

3-8-2011

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Zhixi Tian

Yanjun Yu

Feng Lin

Yeisoo Yu

Phillip J. SanMiguel

See next page for additional authors

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Recommended Citation

Tian, Zhixi; Yu, Yanjun; Lin, Feng; Yu, Yeisoo; SanMiguel, Phillip J.; Wing, Rod A.; McCouch, Susan R.; Ma, Jianxin; and Jackson, Scott A., "Exceptional Lability of a Genomic Complex in Rice and its Close Relatives Revealed by Interspecific and Intraspecific Comparison and Population Analysis" (2011). *Department of Agronomy Faculty Publications*. Paper 2.
<http://dx.doi.org/10.1186/1471-2164-12-142>

Authors

Zhixi Tian, Yanjun Yu, Feng Lin, Yeisoo Yu, Phillip J. SanMiguel, Rod A. Wing, Susan R. McCouch, Jianxin Ma, and Scott A. Jackson

RESEARCH ARTICLE

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Exceptional lability of a genomic complex in rice and its close relatives revealed by interspecific and intraspecific comparison and population analysis

Zhixi Tian¹, Yanjun Yu^{1,5}, Feng Lin¹, Yeisoo Yu², Phillip J SanMiguel³, Rod A Wing², Susan R McCouch⁴, Jianxin Ma^{1*}, Scott A Jackson^{1*}

Abstract

Background: Extensive DNA rearrangement of genic colinearity, as revealed by comparison of orthologous genomic regions, has been shown to be a general concept describing evolutionary dynamics of plant genomes. However, the nature, timing, lineages and adaptation of local genomic rearrangement in closely related species (e. g., within a genus) and haplotype variation of genomic rearrangement within populations have not been well documented.

Results: We previously identified a hotspot for genic rearrangement and transposon accumulation in the *Orp* region of Asian rice (*Oryza sativa*, AA) by comparison with its orthologous region in sorghum. Here, we report the comparative analysis of this region with its orthologous regions in the wild progenitor species (*O. nivara*, AA) of Asian rice and African rice (*O. glaberrima*) using the BB genome *Oryza* species (*O. punctata*) as an outgroup, and investigation of transposon insertion sites and a segmental inversion event in the AA genomes at the population level. We found that *Orp* region was primarily and recently expanded in the Asian rice species *O. sativa* and *O. nivara*. LTR-retrotransposons shared by the three AA-genomic regions have been fixed in all the 94 varieties that represent different populations of the AA-genome species/subspecies, indicating their adaptive role in genome differentiation. However, LTR-retrotransposons unique to either *O. nivara* or *O. sativa* regions exhibited dramatic haplotype variation regarding their presence or absence between or within populations/subpopulations.

Conclusions: The LTR-retrotransposon insertion hotspot in the *Orp* region was formed recently, independently and concurrently in different AA-genome species, and that the genic rearrangements detected in different species appear to be differentially triggered by transposable elements. This region is located near the end of the short arm of chromosome 8 and contains a high proportion of LTR-retrotransposons similar to observed in the centromeric region of this same chromosome, and thus may represent a genomic region that has recently switched from euchromatic to heterochromatic states. The haplotype variation of LTR-retrotransposon insertions within this region reveals substantial admixture among various subpopulations as established by molecular markers at the whole genome level, and can be used to develop retrotransposon junction markers for simple and rapid classification of *O. sativa* germplasm.

* Correspondence: maj@purdue.edu; sjackson@purdue.edu

¹Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA
Full list of author information is available at the end of the article

Background

Comparative genetic mapping and comparison of orthologous genomic sequences of grasses, such as rice, maize, sorghum, barley, wheat, and millet have demonstrated extensive genomic colinearity among species that radiated from common ancestors ~10-60 million years ago [1,2]. Although numerous and different levels of genomic rearrangements, including gene movement, and loss or creation of new genes were uncovered in some grass lineages [3-5], gene content has been shown to be highly conserved between species. For instance, all genes, including single-copy ones, absent in the genomic region surrounding the orange pericarp (*Orp*) gene of rice, in contrast to its orthologous regions of sorghum and maize, were found elsewhere in the rice genome and even in *Arabidopsis* [3,6]. Comparison of homoeologous segments of maize revealed exceptionally high-level of loss of one of the homoeologous gene pairs [3,5-7], which appears to be a general phenomenon in the evolution of any polyploid organism toward a diploid genomic state. These dynamic processes of gene duplication and deletion may explain why rice and *Arabidopsis* share a similar set of genes, although their genomes have undergone separate paleopolyploidy and/or segmental duplication events during their ~120 million year independent evolution [8].

In contrast to genes, intergenic spaces were found to be less or not conserved between grasses, such as maize and sorghum. Intergenic sequences are generally composed of transposable elements or transposable element fragments, primarily long terminal repeat (LTR)-retrotransposons, and other unknown DNA components. Given that most structurally detectable LTR-retrotransposons were amplified within last a few million years [9,10], it is not surprising that substantial differences in intergenic regions have been found between subspecies of rice [11], or even between inbreds of maize [12,13]. On the other hand, LTR-retrotransposons can be partially or completely deleted from the host genomes within very short evolutionary timeframes. For example, it was estimated that ~200 Mb of LTR-retrotransposon DNA was removed from the rice genome by unequal homologous recombination and illegitimate recombination within the past 5 million years [9,10], although neither amplification nor removal of LTR-retrotransposons seems to be absolutely gradual processes [14]. In addition to the gain and loss of transposable elements, intergenic sequences generally diverge more rapidly than genic sequences by nucleotide substitution [10,15]. These dynamic processes have led to the scarcity of conserved intergenic sequences, even between moderately diverged grass lineages such as maize and sorghum [6,16].

Comparison of closely related species, subspecies, and/or different haplotypes or ecotypes is a promising approach to investigate more recent evolutionary events.

A comparative sequence analysis of ~1.1 Mb orthologous regions of two subspecies of rice, *indica* and *japonica*, revealed more than 2% and 6% growth of two respective genomes over the past half million years, primarily by amplification of LTR-retrotransposons [10]. Wang and Dooner presented a comprehensive comparison of seven inbred lines of maize, demonstrating the remarkable haplotype variation of the *bz* genomic regions caused predominantly by insertion of LTR-retrotransposons, helitrons, DNA transposons and other new repetitive components [17]. However, the dynamic variation of transposable elements, their potential interplay with genic rearrangement, and their roles for genomic selection and diversity remain to be investigated, particularly, at the population level.

