normal leaves
L/W ratio of leaf	Ratio of length to width of the three leaves

[a] For details see. (1), Cai and Morishima (2000); (2), Oka and Morishima (1967); (3), Oka (1958)
[b] Evaluated in 2 years

ductivity with many large panicles, high seed fertility and heavy seeds. Wild rice, particularly the perennial type, is partially outbreeding, while cultivated rice is predominantly inbreeding. Since it is difficult to estimate the accurate outcrossing rate for a large number of lines, and given that anther length is positively correlated with outcrossing rate (Oka and Morishima 1967), this latter trait was measured as an indication of outcrossing rate. Because in some characters, such as seed shedding, seed dormancy and regenerating ability, the results of evaluation are greatly affected by the number of days from heading to the test date, each line was harvested 30 days after heading based on the respective line means for heading date. Seed dormancy was evaluated using four different tests in 1997 and are presented in this paper (details in Cai and Morishima 2000).

Heading behavior is an important character in wild and cultivated rice. In rice, growth duration or days to heading from seeding (DTH) is often divided into a basic vegetative phase (BVP) and a photoperiod-sensitive phase (PSP) (Chang et al. 1969). In the present study, BVP was estimated by DTH under the short daylength condition, and PSP was estimated by the difference between DTH under short and long (natural) daylength conditions. The temperature-sensitive phase was estimated by the difference in DTH between 1996 and 1997.

To evaluate Indica versus Japonica variation, we observed the following four diagnostic characters (Oka 1958): $KClO_3$ resistance, low temperature tolerance, apiculus hair length and germination speed. Further, the parameter distance from the panicle neck to the base of the lowest rachis used by Cheng (1993) was added.

Statistical analysis

A linkage map was constructed using MAPMANAGER (Manly 1993) based on the segregation data of 147 markers in F_6 RILs. To determine primary linkage groups, we took known map information (Causse et al. 1994; Harushima et al. 1998) into account. For the segregation data of each marker, deviation from the Mendelian ratios (1:1) was examined by chi-square tests.

The chromosomal locations of putative QTLs were determined by the interval mapping method using QGENE (Nelson 1997). LOD score>3.0 was adopted as a probability threshold for significant QTL. Composite interval mapping using MQTL (Tinker and Mather 1995) was also performed for several traits to test the effect of genetic background. Since the two methods yielded essentially the same results, only the results of QGENE are shown. The proportion of observed phenotypic variance explained by each QTL was estimated by the coefficient of determination (R^2). Additive gene effects were estimated, but dominance effects could not be evaluated in RILs. Genotype-by-year interactions were tested using MQTL software for the traits evaluated in 2 years. Prior to the QTL analysis, frequency distributions of phenotypes among RILs were examined in each character, and for seed shedding (index), seed dormancy (germination %), $KClO_3$ resistance (index) and mesocotyle length, which showed high skewness, original data were transformed to log or arcsine.

Results

Segregation of markers in RILs and map construction

One hundred forty-seven markers were mapped on 12 chromosomes. Our linkage map has a total length of 1,192.7 cM (Fig. 1). The number of the markers mapped on their respective chromosomes ranged from 7 (Chr. 10) to 20 (Chr. 1). The order of the RFLP markers largely coincided with results reported by other workers (Causse et al. 1994; Harushima et al. 1998).

The Mendelian factors controlling the following six qualitative characters were mapped. Panicle shape (OP) was found to be linked to *Est10* on chromosome 1. Since previously identified loci for panicle shape (spreading panicle) are located on chromosome 4 (*spr1*, Kinoshita and Takamure 1986; *Spr3*, Eiguchi and Sano 1990), this might be a different locus. New tiller emergence after heading (NT) was mapped on the end of long arm of chromosome 6. The AW locus controlling the presence or absence of awn in this cross was mapped in the middle of long arm of chromosome 9. A gene for black hull segregating in this cross was mapped on chromosome 5; this is most probably *Bhb* (one of the three complementary genes controlling black hull) because the genotype of the wild parent should be *Bha Bhb Bhc* and that of cultivar parent may be *Bha bhb Bhc* (=*Ph*) (Maekawa et al. 1981). The locus for gold furrows on the hull (*gf*) was mapped on chromosome 1, and it is probably *gf2* which has been assigned to chromosome 1 but not yet mapped (Sanchez and Khush 1994). The second locus of salt-soluble protein (APAGE2) was mapped on chromosome 11. One RAPD marker (RB1) linked to a sterility gene (our unpublished data) was located in the middle of long arm of chromosome 1. Two isozyme loci, *Mal3* and *Amp7*, were newly mapped on chromosomes 1 and 6, respectively.

Segregation ratios of two parental alleles deviated significantly from the 1:1 ratio that is theoretically expected in RILs at many loci. Among 147 markers, 76 (52%) showed an excess of cultivar-derived alleles and 17 (12%) showed an excess of wild rice-derived alleles at the significance level of $P < 0.001$. The frequencies of cultivar-derived alleles at respective markers are shown in parenthesis (Fig. 1). Distorted allele frequencies were observed over all 12 chromosomes with 30% (Chr. 12) to 75% (Chr. 5) of the mapped marker loci.

Detection of putative QTLs

The quantitative traits observed generally showed nearly normal distributions among RILs. However, seed shedding, seed dormancy, shoot regeneration index, germination speed and mesocotyle length distorted towards the cultivar-parent type. Log-transformed data were used for seed shedding and mesocotyle length, and arcsine-transformed data were used for seed dormancy. For shoot regeneration and germination speed, the original data were used because scale transformation did not improve the frequency distribution pattern. Transgressive segregation was observed for heading date.

The association between phenotype and marker genotype was investigated using the interval mapping method. Among 37 traits examined, 24 traits revealed significant QTLs at the level of LOD>3.0. Chromosomal regions of 143 QTLs identified are shown with 1.0-LOD supporting confidence intervals (vertical bar, Fig. 1). QTLs for different traits closely located on the chromosome map were represented in a group corresponding to a confidence interval drawn so as to include all their 1.0-LOD supporting confidence intervals.

