Supporting Information

Yamagata et al. 10.1073/pnas.0908283107

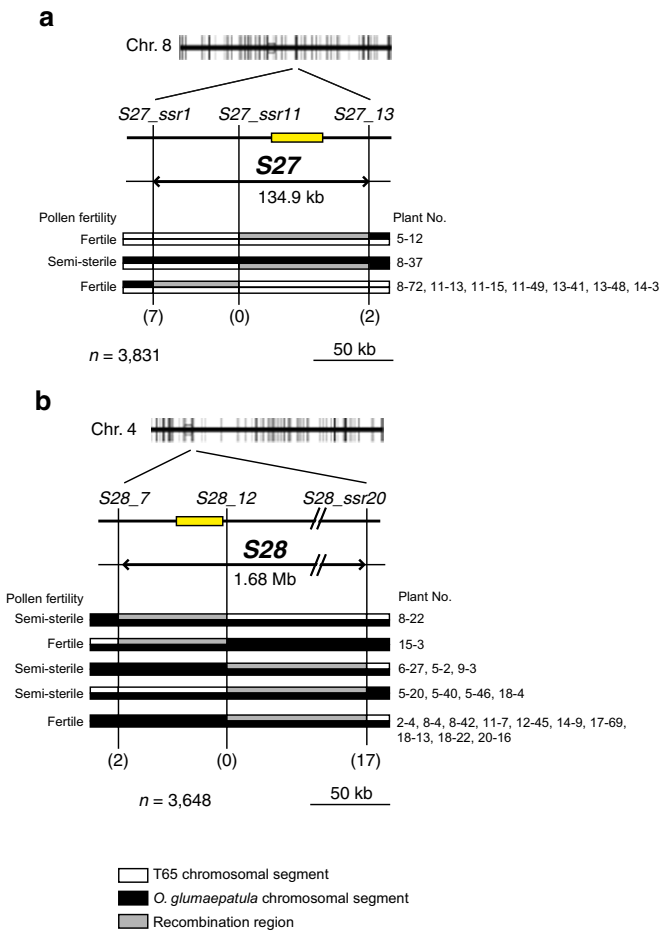

Fig. S1. High-resolution mapping of *S27* (*A*) and *S28* (*B*). Pollen fertility and graphical genotypes of the most informative plants for the high-resolution mapping of *S27* and *S28* are shown. Numbers of recombinants between the genes and markers are indicated in parentheses. Primers of the markers are listed in Table S2. White and black boxes show T65 and *O. glumaepatula* chromosomal segments. Recombination regions are gray. Yellow boxes indicate the location of duplicated genomic segments of *S27* and *S28* found in the rice reference sequence of Nipponbare.