The high-quality genomic sequence of rice [18] and genomic resources (e.g., BAC libraries, BAC end sequences, BAC-based physical maps) generated by the ongoing *Oryza* map alignment project (OMAP) [19] provide an unprecedented opportunity for research community to study the evolution of plant genomes within a genus. To date, three genomic (*Adh1*, *MOC1*, and *Hd1*) regions of multiple *Oryza* species have been investigated [20-22]. Because the *Oryza* species included in OMAP span evolutionary scales from < 1 million years to ~15 million years, as indicated by their phylogeny [23], comparisons of multiple *Oryza* species in these regions have uncovered some specific evolutionary events in specific lineages during the radiation of the *Oryza* species. However, all three regions are gene-rich and repeat-poor, therefore little is known about how transposable elements have affected the instability of the *Oryza* genomes during their speciation and diversification.

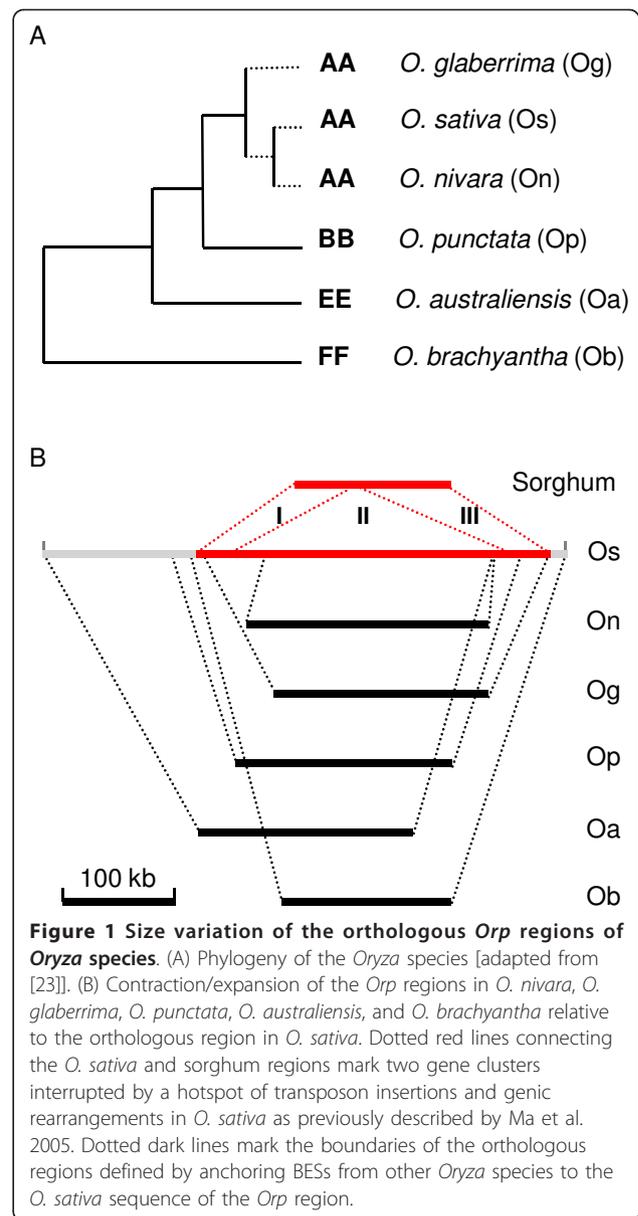
A hotspot of transposable element accumulation that harbors a few truncated and duplicated gene fragments was previously described between two gene clusters of the *Orp* region of rice (*O. sativa* ssp. *japonica*). This hotspot is located near the end of the short arm of chromosome 8 (from 1757 to 1997 kb, rice Pseudomolecule 4.0), and contains a high proportion of LTR-retrotransposons, similar to that observed in the centromeric region (Cen8) of this same chromosome [6,24], but it is absent in the corresponding regions of sorghum and maize [6]. To track the evolutionary history of the formation of this hotspot and the spectrum of genic rearrangements involved, we identified its orthologous regions from AA-, BB-, EE-, and FF-genome *Oryza* species by searching the *O. sativa Orp* region against BAC end sequences (BESs) generated by OMAP. In particular, we sequenced two overlapping BAC clones from *O. nivara* (AA), one of the proposed wild progenitors of Asian rice (*O. sativa*), one BAC clone from *O. glaberrima* (AA), the cultivated rice species domesticated in African, and one BAC clone from *O. punctata* (BB). In

addition, we investigated the haplotype variation of LTR-retrotransposon insertions and an inversion of a genomic segment within the hotspot. We present here the comparative genomic analysis of these orthologous regions and haplotype variation mediated by LTR-retrotransposons, thereby depicting the nature, timing, rate and specificity of DNA changes observed in these regions during the speciation and diversification of these closely related *Oryza* species.

Results and Discussion

Size variation of the *Orp* orthologous regions across *Oryza* species reflects recent genomic expansion

To select the genomic segments that are orthologous to the *O. sativa* *Orp* regions in other *Oryza* species, we searched a set of BAC end sequences (BESs) derived from the *O. nivara*, *O. glaberrima*, *O. punctata*, *O. australiensis*, and *O. brachyantha* genomes (OMAP, <http://www.omap.org>, Figure 1A). Individual BAC clones with two BESs anchored to the unique sequences of the *O. sativa* *Orp* region and/or its flanking regions in opposite orientations were considered to be orthologous segments. One or two overlapping BAC clones from each species that maximally cover the hotspot of insertions of transposable elements in the *Oryza Orp* region [6] were chosen for sequencing and/or further analysis. BAC clones OR_BBa0014L06 from *O. nivara*, two overlapping clones OG_BBa0075G14 and OG_BBa0001L21 from *O. glaberrima*, and clone OP_Ba0008J05 from *O. punctata* were completely sequenced. These sequenced genomic segments are 214 kb, 190 kb, and 192 kb in *O. nivara*, *O. glaberrima*, and *O. punctata*, respectively, corresponding to 202 kb, 309 kb, and 339 kb of the orthologous regions in *O. sativa* (Table 1). Thus, a 12-kb expansion of the *O. nivara* region and 119-kb and 147-kb contractions of the *O. glaberrima* and *O. punctata* regions relative to the corresponding orthologous regions in *O. sativa* were revealed. In addition, 212-kb and 192-kb contractions of the *O. australiensis* and *O. brachyantha* regions relative to the corresponding *O. sativa* orthologous regions (Table 1) were suggested by fingerprinting of BAC clones OA_ABa0108F22 and OB_Ba0050L03 (OMAP, <http://www.omap.org>) that define the orthologous *Orp* regions in *O. australiensis* and *O. brachyantha*, respectively (Figure 1B). The relative contraction of the *Orp* region in sorghum in contrast to the *Orp* region in *O. sativa* is 175 kb (Table 1), and no transposable elements were identified in the sorghum region [6]. These observations, together with the evolutionary relationship of these species as illustrated in Figure 1A, suggest that the *Orp* region was primarily and recently expanded in the Asian rice species *O. sativa* and *O. nivara* [i.e., after the divergence of Asian and African rice approximately 1.2 million years ago