The distributions of the percentage of observed phenotypic variance explained by each QTL (PVE) in respective traits are shown (Table 2). PVE were mostly less than 25%. Two loci with large effects, one for awn length (73%) and the other for ratooning ability (36%), were mapped at the locations of AW (Chr. 9) and NT (Chr. 6) which we used as qualitative markers, respectively.

The number of QTLs per trait varied markedly. One to six QTLs were detected for 16 traits and more than ten QTLs for 8 traits. Among the various domestica-

Fig. 1 Linkage map of rice based on RILs of the cross Pei-kuh × W1944 and locations of putative QTLs (LOD>3.0) affecting 24 traits. *Figures* in *parenthesis following* respective markers to the *left* of chromosomes show the frequencies of the cultivar-derived allele in which *red* and *blue figures* stand for significant excess and deficiency from the theoretical ratio of 0.5, respectively. Approximate positions of centromeres are indicated by the *spindle symbol* according to Singh et al. (1996). *Trait code* to the *right* of chromosomes can be found in Table 2 (*red*, domestication-related traits; *blue*, Indica/Japonica traits; *black*, heading behavior; *green*, morphology). *Underlined QTLs* signify those for which gene effect showed the opposite direction expected from the parental phenotypes. *Vertical bars* to the *right* of chromosomes indicate QTLs defined by the 1.0 LOD supporting confidence intervals ▶