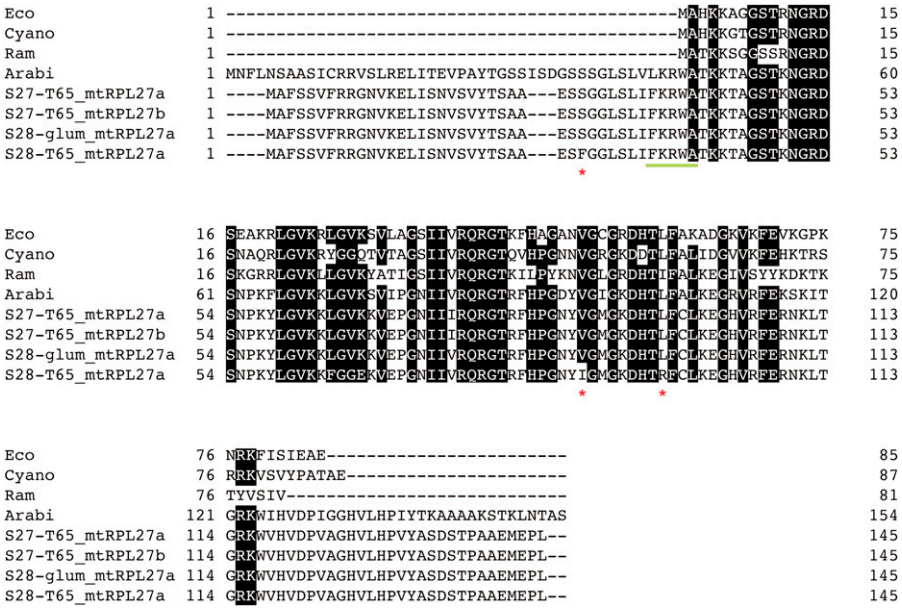

Fig. S2. Multiple alignments of the amino acid sequences of mtRPL27 proteins deduced from the *S27-T65*$^{+}$, *S28-glum*$^{+}$, and *S28-T65*^{s} alleles. The amino acid sequences were deduced from the *S27-T65*$^{+}$ and *S28-glum*$^{+}$ transcripts and the *S28-T65*^{s} predicted gene. Black boxes indicate identical residues in more than seven sequences. Red asterisks show the positions of the amino acid substitutions found only in the mtRPL27 protein deduced from the *S28-T65*^{s} allele. Eco, *Escherichia coli* rpl27 (GenBank accession P02427); Cyano, the cyanobacterium *Synechocystis* sp. RPL27 (NP_441681); Ram, *Reclinomonas americana* (NP_044787); Arabi, *Arabidopsis thaliana* putative mitochondrial RPL27 (BAC43174). The green line indicates the signal peptide sequence for transporting mtRPL27 into mitochondria. The location of the signal peptide sequence is taken from Ueda et al. (1).

1. UedaM et al.Promoter shuffling at a nuclear gene for mitochondrial RPL27. Involvement of interchromosome and subsequent intrachromosome recombinations*Plant Physiol*141702710.

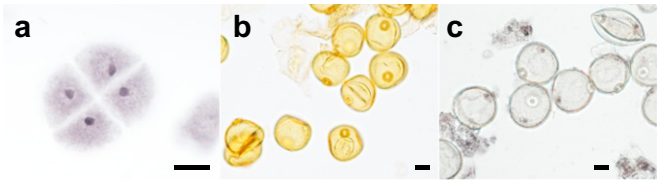

Fig. S3. Observation of the pollen phenotype at the tetrad and unicellular stages. (*A*–*C*) The pollen at the tetrad (*A*) and unicellular (*B* and *C*) stages was observed in *S28* semisterile plants under light microscopy. To detect nuclei and starch accumulation, hematoxylin staining (*A* and *C*) and I_2–KI staining (*B*) were performed. No phenotypic abnormality was observed at these stages. Scale bars: 10 μm.