[25]]. Because this study aimed to decipher the nature and timing of recent genic rearrangements and gain and loss of LTR-retrotransposon in the *Orp* regions of the AA genome species, the orthologous BAC clones from *O. australiensis* and *O. brachyantha* were not further investigated.

Sequence organization and comparison of the orthologous regions

Analysis of the *Orp* orthologous regions in *O. sativa* and sorghum was previously performed [6]. Using these same criteria, we annotated the orthologous regions from *O. nivara*, *O. glaberrima*, and *O. punctata*. Sequence organizations of the five orthologous regions

Table 1 Size variation of the orthologous *Orp* regions of *Oryza* investigated

Species ^a	Genome designation	Genome size ^b (Mb)	BAC or contig	Size of BAC or contig	Size of corresponding region in	Size variation relative to <i>O. sativa</i> (kb)	
				(kb) ^c	<i>O. sativa</i> (kb) ^d	Contraction	Expansion
<i>O. nivara</i>	AA	448	OR_BBa0014L06	214	202		12
<i>O. glaberrima</i>	AA	354	OG_BBa0075G14/ OG_BBa0001L21	190	309	119	
<i>O. punctata</i>	BB	423	OP__Ba0008J05	192	339	147	
<i>O. australiensis</i>	EE	960	OA_Aba0108F22	190	401	212	
<i>O. brachyantha</i>	FF	338	OB__Ba0050L03	150	342	192	
Sorghum	HH	735	SB18C08	138 ^e	313 ^e	175	

^a*Oryza* species refers to Wing et al., 2005 and sorghum refers to Ma et al., 2005.

^bRefers to Bennett and Leitch, 2003.

^cEstimated by gel electrophoreses of restriction enzyme-digested fragments or sequencing.

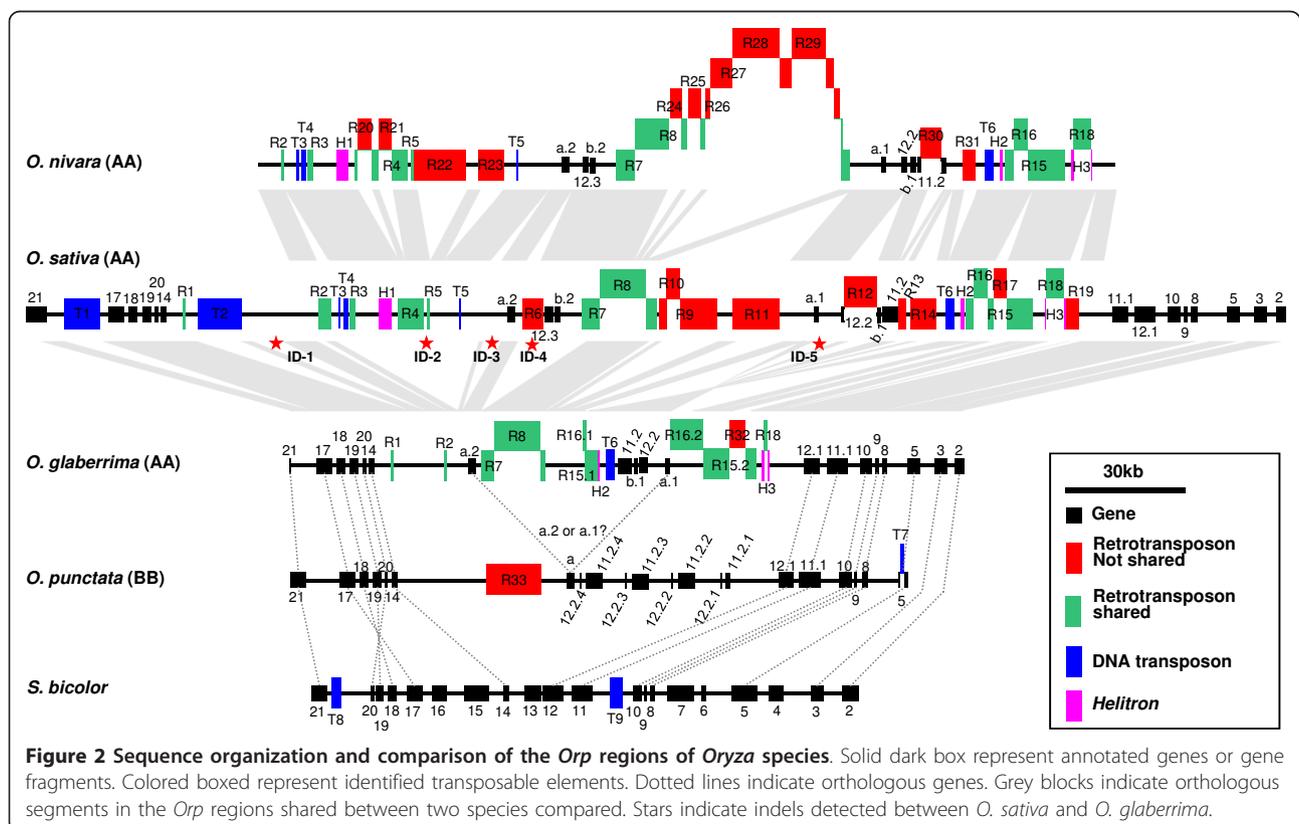
^d*O. sativa* subspecies, *japonica* (c.v., Nipponbare).

^eThe corresponding region comparable.

are illustrated in Figure 2 and detailed in Additional file 1, Table S1.