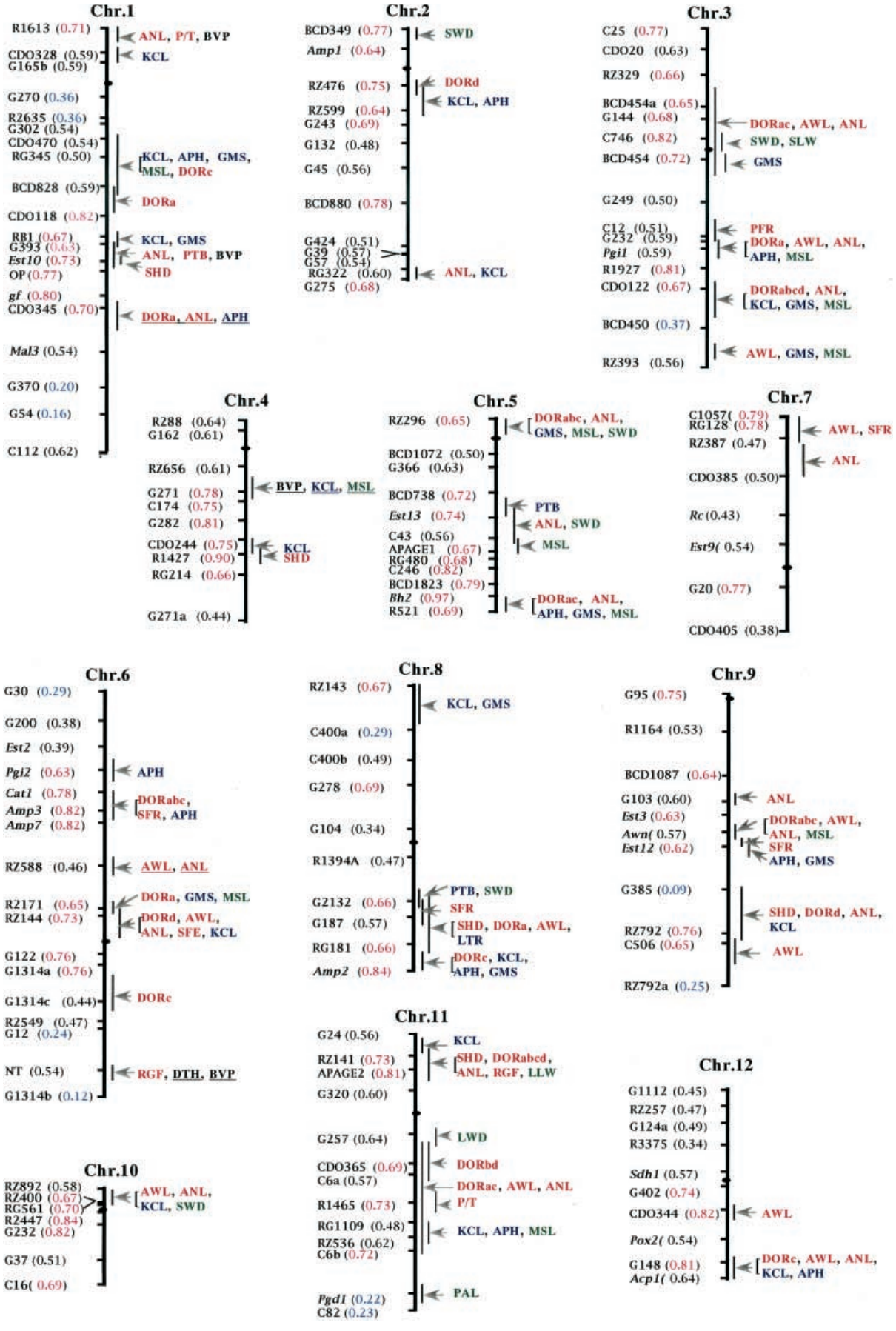
Chr.1
R1613 (0.71)
ANL, P/T, BVP
CDO328 (0.59)
G165b (0.59)
KCL
G270 (0.36)
R2635 (0.36)
G302 (0.54)
CDO470 (0.54)
RG345 (0.50)
KCL, APH, GMS, MSL, DORc
BCD828 (0.59)
DORa
CDO118 (0.82)
RB1 (0.67)
G393 (0.63)
Est10 (0.73)
KCL, GMS
ANL, PTB, BVP
OP (0.77)
SHD
gf (0.80)
CDO345 (0.70)
DORa, ANL, APH
Mal3 (0.54)
G370 (0.20)
G54 (0.16)
C112 (0.62)
Chr.2
BCD349 (0.77)
SWD
Amp1 (0.64)
RZ476 (0.75)
DORd
KCL, APH
RZ599 (0.64)
G243 (0.69)
G132 (0.48)
G45 (0.56)
BCD880 (0.78)
G424 (0.51)
G39 (0.57)
G57 (0.54)
RG322 (0.60)
ANL, KCL
G275 (0.68)
Chr.3
C25 (0.77)
CDO20 (0.63)
RZ329 (0.66)
BCD454a (0.65)
G144 (0.68)
DORac, AWL, ANL
C746 (0.82)
SWD, SLW
BCD454 (0.72)
GMS
G249 (0.50)
C12 (0.51)
PFR
G232 (0.59)
Pgi1 (0.59)
DORa, AWL, ANL, APH, MSL
R1927 (0.81)
CDO122 (0.67)
DORabcd, ANL, KCL, GMS, MSL
BCD450 (0.37)
RZ393 (0.56)
AWL, GMS, MSL
Chr.4
R288 (0.64)
G162 (0.61)
RZ656 (0.61)
BVP, KCL, MSL
G271 (0.78)
C174 (0.75)
G282 (0.81)
CDO244 (0.75)
KCL
R1427 (0.90)
SHD
RG214 (0.66)
G271a (0.44)
Chr.5
RZ296 (0.65)
DORabc, ANL, GMS, MSL, SWD
BCD1072 (0.50)
G366 (0.63)
BCD738 (0.72)
PTB
Est13 (0.74)
ANL, SWD
C43 (0.56)
MSL
APAGE1 (0.67)
RG480 (0.68)
C246 (0.82)
BCD1823 (0.79)
Bh2 (0.97)
R521 (0.69)
DORac, ANL, APH, GMS, MSL
Chr.7
C1057(0.79)
RG128 (0.78)
AWL, SFR
RZ387 (0.47)
ANL
CDO385 (0.50)
Rc (0.43)
Est9(0.54)
G20 (0.77)
CDO405 (0.38)
Chr.6
G30 (0.29)
G200 (0.38)
Est2 (0.39)
Pgi2 (0.63)
APH
Cat1 (0.78)
Amp3 (0.82)
Amp7 (0.82)
DORabc, SFR, APH
RZ588 (0.46)
AWL, ANL
R2171 (0.65)
RZ144 (0.73)
DORa, GMS, MSL
DORd, AWL, ANL, SFE, KCL
G122 (0.76)
G1314a (0.76)
G1314c (0.44)
DORc
R2549 (0.47)
G12 (0.24)
NT (0.54)
RGF, DTH, BVP
G1314b (0.12)
Chr.8
RZ143 (0.67)
KCL, GMS
C400a (0.29)
C400b (0.49)
G278 (0.69)
G104 (0.34)
R1394A (0.47)
G2132 (0.66)
PTB, SWD
SFR
G187 (0.57)
SHD, DORa, AWL, LTR
RG181 (0.66)
Amp2 (0.84)
DORc, KCL, APH, GMS
Chr.9
G95 (0.75)
R1164 (0.53)
BCD1087 (0.64)
G103 (0.60)
ANL
Est3 (0.63)
Awn(0.57)
Est12 (0.62)
DORabc, AWL, ANL, MSL
SFR
APH, GMS
G385 (0.09)
SHD, DORd, ANL, KCL
RZ792 (0.76)
C506 (0.65)
AWL
RZ792a (0.25)
Chr.10
RZ892 (0.58)
RZ400 (0.67)
RG561 (0.70)
R2447 (0.84)
G232 (0.82)
AWL, ANL, KCL, SWD
G37 (0.51)
C16(0.69)
Chr.11
G24 (0.56)
KCL
RZ141 (0.73)
APAGE2 (0.81)
SHD, DORabcd, ANL, RGF, LLW
G320 (0.60)
G257 (0.64)
LWD
CDO365 (0.69)
DORbd
C6a (0.57)
DORac, AWL, ANL
R1465 (0.73)
P/T
RG1109 (0.48)
KCL, APH, MSL
RZ536 (0.62)
C6b (0.72)
Pgd1 (0.22)
PAL
C82 (0.23)
Chr.12
G1112 (0.45)
RZ257 (0.47)
G124a (0.49)
R3375 (0.34)
Sdh1 (0.57)
G402 (0.74)
CDO344 (0.82)
AWL
Pox2(0.54)
G148 (0.81)
Acp1(0.64)
DORc, AWL, ANL, KCL, APH

Table 2 Percentages of observed phenotypic variance explained by each QTL for 24 traits

Trait (Code)	Percentage												Total no. of QTLs
	12	14	16	18	20	22	24	26	28	30	32	>35	
Life-history traits													
Seed shattering '96 (SHD)	1	3	1										5
Seed dormancy (DOR)[a] :a		2	4	3	1	2		1					13
b		3	2		1								6
c		2	2	1	2	3		1		1			12
d		1	1	2	1					1			6
Awn length (AWL)		7	1		2	1		1				1	13
Anther length (ANL)		4	3		5	4	3	1			1		20
Pollen fertility (PFR)		1											1
Seed fertility '97 (SFR)			1	2	1		1						5
Ratooning ability (RGF)	1											1	2
Panicle/tiller ratio (P/T)		1	1										2
Heading behavior													
Heading date '97 (DTH)		1	1										1
Basic vegetative phase (BVP)	2	1	1										4
lndica/Japonica diagnostic traits													
$KClO_3$ resistance '96 (KCL)	1	4	5	3	1		2						16
Apiculus hair length (APH)	2	3	3		1		1	1					11
Germination speed (GMS)	1	4	4		1	1							11
Low temperature resistance (LTR)	1												1
Panicle base to lowest branch (PTB)	3												3
Plant morphology													
Mesocotyl length (MSL)	6		3		2								11
Panicle length (PAL)				1									1
Spikelet width (SWD)	2	1	1	2									6
L/W ratio of spikelet (SLW)		1											1
Leaf width (LWD)	1												1
L/W ratio of leaf (LLW)	1												1