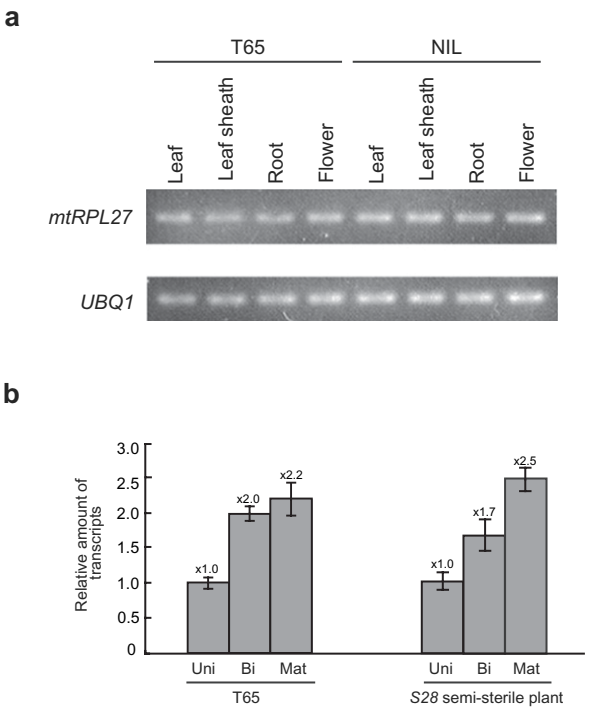

Fig. S4. Expression analysis of *mtRPL27*. (*A*) RT–PCR of *mtRPL27* using RNA extracted from leaf, leaf sheath, root, and flower in T65 and NIL carrying the genotype *S27-glum*^{s}/*S27-glum*^{s}/*S28-glum*$^{+}$/*S28-glum*$^{+}$. Transcriptions of *mtRPL27* were observed in all these tissues. (*B*) Quantitative RT–PCR analysis of *mtRPL27* at each pollen developmental stage. About twofold greater expression of *mtRPL27* was detected at the bicellular and mature stages compared to the unicellular stage in both T65 and the *S28* semisterile plants. Total RNA was extracted from anthers. The genotypes of T65 and the *S28* semisterile plants were *S27-T65*$^{+}$/*S27-T65*$^{+}$/*S28-T65*^{s}/*S28-T65*^{s} and *S27-glum*^{s}/*S27-glum*^{s}/*S28-T65*^{s}/*S28-glum*$^{+}$, respectively. The relative expression of each allele was normalized using the expression of *UBQ1* (*Os03g0234200*). Data represent the average of three replicates of the first-strand cDNA. Error bars indicate the standard error of the mean (SEM).

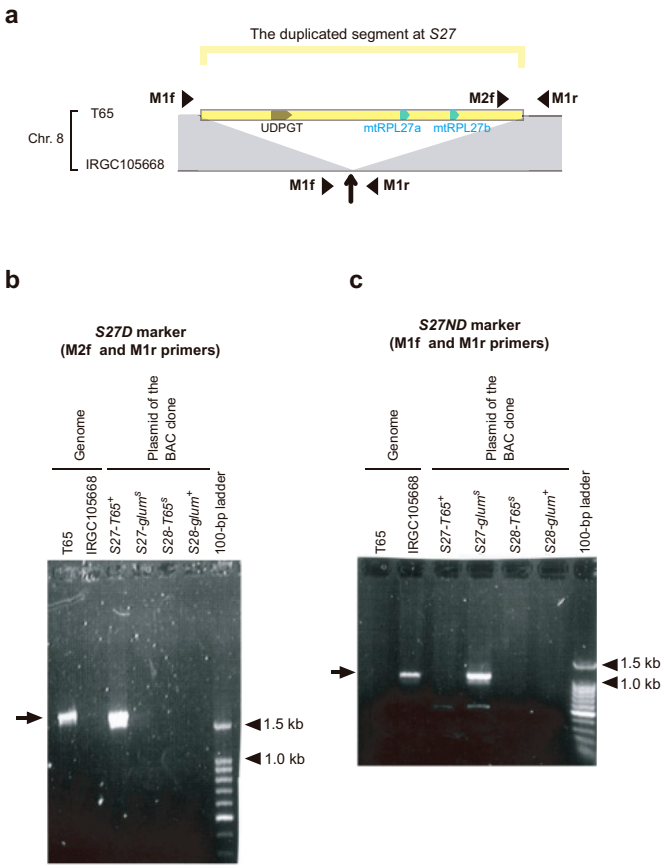

Fig. S5. PCR markers used to detect the presence and absence of the duplicated segment at *S27*. (*A*) Locations of the primers in the T65 and IRGC105668 genomic regions. Arrowheads indicate the location and direction of the primers. An arrow shows the location of the absence of the duplicated segment at *S27-glum*^{s}. For the *S27D* marker used to detect the presence of the duplicated segment at *S27*, primers M2f and M1r were used. For the *S27ND* marker used to detect the absence of the duplicated segment at *S27*, primers M1f and M1r were used. (*B* and *C*) PCR amplification using the *S27D* and *S27ND* markers. The BAC clones GN21L06 and GN27D10, derived from T65, and GL12L12 and GL17D14, derived from IRGC105668, are represented by *S27-T65*$^{+}$, *S28-T65*^{s}, *S27-glum*^{s}, and *S28-glum*$^{+}$, respectively. (*B*) The PCR product obtained from the T65 genome using the *S27D* marker was amplified from the genomic sequence at the *S27* region (arrow). (*C*) The PCR product obtained from the IRGC105668 genome using the *S27ND* marker was amplified from the genomic sequence at the *S27* region (arrow).