The 214-kb *O. nivara* region is comprised of 21 LTR-retrotransposons, 3 DNA transposons, 3 Helitrons, and 6 genes/pseudogenes. LTR-retrotransposons alone make up 121 kb of DNA sequence, accounting for 56.6% of the region. Although the *O. nivara* region is only 12 kb larger than its corresponding orthologous region in

O. sativa, it was found that 12 LTR-retrotransposons (74 kb of DNA) in the former and 9 LTR-retrotransposons (55 kb of DNA) in the latter, were not shared in the two regions. Each of these unshared elements is flanked by 5-bp target site duplication in its host region with a single copy of the 5-bp “target site” in the other orthologous region, suggesting that these elements inserted into the current positions after the divergence



of the two regions from their ancestral form. By contrast, all of the 3 DNA transposons and 3 *Helitrons* are shared in the two regions, suggesting that they were integrated before the divergence of the two regions. Overall, these two regions exhibit perfect colinearity in gene order and orientation, although gene 11.2 in *O. nivara* and gene 12.2 in *O. sativa* each had a LTR-retrotransposon insertion (Figure 2).

The 190-kb *O. glaberrima* region is comprised of 7 LTR-retrotransposons, 1 DNA transposon and 2 *Helitrons*. Nine out of these 10 transposable elements are shared by *O. glaberrima* and *O. sativa*, indicating that the *O. glaberrima* region did not expand substantially by the amplification of LTR-retrotransposons after its divergence from the *O. sativa* lineage. In addition to indels (insertions/deletions) generated by insertions of 2 DNA transposons (T1 and T2) and 8 LTR-retrotransposons (R9, R10, R11, R12, R13, R14, R17, and R18), and by the formation of a solo LTR (R2) through unequal recombination [26] in *O. glaberrima*, 5 relatively large indels (> 3 kb), ID-1, ID-2, ID-3, ID-4 and ID-5 (Figure 2) were observed between the two regions. Among these 5 indels, ID-2 is the largest (37 kb) and harbors 3 DNA transposons, 1 *Helitron*, and 3 LTR-retrotransposons present in *O. sativa*. Genes 12.3 and b.2 involved in ID-4 were present in *O. sativa* but absent in *O. glaberrima*. ID-5 appears to be a (4.3 kb) deletion that led to partial truncation of gene a1 and removal of a DNA transposon (4.3 kb) fragment in *O. glaberrima*. This deletion flanks the inverted segment in the *O. glaberrima* region that harbors genes 11.2, b1 and 12.2. The other breakpoint for this inversion is located within LTR-retrotransposon R16 (belonging to family *Osr14* [27,28]) and as a result R16 was separated into two fragments (R16.1 and R16.2). Interestingly, a deletion of ~4.3 kb internal sequence of R16 at this breakpoint was deduced by comparing R16.1 and R16.2 with typical intact elements of the *Osr14* family [27,28]. It is unclear whether the inversion led to the two flanking deletions or the latter caused the former. The *O. glaberrima* and *O. sativa* regions show overall colinearity except for the genic rearrangements described above.

The 148-kb *O. punctata* region has a single LTR-retrotransposon (R33), which is not shared by the three A-genome species. Based on the divergence of two LTRs of R33, it was estimated that this element was integrated into the region ~0.038 mya. This region shares perfect colinearity at the two gene clusters (i.e., genes 5, 8, 9, 10, 11, 12, and genes 14, 20, 19, 18, 17, 21) with the A-genome species, with the exception of a recent quadruplication of a segment containing two gene fragments (genes 11.2 and 12.2), which resulted in a substantial size increase of the interval between the two gene clusters in *O. punctata*.

The nature and history of genic rearrangements

Most duplicated genes interspersed in the intervals of the two highly conserved gene clusters are pseudogenes or gene fragments, in which the protein-coding sequences cannot be accurately predicted. Thus, the gene duplication events observed in this study could not be dated based on protein-coding sequences. To illuminate the history of the duplication events, we performed phylogenetic analysis of the duplicated genes within and across species using their genomic sequences (Figure 3). As shown in Figure 3A, gene 11.1 and gene 11.2 from the three species, *O. sativa*, *O. glaberrima*, and *O. punctata*, were grouped into two distinct branches (i.e., gene 11.1 branch and gene 11.2 branch), and the phylogeny reflected by either branch is consistent with the evolutionary relationship among the three species [23]. These data suggest that gene 11.1 and gene 11.2 were duplicated before the divergence of AA and BB genome species from a common ancestor. Gene 12.2 (12.2.1, 12.2.2, 12.2.3 and 12.2.4) in *O. punctata* was largely truncated, and thus excluded in the phylogenetic analysis. Gene 12.1 in the three species shows a similar phylogenetic pattern (Figure 3B) as revealed by gene 11.1 and 11.2,

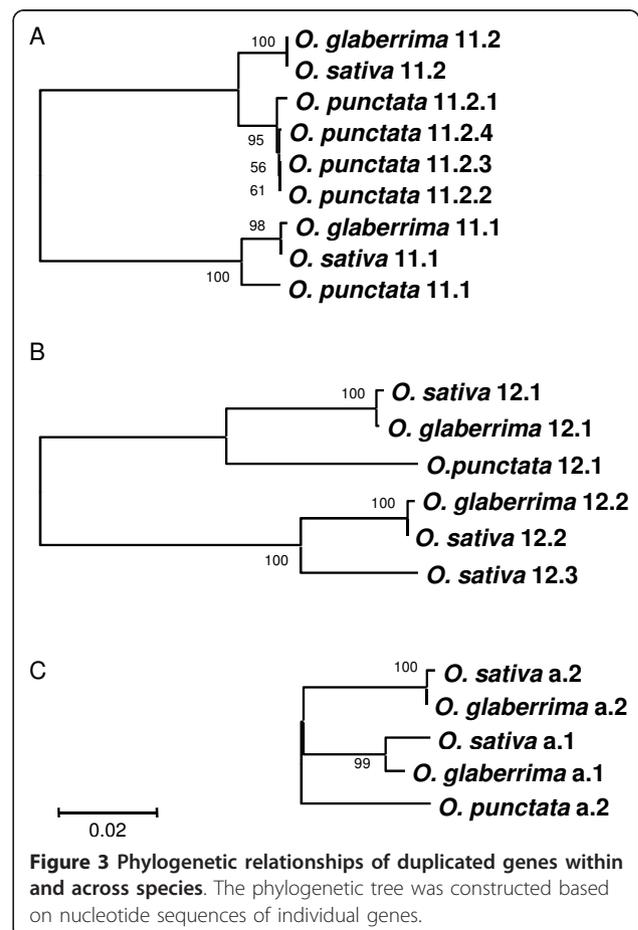


Figure 3 Phylogenetic relationships of duplicated genes within and across species. The phylogenetic tree was constructed based on nucleotide sequences of individual genes.

suggesting that the duplication of gene 12.1 and 12.2 also occurred before the divergence of the AA and BB genomes. The genetic distances between genes 11.1 and 11.2 and between genes 12.1 and 12.2 differ (Figure 3A and 3B), but the genes 11.1 and 12.1 and genes 11.2 and 12.2 were found to be arranged in the same order and orientation in the duplicated fragments. Thus, it is most likely that the duplication of both genes was caused by a single event.