[a] Germination percentage evaluated at 30 days after heading for hulled seeds (a), 30 days after heading for de-hulled seeds (b), 60 days after heading for hulled seeds (c) and 60 days after heading for de-hulled seeds (d).

tion-related traits, seed dormancy, awn length and anther length had a large number of QTLs. Another, and probably the most important character for domestication, seed shedding, proved to be controlled by a relatively small number of loci (five). Regarding perennial/annual variation, two QTLs were detected for ratooning ability in the field and zero for regenerating ability of excised stem cut. Two QTLs were found for panicle number / tiller number (degree of sexual/vegetative reproduction). Regarding heading behavior, one and four QTLs were detected for days to heading and basic vegetative phase, respectively. No QTL for photoperiod and temperature sensitivity were detected. This may be because the wild parent is photoperiod sensitive and the late flowering plants were selected against during establishing RILs.

Among five Indica/Japonica diagnostic traits, $KClO_3$ resistance, apiculus hair length and germination speed had between 11 and 16 QTLs, in contrast to the other two traits, low-temperature tolerance and distance from panicle base to the lowest branch, which were controlled by one and three QTLs, respectively.

Size differences between the parents seemed to be controlled by a relatively small number of genes except for seed width (6 QTLs) and mesocotyl length (11 QTLs). Five morphological traits failed to reveal QTLs at the significance level of LOD>3.0.

Distribution of QTLs on the 12 chromosomes is summarized with LOD values in Table 3. The number of QTLs detected on each chromosome varied from 3 (Chr. 7) to 21 (Chr. 11). This distribution significantly deviated from the values expected on the basis of the length of the respective chromosomes (chi-square = 71.9, $P < 0.0001$). In particular, chromosome 8 carried a much larger number of QTLs than expected.

For most QTLs, parental alleles had the expected effect on traits. For example, alleles from the wild parent increased wild traits such as awn length and seed dormancy, while alleles from the cultivar parent had the opposite effect. However, a few QTLs exhibited effects in the opposite direction from that expected. For example, alleles from the wild parent decreased awn length and seed dormancy, and alleles from the cultivar parent had the opposite effect. These QTLs (10) were found to concentrate in the long arm of chromosome 1 and 4, and in both arms of chromosome 6 (underlined in Fig. 1).

Genotype-by-year interactions were analyzed for seed shedding, seed fertility and $KClO_3$ resistance in 1996 and 1997. No significant interactions were detected.

Table 3 Distribution of QTLs on 12 chromosomes (LOD scores are shown)

Trait code[a]	Chromosome												Total no.
	1	2	3	4	5	6	7	8	9	10	11	12	
SHD	3.36			3.37				3.70	3.97		3.85		5
DORa	3.68 3.22		4.92 3.46 6.7		5.52 4.45	4.17 4.45		3.64	5.40		3.76 4.17		13
DORb			3.35		3.78	3.27			3.92		4.67 3.48		6
DORc	3.09		5.68 6.92		5.71 4.75	7.97 3.97		5.24	3.66		4.60 5.12	3.10	12
DORd		4.37	3.14			4.60			5.02		8.18 3.78		6
AWL			3.59 4.99 3.29			3.36 3.40	3.88	7.04	29.40 3.34	3.24	5.92	3.27 5.11	13
ANL	5.72 8.49 4.85	5.19	3.98 5.10 6.87		5.94 3.29 4.80	3.63 4.11	3.56		3.46 3.45 5.72	6.28	6.53 6.28	5.18	20
PFR			3.06										1
SFR						3.82 3.54	3.17	3.18	4.58				5
RGF						10.28					3.09		2
P/T	3.60										3.39		2
DTH						3.58							1
BVP	3.91 3.39			3.05		4.36							4
KCL	3.78 4.91 6.52	4.13 4.18	3.83	3.68 4.58		3.89		3.38 6.40	3.42	3.64	4.53 3.04	4.82	16
APH	3.11 3.23	3.76	6.88		3.63	4.04 3.53		4.54	6.14		3.08	3.99	11
GMS	3.80 3.37		3.81 5.99 3.22		3.44 5.13	3.77		3.45 3.43	3.80				11
LTR								3.03					1
PTB	3.26				3.09			3.21					3
MSL	3.02		3.11 5.51 3.04	3.34	4.06 3.03 3.10	4.31			4.21		5.40		11
PAL											4.72		1
SWD		4.46	4.77		3.14 3.20			4.60		3.51			6
SLW			3.75										1
LWD											3.19		1
LLW											3.00		1
Total no.	19	6	24	5	17	20	3	13	15	4	21	6	153

[a] See Table 2 for abbrevations and code for DOR

Gene clusters

Genomic regions of the QTLs of various traits were compared. QTLs for different traits were often mapped on the same or adjacent intervals, forming several clusters over the 12 chromosomes (Fig. 1). To test whether or not QTLs were randomly distributed along a chromosome, we carried out the null hypothesis in a Poisson goodness of fit. The chi-square test indicated that the distribution of QTLs on each chromosome was nonrandom except for chromosome 5.