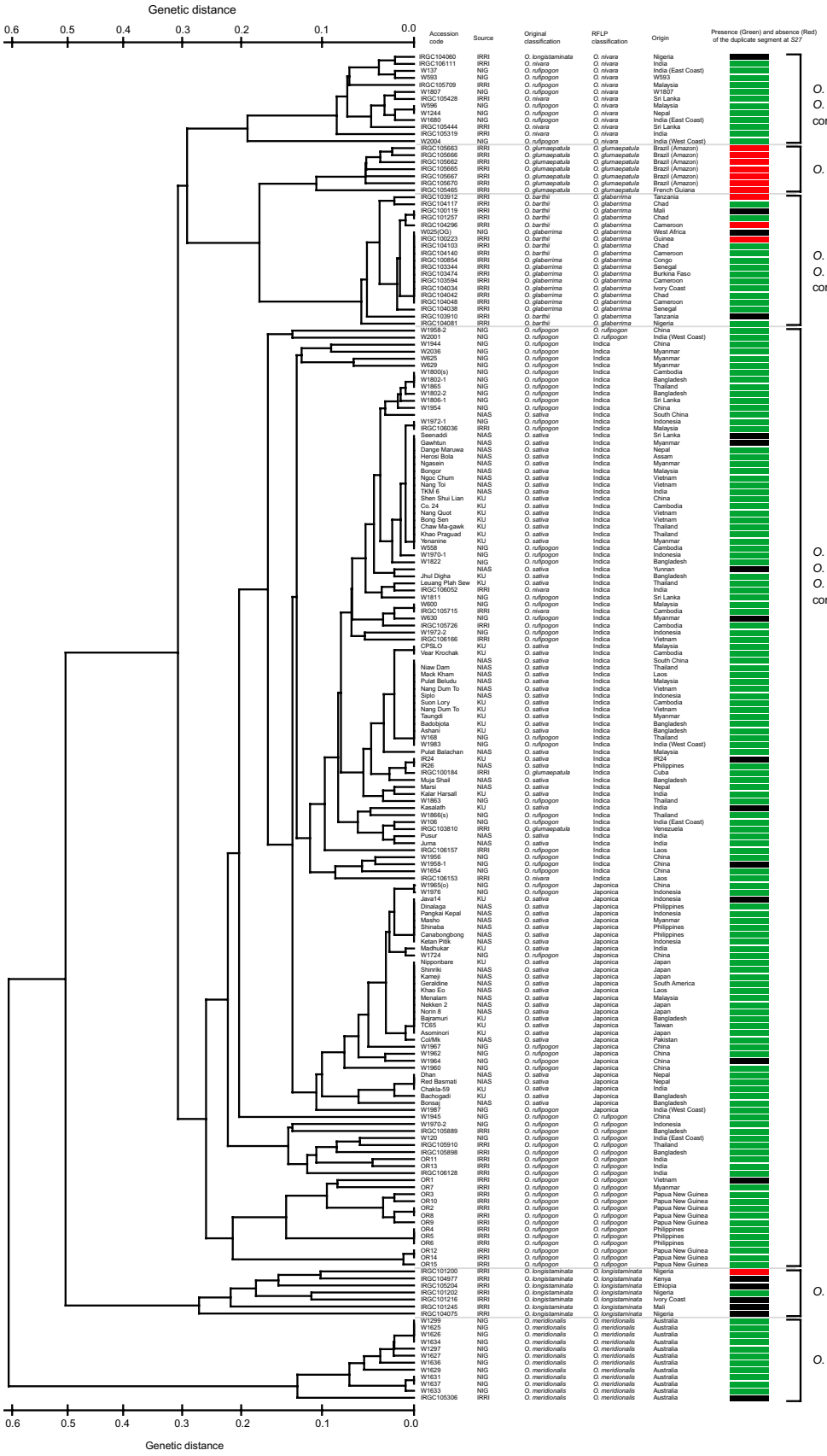

Fig. S6. Detailed distribution of the duplicated segment at *S27* among the AA genome species. Green and red boxes indicate the accessions in which the duplicated segment at *S27* was present and absent, respectively. Black boxes indicate the accessions for which PCR amplification was not obtained or for which

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DNA samples were not available. AA genome species accessions were classified on the basis of restriction fragment length polymorphisms (1). The seeds or DNA were provided by the National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan; the National Institute of Genetics (NIG), Mishima, Japan; the International Rice Research Institute (IRRI), Los Baños, Philippines; and Kyushu University (KU), Fukuoka, Japan.

2. DoiKNonomuraMNYoshimuraAIwataNVaughanDARFLP relationships of A-genome species in the genus Oryza*J Fac Agr Kyushu Univ*458398.

Table S1. Chi-square test of the observed ratio of genotypes in the progenies of the NIL113

Genotype*			No. of individuals		
S27_ssr1	*S28_10*	Expected ratio	Expected	Observed	χ^2
TT	TT	1	10.2	15	2.3
	TG	2	20.3	23	0.3
	GG	1	10.2	10	0.0
TG	TT	1	10.2	8	0.5
	TG	3	30.5	20	3.6
	GG	2	20.3	30	4.6
GG	TT	—	—	0	—
	TG	1	10.2	10	0.0
	GG	1	10.2	6	1.7
				χ^2_{Total} = 13.0	