Genes 12.2 and 12.3 in *O. sativa* grouped in the same branch, distinct to the branch of gene 12.1, suggesting that the duplication of 12.2 and 12.3 occurred after the first duplication event that predates the divergence of the AA and BB genomes. Gene b.1 is present in the AA genome species, but absent in the BB genome and sorghum. If the conserved segments containing genes 11.1 and 12.1 are ancestral copies of genes 11 and 12, the insertion of gene b (b.1 or b.2) must have occurred after the first duplication event. Because the three genes in each of the two gene clusters (a.2, 12.3 and b.2 cluster, and a.1, 12.2, and b.1 cluster) are arranged in the same order and orientation, it is likely that these three genes were duplicated by a single event before the divergence of the Asian and African AA genomes. The levels of sequence divergences between genes 12.2 and 12.3 and between genes a.1 and a.2 in *O. sativa* are similar (Figure 3B and 3C), reinforcing this conclusion. Assuming this deduction is correct, the absence of genes 12.3 and b.2 in *O. glaberrima* must be the outcome of deletion(s) at ID-4 site (Figure 2).

Phylogenetic analysis revealed that gene a (a.1 or a.2) in *O. punctata* is nearly equally distinct to genes a.1 and a.2 in either AA genome species (Figure 3C), suggesting that the duplication of the gene a (i.e., a.1 and a.2) occurred near the split of the AA and BB genome species. Thus, the orthologous copy/copies of genes a between the AA and BB genomes cannot be deduced based on their sequence similarities. Phylogenetic analysis indicates that the four recently amplified copies of gene 11.2 in *O. punctata* are orthologous to gene 11.2 in *O. sativa* and *O. nivara* (Figure 3A). In comparison with the proposed two orthologous regions in the AA genomes, genes 12.3, b.2, b.1 and a.1 were absent in the BB genome (Figure 2).

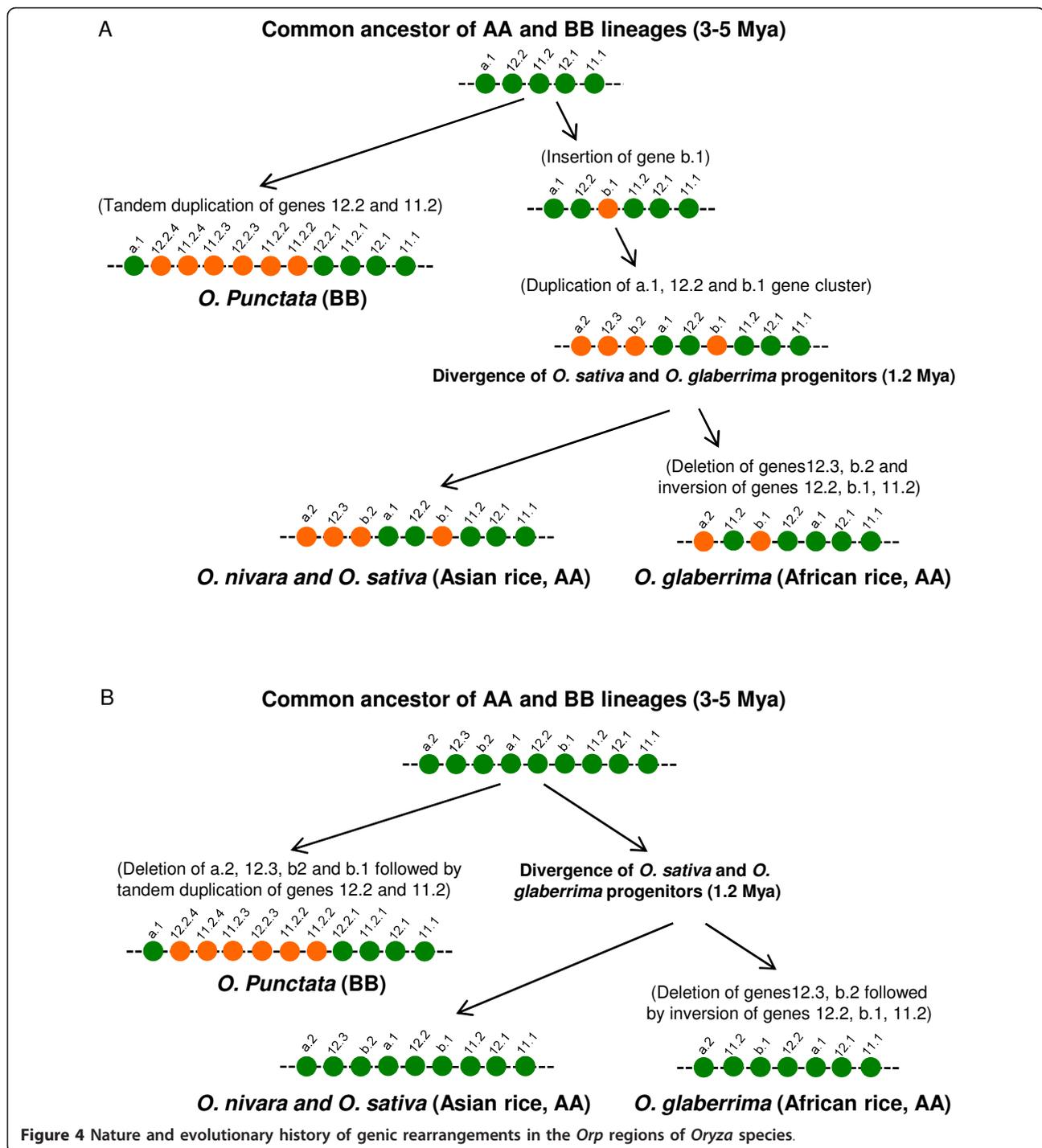
According to the analyses above, we propose two evolutionary scenarios regarding genic arrangements and rearrangements in the *Oryza Orp* regions. The first scenario, as illustrated in Figure 4A, proposes that the initial copy of gene a (i.e., a.1) inserted before the divergence of the AA and BB genome, and the initial copy of gene b (i.e., b.1) inserted only in the AA species after the AA and BB species divergence. The duplication event was followed by the duplication of the gene cluster (a.1, 12.2 and b.1) that generated genes a.2, 12.3, and

b.2 in the AA species. Based on this hypothesis, the absence of genes 12.3, b.2, b.1 and a.1 in the BB genome can be explained solely by “gain” of these genes in the *Orp* regions of the AA species. Of course, it is also possible that the insertions of initial copies of genes a and b and the subsequent duplication of the gene cluster (a.1, 12.2 and b.1) occurred before the divergence of the AA and BB species (Figure 4B). In this scenario case, the absence of genes seen in the BB genome could be explained by multiple gene deletion events, which is less parsimonious than the first scenario.