QTLs for domestication-related traits (shown in red in Fig. 1) tended to be concentrated on particular regions of chromosomes 3, 6, 8, 9, 11 and 12. Smaller but similar clusters were found in other regions. Further, QTLs for Indica/Japonica diagnostic characters (blue in Fig. 1)

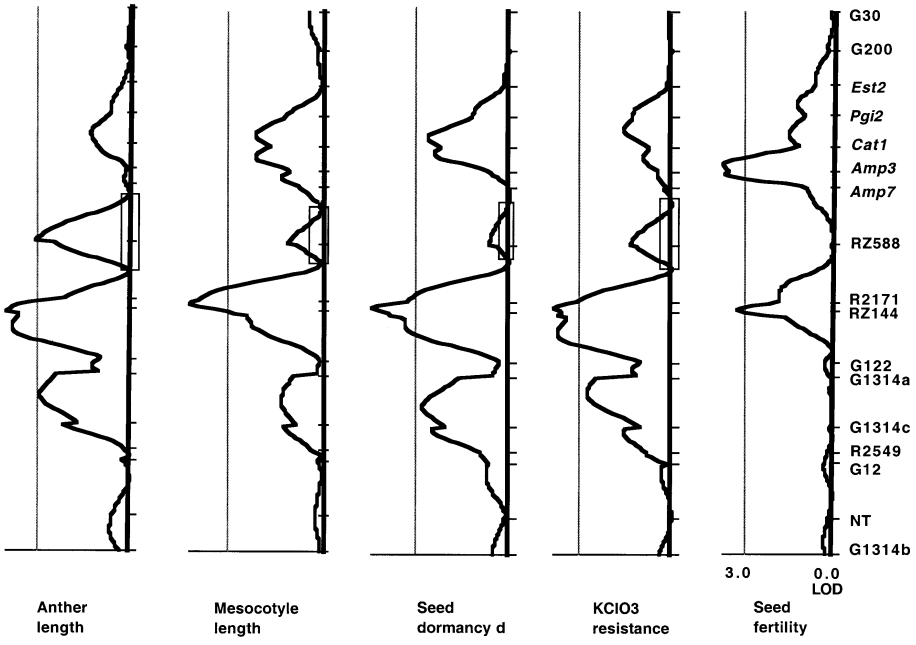

Fig. 2 LOD profiles for QTLs of five traits along chromosome 6. *Abscissa* indicates LOD value. The regions on the chromosomes indicated by *rectangles* signify those in which the direction of gene effect is opposite to that expected from the parental phenotypes

tended to be mapped near the above-mentioned clusters. For instance, the distal end of the long arm of chromosome 8 harbors QTLs for domestication-related traits (SHD, DOR, AWL, SFR) as well as Indica/Japonica QTLs (KCL, APH, GMS, LTR, PTB). QTLs for mesocotyl length (MSL) also tended to be part of the clusters. Near the centromere of chromosome 3 and in the middle of the long arm of chromosome 11, the LOD profile of seed dormancy, awn length and anther length showed contiguously distributed multi-peaks. Whether there are several linked QTLs or a broad region (shown by a confidence interval in Fig. 1) affecting the trait is not known from the present study.

Several traits, each controlled by more than 11 QTLs (DOR, AWL, ANL, KCL, APH, GMS, MSL), were found to exhibit a similar LOD profile along a chromosome. As an example, LOD profiles for anther length, dormancy, $KClO_3$ resisitance and mesocotyle length along chromosome 6 are shown (Fig. 2). Awn length, apiculus hair length and seed width also showed a similar pattern. In these traits, QTLs detected near marker RZ588 on chromosome 6 consistently showed a different direction of genic effect from QTLs in other regions. Seed fertility given in Fig. 2 for comparison, however, showed a quite different pattern. These results suggest that an unknown key factor with pleiotropic effect is involved in the expression of several traits.

The locations of the gene clusters mentioned above were not near the centromeres. Rather, they seemed to be in the regions where cultivar-derived alleles significantly exceeded wild alleles in frequencies. The predominance of cultivar-derived alleles in this population may be accounted for by, in addition to segregation distortion usually observed in the F_2, the selective advantage of the plants with cultivar-like traits during establishment of RILs. Data on the allele frequencies of some isozyme and pigmentation genes observed for F_2 are available. Table 4 shows a comparison of allele frequencies between F_2 and F_7 RILs. Six marker loci linked to domestication QTLs tended to show an increase in cultivar alleles from the F_2 to F_7 generation. In contrast, eight marker loci unlinked to domestication QTLs did not show such a trend. This suggests that

Table 4 Frequencies of cultivar-derived alleles observed in F_2 (estimated from the genotypes of the mixed seeds on F_2 plants) and F_7 RILs for 12 isozyme and two coloration genes. Figures in the parentheses are the chromosome number

Loci	F_2	F_7	Difference[a]
Loci linked to domestication QTLs			
Acp1 (12)	0.50	0.64	*
Amp2 (8)	0.79	0.84	NS
Amp3 (6)	0.49	0.82	**
Bh (5)	0.68	0.97	**
Cat1 (6)	0.71	0.78	NS
Pgi1 (3)	0.62	0.59	NS
Loci unlinked to domestication QTLs			
Amp1 (2)	0.65	0.64	NS
Est2 (6)	0.45	0.39	NS
Est9 (7)	0.57	0.54	NS
Pgd1 (11)	0.24	0.22	NS
Pgi2 (6)	0.65	0.63	NS
Pox2 (12)	0.61	0.54	NS
Rc (7)	0.45	0.43	NS
Sdh1 (12)	0.52	0.57	NS

[a] Difference between F_2 and F_7. * significant at 5%; ** significant at 1%; NS, non-significant

gene blocks of domestication-related QTLs carrying cultivar-alleles might have been favored through unconscious selection.

Discussion

Related species or ecotypes can be recognized by a set of traits or genes in which the states of different traits or alleles at different loci are non-randomly associated with each other; these association patterns characterize the taxa. Major factors causing such non-random association or gametic disequilibrium are natural selection for co-adapted traits, linkage, pleiotropy and/or the founder effect (Hedrick et al. 1979). Though these factors are barely distinguishable, comparison of association patterns between different generations could be an effective approach to dissect its genetic mechanism. Non-random associations that exist in nature and disappear in the interpopulation F_2 generation are largely the products of natural selection for co-adapted traits. On the other hand, those remaining in the F_2 (so-called character coherence at the phenotypic level) may be due to pleiotropy or close linkage. Recent advances in genome analysis enabled us to open the door to look into the genetic bases of character association at the genic level.