				n = 8	
				df = 7	
				P = 0.072†	

*TT, TG, and GG indicate T65 homozygous, heterozygous, and IRGC105668 homozygous, respectively.
†The observed segregation ratio of the genotype fit the expected ratio at the 5% significance level.

Table S2. PCR primers used in the high-resolution mapping of *S27* and *S28*

Marker	Marker type	Forward primer sequence	Reverse primer sequence	Restriction enzyme for CAPS marker
S27_ssr1	SSR	ATGCGAAGGCAATGAAAAAG	TGAAGCACAACGCTAACAGAG	
S27_ssr11	SSR	TGGTGTTAGCTAGGAGGCTAAA	GGGATTTAAAGCCAACATTGA	
S27_13	CAPS	GTGCTAGCTTGGGCCTCTATTC	CCAAACGAGGCACTTTTCATAC	*Eco*RI
S28_7	SNP	TGTCATTTGTGTGGTCTGGTTC	ATGCAGAGCACATCATGGATAC	
S28_12	SNP	GAAGCGCTCCTAGCTGTTTCTC	ATGGTACTTTGGAGGGCAAGTC	
S28_ssr20	SSR	CATGTCCAAAAGTCAACAACG	AAAGTGGGCTTTTTCCTTGG	

SSR, simple sequence repeat; CAPS, cleaved amplified polymorphic sequence; SNP, single nucleotide polymorphism.

Table S3. Locked-nucleic-acid-containing primers used for allele-specific RT–PCR

		Primer sequence	
Target allele	Allele type	Forward primer sequence*	Reverse primer sequence*
S27_T65$^+$	Fertile allele	CATCATCATCCGCCAAAGAG	CTTGTTGCGCTCGAATCGC
S28-T65^s	Sterile allele	CATCATCGTCCGTCAAAGAG	CTTGTTGCGCTCGAATCGC
S28-glum$^+$	Fertile allele	CATCATCGTCCGCCAAAGAG	CTTGTTGCGCTCGAATCGC

*The locations of the locked nucleic acids are underlined.