Regardless, our data revealed unusual structural instability in the *Oryza Orp* regions, including recent and rapid accumulation of LTR-retrotransposons and recent genic rearrangements. These genomic changes took place within an originally gene-rich euchromatic chromosome arm, reflecting a general plasticity of the *Oryza* genomes under the umbrella of local genic colinearity. Given that the structural variation of genomic regions can substantially affect chromatin states [29], frequencies of local recombination [13], and the expression/functionality of genes within or flanking the regions [30], the genomic plasticity revealed in this region, and probably many other genomic regions, as a general pattern, may have played a significant role, as proposed by Ginzburg et al. [31], in the processes of *Oryza* genome speciation.

Population analysis of haplotype variation of LTR-retrotransposon insertions and segmental inversion in the AA species

Previous investigation of the *bz* genomic region in seven different maize inbred lines revealed remarkable variation in the maize genome, structure mediated by transposable elements [17,32]. Similar to the *bz* region, the *Orp* regions of the three *Oryza* AA genomes show a high level of polymorphisms of LTR-retrotransposon insertions (Figure 2). To further track whether a particular LTR-retrotransposon is present at high frequencies or fixed within a species/subspecies at population levels, we investigated the presence or absence of a set of LTR-retrotransposons identified in the *Orp* regions by PCR amplification of transposon insertion junctions in 95 AA genome varieties, following a protocol previously described by Devos et al. [1] (see details in Materials and Methods). These 95 varieties, including 46 *O. sativa*, 20 *O. nivara*, 24 *O. rufipogon* and 4 *O. glaberrima* and 1 *O. barthii* accessions (Additional file 2, Table S2) were chosen based on their geographic distribution and genetic diversity estimated by SSR and SNP markers [33-35]. The results of PCR analysis are illustrated in Figure 5 and Additional file 3, Figure S1. The primers designed for PCR analysis are listed in Additional file 4, Table S3.



R7 and R15, two representative LTR-retrotransposons shared by the three AA genomic regions, were detected in all the 94 AA genome varieties (Figure 5). In other words, these two insertions were fixed during the evolution and divergence of the AA genome species. It was estimated that these two elements were inserted approximately 2.1 and 1.2 million years ago. In general, LTR-retrotransposons tend to accumulate in low

recombination heterochromatic regions where selection is expected to be less efficient in removing them [36]. Thus, the fixation of the insertion of these two elements inserted before the divergence of African and Asian rice lineages in the “originally” gene-rich *Orp* region with high rate of recombination [28] would suggest that these elements may have played or be playing an adaptive role.