Classical evidence for character coherence was reported by Clausen and Hiesey (1958) in *Potentilla glandulosa*. In the F_2 population of an interracial cross, they found that most pairs of characters showed weak but significant correlations in such a way as observed between the two parents in nature. Grant (1981) inferred that the character coherence is due to "multifactorial linkages". It is a phenomenon inevitably caused by the random distribution of multiple factors over the "limited number" of chromosomes determining the differences of two or more quantitative traits. Some of the genes for different traits occur on the same chromosome. Grant (1981) described that "all multifactorial characters (quantitative traits) differentiating the two races or species are expected to be tied together in an interlocking system of weak linkages". Another example of character coherence was reported by Joly and Sarr (1985); they found a preferential association of characters in the progeny of a cross between pearl millet (*Pennisetum typhoides*) and its wild relatives and discussed a role for natural selection, which supposedly worked on the gametophyte to preserve the domestication syndrome.

The recently observed clustering of functionally related genes has been of great interest in many species. Koinange et al. (1996) found that the distribution of the domestication syndrome genes in common bean appears to be concentrated on specific genomic regions. Khavkin and Coe (1997) examined the map locations of many maize genes reported to date and pointed out that not only QTLs but also classical mutant genes for growth or development and homeotic genes mapped with cDNA are distributed non-randomly in gene clusters along the ten maize chromosomes. This cluster phenomenon was explained by the adaptive significance of the functional gene network. In rice, Xiong et al. (1999) and Bres-Patry et al. (2001) performed QTL analysis of domestication-related traits using the crosses between *O. sativa* ×*O. rufipogon* and between *O. sativa* ×weedy type rice, respectively. Both studies demonstrated clusters of QTLs related to domestication, though a comparison between their results and ours is difficult due to insufficient common markers.

Regarding the three types of differentiation targeted in the present study (wild/cultivated types, perennial/annual types, and Indica/Japonica types), a number of QTLs responsible for wild/cultivated differentiation as well as for Indica/Japonica differentiation were identified, and clustering phenomena were demonstrated.

As reviewed by Oka (1988), domestication syndromes or character associations differentiating wild and cultivated rice are mostly broken in the F_2 of a cross between wild and cultivated plants. In our RILs, trait associations characterizing the two parents were partly significant but generally weak (data not shown). This may imply that non-random character associations differentiating wild and cultivated rice are mainly due to natural selection for co-adapted traits and not due to linkage or pleiotropy of a few genes. Gene clusters for the domestication syndrome are probably due to multifactorial linkages as argued by Grant (1981), followed by natural selection favoring co-adapted gene blocks. Pernes (1983), predicted that linkages of the components of the domestication syndrome could be adaptive, particularly in outbreeding species in which the recovery of cultivated type following frequent outcrossing between wild and cultivated forms might be facilitated by such linkages. In rice, though the current domesticates are predominantly inbreeding, natural hybridization must have played an important role in the domestication process, preserving chromosome blocks carrying co-adapted genes.

In Indica/Japonica hybrids of rice cultivars, character associations differentiating the two types are broken in the F_2 and tend to recover in later generations (Oka 1988). We detected a number of QTLs affecting Indica/Japonica diagnostic characters such as KCL, APH and GMS and found that those QTLs were distributed over the genome forming the clusters. Unexpectedly, the Indica/Japonica clusters and domestication clusters were mapped on the same or adjacent regions of the chromosomes. QTLs for the traits controlled by multiple factors showed quite similar variation patterns of gene effects along the chromosome (Fig. 2). It should be noted that QTLs for mesocotyl elongation (MSL) in the dark, a trait considered to reflect endogenous hormone level were mapped near QTL clusters. Plant hormones such as abscisic acid and giberellic acid are known to be responsible for seed dormancy and germination speed (Takahashi 1997). This suggests the possibility that an unknown key factor(s) controlling some metabolic path for differentiation and development of many traits is involved in this clustering phenomenon.

QTLs of a cluster might be loosely linked to each other as argued by Grant (1981), or some of them are functionally co-adapted or some others might be related by unknown factor(s) with pleiotropic effect. Such chromosomal regions are inherited as a block. Since they are distributed throughout the genome, they recombine showing a decrease in character association in the early generations of the hybrid and subsequently tend to recover parental types in the later generations through natural selection and/or some internal mechanism.

Our study failed to elucidate the genetic basis of another targeted differentiation – perennial/annual variation associated with mating system – since few related QTLs were detected. One of the two loci for P/T (proportion of panicle number to tiller number) was mapped together with a locus for BVP near the distal end of short arm of chromosome 1 (flanked with R1613 and CDO328). This might correspond to a QTL reported by Kohn et al. (1997) based on a preliminary study of a cross between an annual and perennial plant of *O. rufipogon*. It was found to be located near G1184Ca and controls several component traits related to perennial/annual differentiation, including heading date. The mapping population used in the present study was probably inappropriate to elucidate the whole scope of perennial versus annual differentiation that primarily occurred in *O. rufipogon*.

Our markers consisted of a number of isozyme and protein genes for which we have conducted a variation survey in many accessions of *O. sativa* and *O. rufipogon*. It enabled us to evaluate the present results by comparison with our previous studies. One of the gene clusters harboring domestication QTLs and Indica/Japonica QTLs was found on the region marked by *Est10* on chromosome 1. *Est10* is a rare isozyme locus known to be diagnostic for distinguishing not only between Indica and Japonica cultivars but also between wild and cultivated races (Wang et al. 1992). This provides evidence that a gene block related to phylogenetic differentiation really exists.