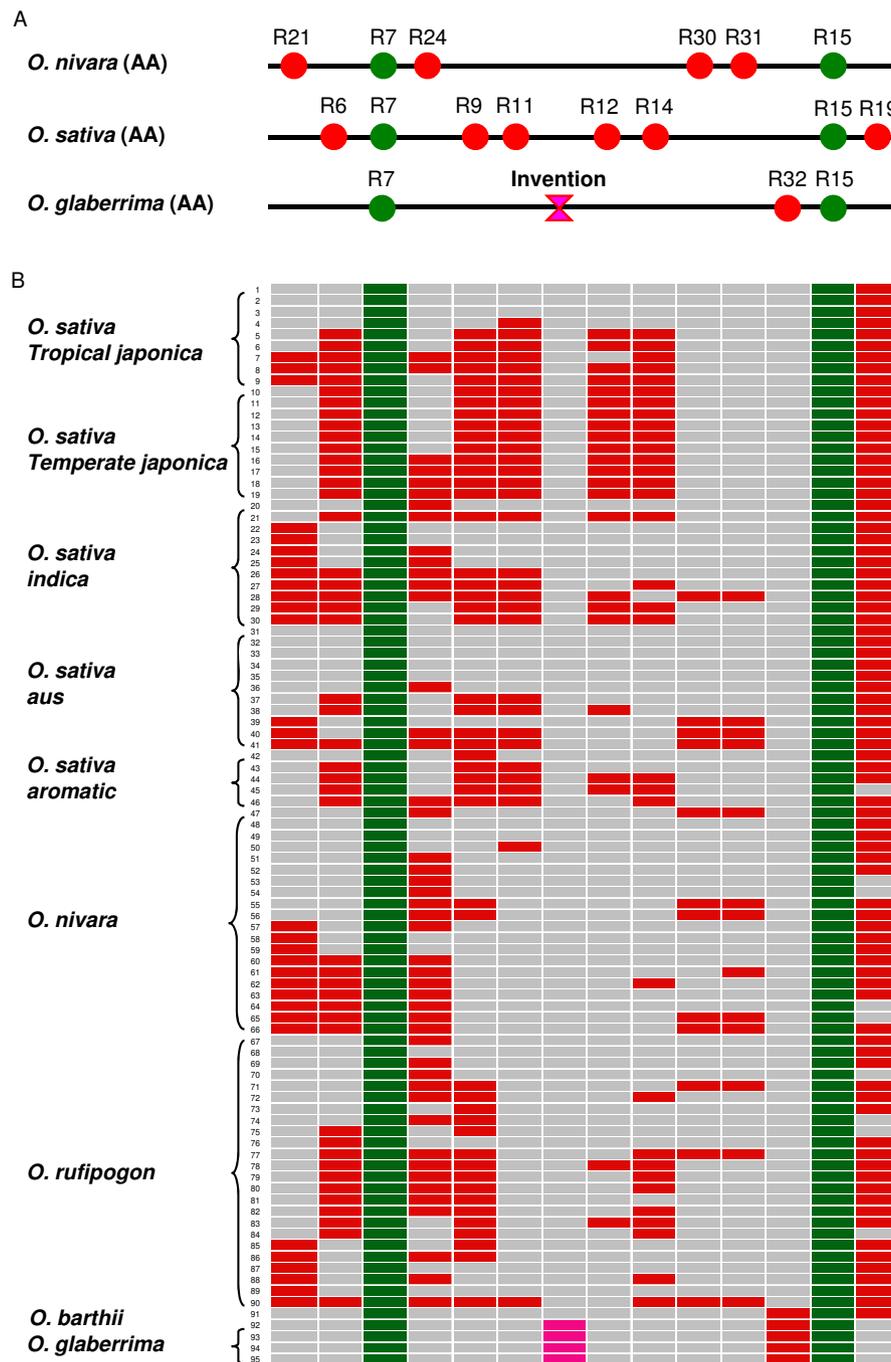


Figure 5 Presence and absence of LTR-retrotransposons in the AA-genome variety populations. (A) Elements investigated by PCR and their distribution in the three sequenced *Orp* regions. (B) The presence (red or green) or absence (grey) of individual LTR-retrotransposons detected by amplification of retrotransposon junctions. Varieties from top to bottom are listed in the same orders as shown in Additional file 2, Table S2.

We also investigated 11 LTR-retrotransposons that are not shared among the three sequenced *Orp* regions, including 6 (R6, R9, R11, R12, R14, and R19) unique to the *O. sativa* region, 4 (R21, R24, R30, and R31) unique to the *O. nivara* region, and 1 (R32) unique to the *O. glaberrima*

region. These elements were relatively young (Additional file 1, Table S1) with an average age of 0.15 million years. R32 was found in the four *O. glaberrima* varieties, but absent in all Asian AA varieties, suggesting that this element was inserted into the African AA lineage after

its divergence from the Asian AA lineage. R19 was found in 47 out of the 48 *O. sativa* varieties, being absent from a single *aromatic/GroupV* accession, and it was detected in 16 out of the 20 *O. nivara* varieties and 20 out of 24 *O. rufipogon* varieties. Interestingly, R6, R9, R11, R12 and R14, which distinguished Nipponbare from *O. nivara*, were fixed in the 10 *temperate japonica* varieties, while R21, R30 and R31, which were present in the sequenced *O. nivara* BAC, were completely absent from the *temperate japonica* subpopulation (Figure 5). A similar pattern was seen for R21, R30 and R31 (all absent), and for R6, R9 and R11 (all present) in the *aromatic/GroupV* accessions, which are known to be closely related to *temperate japonica* at the genetic level [37]. On the other hand, the *tropical japonica* varieties, as a group, were more similar to the *indica* and *aus* varieties across this region, with varying frequencies of LTRs matching those found in Nipponbare and/or *O. nivara*. Interestingly, the *indica* variety, 9311, which is known to have *japonica* parentage, shares a regional haplotype with *temperate japonica*, as do the two Indonesian *tropical japonica* varieties, Gotak Gatik and Trembese, while most of the others in this group carry the *O. nivara* alleles at R9, R112, R12 and R14. The *indica* subpopulation is almost fixed for the *O. nivara* allele at R21, and is highly variable across all other markers. The *aus* subpopulation is distinguished from the other *O. sativa* groups by a higher frequency of accessions carrying R30 and R31 and the complete absence of accessions carrying both R12 and R14. In these ways, *aus* more closely resembles *O. nivara* than other *O. sativa* varieties across this region. These observations are consistent with previous studies showing an intense genetic bottleneck in *temperate japonica* but greater variation in the *indica* and *aus* subgroups [33,38] and substantial admixture among the various subpopulations, most notably in *tropical japonica* [35].

Based on the presence/absence of all these LTR-retrotransposons, "phylogenetic relationships" of these varieties were analyzed. As shown in Additional file 5, Figure S2, the analysis clearly separated the African species, *O. glaberrima* and *O. barthii* (the proposed wild progenitor of *O. glaberrima*) from the Asian species, and the *indica* and *aus* subpopulations clustered at one end of the graph along with most of the *O. nivara* and some of the *O. rufipogon* accessions, well separated from the majority of *aromatic/Group V*, *temperate* and *tropical japonica* varieties that clustered with a different set of *O. rufipogon* accessions. This analysis further identifies admixed *tropical japonica*, *indica* and *aus* varieties that cluster with several *O. nivara* and *O. rufipogon* accessions in the middle of the graph. Several *indica* varieties are seen clustering with the *japonica* group, reflecting the greater genetic variation and mixed parentage of many *indica* varieties, as previously noted for *c.v.* 9311. Thus, this graphical display reflects the

taxonomy of these species and subspecies as established by SNP and/or SSR analyses, providing an interesting window on a highly variable region of the rice genome [33-35].

Several hypotheses can explain the observed lability of LTR-retrotransposons. 1) Since the regions are highly instable and plastic, there may be a high level of lineage sorting going on in present-day populations derived from a very diverse set of ancestral haplotypes. Each descendant population may inherit a large subset of the ancestral haplotypes, which continue to segregate in the descendants. In theory, over evolutionary time they should sort out such that each group has its own distinct haplotype/haplotypes that are more closely related to each other than to haplotypes from other species. However, because of the relatively short time that has elapsed for the speciation of the Asian AA genomes, the haplotypes of LTR-retrotransposons remain largely unsorted. 2) The lability can be explained by intra-specific and inter-specific introgression, which may have occurred during speciation of these genomes [35,39,40]. 3) Balancing selection for recent LTR-retrotransposon insertions may contribute to the high level of insertion polymorphisms, although adaptive selection and/or genetic bottlenecks affecting the two relatively old elements, R7 and R15, was suggested. Further investigation of a larger collection of wild and cultivated germplasm and more LTR-retrotransposon insertions at a larger genomic scale would help to reveal the dynamics of retention and/or removal of LTR-retrotransposons and their contributions to genomic diversity and speciation.

The inverted segment harboring genes 11.2, b1 and 12.2 in the sequenced *O. glaberrima* region (Figure 2) was detected in all other *O. glaberrima* accessions analyzed by PCR approach, but absent in the *O. barthii* accession and all Asian AA-genome *Oryza* species/subspecies (Figure 5 and Additional file 3, Figure S1). This suggests that the inversion occurred in African rice after its divergence from Asian rice. Because only a single *O. barthii* accession was included in this analysis, it remains unclear whether the inversion took place before or after the domestication of *O. glaberrima* from *O. barthii*.