It is known that allelic variations at several isozyme loci such as *Acp1*, *Amp2*, *Cat1*, *Pgi2* and *Pox2* are strongly associated with Indica versus Japonica differentiation (Second 1982; Glaszmann 1987). Our study demonstrated that chromosome regions marked by those diagnostic isozymes harbor Indica/Japonica QTLs. This may explain why independent isozyme loci, that are supposedly neutral, are in a strong gametic disequilibrium state among rice varieties. In contrast, no gene cluster was found near isozyme loci that do not show distinct association with Indica versus Japonica differentiation (*Amp1*, *Mal3*, *Pgd1*, *Sdh1*).

Segregation distortion in rice was recently investigated by molecular markers in different types of populations and crosses, and a number of segregation distortion loci distributed over the genome were postulated (Harushima et al. 1996; Xu et al. 1997; Virk et al. 1998). In RI populations it is difficult to distinguish genetic causes of distorted allele frequencies from environmental ones (Xu et al. 1997). In our RI population, alleles from the female Indica cultivar parent were generally predominant. Yet, allele distortion in F_2 was not so severe compared with F_7 RI population (Table 4). Segregation distortion in the F_2 might have been blurred in the RI population by various factors. The chromosomal regions showing apparent over-representation of cultivar alleles in RILs were associated with QTL clusters. It can be inferred that a predominance of cultivar-derived alleles might largely reflect its selective advantage under the cultivation environment in which the RI population was developed.

Recent advances in synteny studies suggest the common genes or gene blocks have been conserved in different grass species. QTL studies carried out in several cereal crops have demonstrated that independent domestication of different species involved convergent selection for the domestication syndrome (Paterson et al. 1995). Comparison between our results and the synteny map given by Paterson et al. (1995) suggested that locations of some of our QTLs for seed dormancy, seed shattering and seed width seemed to correspond to the regions harboring QTLs for the same (or related) traits on maize and sorghum chromosomes, although precise examination is difficult due to the insufficient common markers.

QTLs detected in the present study were significant at LOD>3.0 but still putative. To what extent the results obtained from this mapping population can be generalized for understanding the genetic mechanisms in the evolutionary history of rice remains a question. The relative importance of linkage and pleiotropy to explain QTL clusters is also unknown. Further analysis based on a finer map or isogenic lines is necessary to determine the precise location of each QTL.

Acknowledgements We are greatly indebted to S.R. McCouch, Cornell University, USA and T. Sasaki, Rice Genome Research Program, NIAR, Japan, for their kind offer of RFLP probes. We are also thankful to D.A. Vaughan of NIAR, Japan, and two anonymous reviewers for their valuable comments to this manuscript. This study was financially supported by a grant-in-aid from the Japanese Ministry of Education, Science, Culture and Sports (Grant No. 084456006). This experiment complies with the current laws of Japan.

References

Bres-Patry C, Lorieux M, Clement G, Bangratz M, Ghesquiere A (2001) Heredity and genetic mapping of domestication-related traits in a temperate *japonica* weedy rice. Theor Appl Genet 102:118–126

Cai HW, Morishima H (1997) New storage protein genes detected by acidic formate PAGE method. Rice Genet Newsl 14:76–78

Cai HW, Morishima H (2000) Genomic regions affecting seed shattering and seed dormancy in rice. Theor Appl Genet 100: 840–846

Cai HW, Wang XK, Cheng KS, Zhang YZ (1995) Classification of Asian rices by esterase isozymes (in Chinese). S W China J Agric Sci 5:19–23

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwonse J, Wu K, Xiao JJ, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Chang TT, Li CC, Vergara BS (1969) Component analysis of duration from seeding to heading in rice by the basic vegetative phase and the photoperiod-sensitive phase. Euphytica 18:79–91

Chen WB, Nakamura I, Sato YI, Nakai H (1993) Distribution of deletion type in cpDNA of cultivated and wild rice. Jpn J Genet 68:597–603

Cheng KS (1993) Discrimination of Indica and Japonica subspecies in Asian cultivated rice (in Chinese). Yunnan Sci Tech Press, China

Clausen J, Hiesey WM (1958) Experimental studies on the nature of species. V. Genetic structure of ecological races. Carnegie Institute, Washington D.C. Publ 615

Dally AM, Second G (1990) Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza*, section *Oryza*). Cladistic-mutation and genetic-distance analysis. Theor Appl Genet 80:209–222

Doi K, Nakano-Nonomura M, Yoshimura A, Iwata N, Vaughan DA (2000) RFLP relationships of A-genome species in the genus *Oryza*. J Fac Agric Kyushu Univ 45:83–98

Eiguchi M, Sano Y (1990) A gene complex responsible for seed shattering and panicle spreading found in common wild rice. Rice Genet Newsl 7:105–107

Grant V (1981) Plant speciation. Columbia University Press, New York

Glaszmann JC (1987) Isozymes and classification of Asian rice varieties. Theor Appl Genet 74:21–30

Harlan JR(1975) Crops and man. American Society of Agronomy, Madison, Wis.

Harushima Y, Kurata N, Yano M, Nagamura, Y, Sasaki T, Minobe, Y, Nakagahra M (1996) Detection of segregation distortions in an indica-japonica rice cross using a high-resolution molecular map. Theor Appl Genet 92:145–150

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148: 479–494

Hedrick P, Jain S, Holden L (1978) Multilocus systems in evolution. Evol Biol 11:101–184

Ishii T, Brar DS, Second G, Tsunewaki K, Khush GS (1995) Nuclear genome differentiation in Asian cultivated rice as revealed by RFLP analysis. Jpn J Genet 70:643–652

Ishii T, Nakano T, Maeda H, Kamijima O (1996) Phylogenetic relationships in A-genome species of rice revealed by RAPD analysis. Genes Gene Syst 71-195–201

Ishikawa R, Morishima H, Mori K, Kinoshita T (1989) Chromosomal analysis of isozyme loci and the allelic expression at cellular level in rice – genetical studies of rice plants. XCVII. J Fac Agric Hokkaido Univ 64:85–98

Joly H, Sarr A (1985) Preferential associations among characters in crosses between pearl millet (*Pennisetum typhoides*) and its wild relatives. In: Jacquard P et al (ed) Genetic differentiation and dispersal in plants. NATO ASI Ser G5, Brussels, pp 95–111

Kato S, Kosaka H, Maruyama Y, Takiguchi Y (1930) On the affinity of cultivated varieties of rice, *Oryza sativa* L. J Dep Agric Kyushu Univ 2:241–276

Khavkin E, Coe E (1997) Mapped genomic locations for developmental functions and QTLs reflect concerted groups in maize (*Zea mays* L.). Theor Appl Genet 95:343–352

Kinoshita T, Takamure I (1986) Linkage studies by the use of backcross data in rice – genetical studies on rice plant. XCV. J Fac Agric Hokkaido Univ 63:136–143

Kohn JR, Leyva N, Dossey R, Sobral B, Morishima H (1997) Quantitative trait locus analysis of trait variation among annual and perennial ecotypes of *Oryza rufipogon*. Int Rice Res Notes 22:4–5

Koinange EMK, Singh SP, Gepts P (1996) Genetic control of the domestication syndrome in the common bean. Crop Sci 36: 1037–1045

Maekawa M, KinoshitaT, Takahashi M (1981) Geographical distribution of the genes for black hull coloration. Genetical studies on rice plant. LXXVII (in Japanese). Res Bull Univ Farm Hokkaido Univ 22:20–28

Manly KF (1993) A MacIntosh program for storage and analysis of experimental genetic maping data. Mammal Genome 4: 303–313

McCouch SR, Kochert G, Yu ZH, Wang ZW, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Morishima H, Gadrinab LU (1987) Are the Asian common wild rice differentiated into the Indica and Japonica types? In: Hsieh SC (ed) Crop exploration and utilization of genetic resources. Taichung Distr Agric Improv Stat, Changhua, Taiwan, pp 11–20

Morishima H, Sano Y, Oka HI (1984) Differentiation of perennial and annual types due to habitat conditions in the wild rice *Oryza perennis*. Plant Syst Evol 144:119–135

Morishima H, Sano Y, Oka HI (1992) Evolutionary studies in cultivated rice and its wild relatives. Oxford Surv Evol Biol 8: 102–109

Nelson JC (1997) QGENE: Software for marker-based genomic analysis and bredding. Mol Breed 3:239–245

Oka HI (1958) Intervarietal variation and classification of cultivated rice. Ind J Genet Plant Breed 18:79–89

Oka HI (1988) Origin of cultivated rice. Japanese Scientific Society Press/Elsevier, Tokyo, Amsterdam

Oka HI, Morishima H (1967) Variations in the breeding systems of a wild rice, *Oryza perennis*. Evolution 21:249–258

Paterson AH, Lin YR, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu SC, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714–1718

Pernes J (1983) The genetics of domestication in cereals (in French). Recherche 14:910–919

Sanchez AC, Khush GS (1994) Chromosomal location of some marker genes in rice using the primary trisomics. J Hered 85: 297–300

Sano Y, Morishima H (1982) Variation in resource allocation and adaptive strategy of a wild rice, *Oryza perennis* Moench. Bot Gaz 143:518–523

Sano Y, Sano R (1990) Variation in the intergenic spacer region of ribosomal DNA in cultivated and wild rice species. Genome 33:209–218

Sano Y, Yi HX, Shao QQ, Iyama S (1989) Ribosomal DNA spacer-length variations in a wild rice population from DongXian, China. In: Breeding research: the key to the survival of the earth. Proc 6th Intl Congress SABRAO, pp 493–496

Second G (1982) Origin of the genic diversity of cultivated rice (*Oryza* spp.): study of the polymorphism scored at 40 isozyme loci. Jpn J Genet 57:25–57

Second G (1985) Evolutionary relationships in the *Sativa* group of *Oryza* based on isozyme data. Genet Sel Evol 17:89–114

Second G, Wang ZY (1992) Mitochondrial DNA RFLP in genus *Oryza* and cultivated rice. Genet Resour Crop Evol 39: 125–140

Singh K, Ishii T, Parco A, Huang N, Brar DS, Khush GS (1996) Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.). Proc Natl Acad Sci USA 93:6163–6168

Sun CQ, Wang XK, Li, ZC, Yoshimura A (2001) Comparison of the genetic diversity of common wild rice (*Oryza rufipgon* Griff.) and cultivated rice (*O. stiva* L.) using RFLP markers. Theor Appl Genet 102:157–162

Takahashi N (1997) Inheritance of seed germination and dormancy (in Japanese). In: Matsuo T (ed) Science of rice plant 3. Genetics, Food and Agriculture Policy Research Center, Tokyo, pp 348–359

Tinker NA, Mather DE (1995) MQTL: software for simplified composite interval mapping of QTL in multiple environments. J Agric Genomics 1, article 2

Virk PS, Ford-Lloyd BV, Newbury HJ (1998) Mapping AFLP markers associated with subspecific differentiation of *Oryza sativa* (rice) and an investigation of segregation distortion. Heredity 81:613–620

Wang ZY, Tanksley SD (1989) Restriction fragments length polymorphism in *Oryza sativa* L. Genome 32:1113–1118

Wang XK, Cai HW, Cheng KS (1992) The discovery of an *Est* locus related to the origin, evolution and classification of Asian rice. Chin Rice Res Newsl 7:1–2

Xiong LZ, Liu KD, Dai XK, Xu CG, Zhang Q (1999) Identification of genetic factors controlling domestication-related traits of rice using an F_2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor Appl Genet 98: 243–251

Xu Y, Zhu L, Xiao J, Huang N, McCouch S R (1997) Chromosomal regions associated with segregation distortion in molecular markers in F_2, backcross, double haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Genet 253:535–545

Zhang Q, Maroof MAS, Lu TY, Shen BZ (1992) Genetic diversity and differentiation of indica and japonica rice detected by RFLP analysis. Theor Appl Genet 83:495–499