The *Orp* region is located near the end of the short arm of rice chromosome 8, but harbors a high proportion of LTR-retrotransposons similar to that observed in the centromeric region of this same chromosome. Thus, it is likely that the region has recently switched from euchromatic to heterochromatic states.

Conclusions

Our data indicate that the *Orp* genomic complex in rice cultivars and their wild progenitors have been recently, independently and concurrently formed from a gene-rich region by differential insertion of LTR-retrotransposons

and genic rearrangement, and that the overall haplotype variation of LTR-retrotransposon insertions in this region echoes to the admixture pattern of genomic diversity and introgression of AA-genome populations/subpopulations revealed by genome-wide SSR and SNP genotyping, thus highlighting the evolutionary roles of LTR-retrotransposons in plant speciation and diversification. Genome-wide profiling of LTR-retrotransposon insertions among the AA-genome cultivars at larger population levels would enhance our understanding of the evolutionary processes and dynamics of the rice genomes.

Methods

Identification of BAC clones

The entire *Orp* region of *O. sativa* and it flanking 150-kb sequences from both ends of the region were searched against the BESs of other *Oryza* species generated by OMAP [19]. Single BAC clones meeting the following criteria: 1) at least one unique end; 2) both ends aligned to the extended *Orp* region of *O. sativa* in forward/reverse pairs; and 3) both ends spanning 100 to 500 kb of *O. sativa* sequences, were considered to be the orthologous segments from the respective *Oryza* species. As the major objective of this study was to target the genomic space corresponding to the hotspot of the transposable element accumulation and genic rearrangement in *O. sativa*, we only selected and analyzed a minimum number of clones from *Oryza* species that maximally cover the target region, as shown in Figure 1.

BAC Sequencing

Shotgun libraries for selected BAC clones were constructed as described previously [41]. Subclones were sequenced from both directions using ABI PRISM Big-Dye Terminator Chemistry (Applied BioSystems, Foster, CA) and run on an ABI3730 capillary sequencer. BAC clones were sequenced at approximately 8-10-fold redundancies, and then were assembled and finished to standard high quality sequences (PHASE III) by primer walking [6]. The assemblies of sequenced BAC clones were confirmed by restriction map analysis similar to the method described by Dubcovsky et al. [41].

Sequence annotation

Putative gene models were predicted using the FGENESH program with the monocot training set (<http://www.softberry.com>), and were further investigated to determine whether they are actually genes following described previously criteria [6]. Truncated gene fragments were identified by sequence homology comparison using BLAST2 [42], DOTTER [43] and CROSS_MATCH (<http://www.phrap.org>). LTR-retrotransposons were identified and classified as described previously [9]. DNA transposon fragments were identified by homology

searches against the TIGR plant repeat database [44], GenBank non-redundant protein database, and *pack_MULE* database [45]. *Helitrons* were identified using a perl script described previously [46].

Dating of segmental duplication and retrotransposon insertions

The alignments of homologous nucleotide sequences were generated by using ClustalX [47]. The dates of segmental duplication and amplification of LTR-retrotransposons were estimated as described previously [10]. The phylogenetic trees of duplicated genes were constructed based on pair-wise comparison of nucleotide sequences using the Kimura two-parameter method provided by MEGA4 program [48]. The Neighbor-Joining tree based on the presence/absence of LTR-retrotransposon insertions were obtained using MEGA4.

The genomic sequences generated in this study has been deposited in GeneBank (Nos. HM999006-HM999008).

Additional material

Additional file 1: Sequence annotation of different BACs in this study. * 1605848 to 2060871 region of *O. sativa* subspecies *japonica* (c.v., Nipponbare) genome sequence (Pseudomolecule 4.0). ** Intact-td, intact element flanked by target site duplication; solo-td; solo LTR flanked by target site duplication; intact-notsd, intact element without target site duplication; solo-notsd; solo LTR without target site duplication; &, internal sequence of LTR-retrotransposon; s, truncated fragment.

Additional file 2: The AA-genome *Oryza* varieties used in this study. These 95 varieties, including 46 *O. sativa*, 20 *O. nivara*, 24 *O. rufipogon*, 4 *O. glaberrima*, and 1 *O. barthii* accessions, were chosen based on their geographic distribution and genetic diversity.

Additional file 3: PCR verification of a segmental inversion present in *O. glaberrima*, but absent in *O. sativa* and *O. nivara* varieties. (A) Schematic primer design for amplification of inversion boundaries. (B) PCR products of primer pairs F1/R1 (panel b) and F2/R2 (panel c), and *Waxy* genes (panel a, control). Primers are shown in Additional file 4. Varieties are numbered according to their orders in Additional file 2.

Additional file 4: Primers used in this study. Primers used to check the polymorphisms of LTR-retrotransposon insertions and the inversion in different species/subspecies.

Additional file 5: Neighbor-Joining "Phylogeny" of the AA-genome varieties constructed using MEGA4 based on the presence/absence of a set of LTR-retrotransposons in individual varieties as illustrated in Figure 5. Varieties are numbered according to their orders shown in Figure 5 and Additional file 2.

Acknowledgements

We thank Jennifer Kimball of Cornell University for sending DNA extractions of the 95 *Oryza* accessions used in this analysis, Michael Purugganan for constructive comments and discussion on this work, and Teri Rambo and Paul Parker for technical support. This study was supported by National Science Foundation grant nos. IOS_0424833, DBI 0321678, DBI 0501877, and DBI 0606461) and the Purdue University College of Agriculture.

Author details

¹Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA.

²Arizona Genomics Institute, The University of Arizona, Tucson, AZ 85721,

USA. ³Genomics Core Facility, Purdue University, West Lafayette, IN 47907, USA. ⁴Department of Plant breeding and Genetics, Cornell University, NY 14853, USA. ⁵Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA.

Authors' contributions

ZT, YaY, FL, YeY, and PJS carried out the BAC sequencing and haplotype variation experiments. ZT, PJS, RAW, SRM, JM and SAJ participated in data analysis, ZT, SRM and JM contributed to the manuscript preparation. All authors read and approved the final manuscript.

Received: 18 November 2010 Accepted: 8 March 2011

Published: 8 March 2011

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doi:10.1186/1471-2164-12-142

Cite this article as: Tian *et al.*: Exceptional lability of a genomic complex in rice and its close relatives revealed by interspecific and intraspecific comparison and population analysis. *BMC Genomics* 2011 **12**:142.

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