Asian rice (Oryza sativa L.) is consumed by more than half of the world’s population. Despite its global importance, the process of early rice domestication remains unclear. During domestication, wild rice (Oryza rufipogon Griff.) acquired non-seed-shattering behavior, allowing humans to increase grain yield. Previous studies argued that a reduction in seed shattering triggered by the sh4 mutation led to increased yield during rice domestication, but our experiments using wild introgression lines show that the domesticated sh4 allele alone is insufficient for shattering loss in O. rufipogon. The interruption of abscission layer formation requires both sh4 and qSH3 mutations, demonstrating that the selection of shattering loss in wild rice was not as simple as previously suggested. Here we identified a causal single-nucleotide polymorphism at qSH3 within the seed-shattering gene OsSh1, which is conserved in indica and japonica subspecies but absent in the circums-aus group of rice. Through harvest experiments, we further demonstrated that seed shattering alone did not significantly impact yield; rather, yield increases were observed with closed panicle formation controlled by SPR3 and further augmented by nonshattering, conferred by integration of sh4 and qSH3 alleles. Complementary manipulation of panicle shape and seed shattering results in a mechanically stable panicle structure. We propose a stepwise route for the earliest phase of rice domestication, wherein selection of visible SPR3-controlled closed panicle morphology was instrumental in the sequential recruitment of sh4 and qSH3, which together led to the loss of shattering.

Oryza sativa | Oryza rufipogon | domestication | seed shattering | closed panicle

The selection of naturally occurring variations in wild plants that provide useful agronomic traits is an essential step in crop domestication. These traits are often related to yield, such as seed number, seed size, and a loss of seed dispersal, or to ease of cultivation, including plant architecture, seed dormancy, and photoperiod sensitivity. Plants with these traits provided the necessary impetus for humans to shift from hunting and gathering to cultivation. Among several domestication-related traits, the suppression of seed shattering is reported to be the most important genetic change that allowed humans to increase harvestable grain yield differentiating domesticated from wild plants (1, 2). However, the role of visible morphological changes in facilitating domestication is debated.

Rice (Oryza sativa L.) is consumed by almost half of the world’s population and is particularly important in Asia. During its domestication, wild rice (Oryza rufipogon Griff.) was transformed by changes in plant and panicle architecture as well as by acquiring non-seed-shattering behavior. Investigations of prehistoric rice spikelet bases from archaeological sites demonstrate that suppression of seed shattering leaves a phenotypic trace that can be observed through the examination of the abscission layer (3). Archaeological rice spikelet bases with rough and deeply torn scars in the spikelet base (rachilla) indicate that humans had to actively detach the spikelets from the pedicels by threshing. More than a decade ago, two seed-shattering loci, sh4 and qSH1, were identified as essential to rice domestication (4, 5). The mutation at sh4 was largely viewed as the principal genetic change that led to rice domestication. Quantitative trait locus (QTL) analysis identified sh4 as the locus responsible for the difference in seed-shattering behavior between an accession of wild rice Oryza nivara (an annual form of O. rufipogon) and O. sativa ssp. indica. A single-nucleotide polymorphism (SNP) at sh4 was found to affect the function of the trihelix transcription factor due to an amino acid change in its DNA-binding domain, resulting in suppression of seed shattering (4) (SI Appendix, Table S1). qSH1 was also detected as the QTL largely responsible for the difference in seed-shattering degree between the rice cultivars O. sativa ssp. japonica and O. sativa ssp. indica. A SNP of qSH1 resulted in suppression of seed shattering in O. sativa ssp. japonica cv. Nipponbare by downregulating
expression of the downstream gene OsRIL1 encoding a BEL1-type homeobox family protein (5) (SI Appendix, Table S1). Since sh4 and qSH1 display large phenotypic variances, and rice cultivars with functional alleles of sh4 or qSH1 promote seed shattering, they are recognized as crucial to rice domestication.

To better understand if the domestication process of Asian rice was promoted mainly by a single domestication gene, such as sh4, and also to see how qSH1 and sh4 are involved in the loss of seed shattering in wild rice, we previously developed introgression lines (ILs) containing small chromosomal segments from O. sativa Nipponbare at the qSH1 and sh4 loci, referred to as IL(qSH1-N) and IL(sh4-N), respectively, in the genetic background of wild rice O. rufipogon W630 (6). Complete seed shattering was still observed in these lines (6–8), indicating that neither of the single mutations at qSH1 or sh4 on its own could account for nonshattering leading to rice domestication. As the sh4 mutation is commonly observed in cultivated rice, while the qSH1 mutation is only found in some japonica cultivars (5), additional mutation(s) together with sh4 must have played a role in reducing seed shattering during the early stages of rice domestication. Such quantitatively inherited mutations reducing shattering behavior represent an impediment to selection of shattering loss, which does not occur on the basis of a simple recessive mutation. Thus, it is plausible that the domestication process in rice is more complicated than the previously proposed single domestication allele model.

A change in panicle structure from open to closed may have played a crucial role in mitigating seed-shattering behavior prior to the selection of nonshattering rice (7). SPRO3 is responsible for closed panicle formation and acts as a cis-regulatory element controlling expression of the downstream gene OsL1G1, which encodes a SQUAMOSA promoter-binding protein (7). A closed panicle structure encases the mature seeds, which are retained in the upper part of the panicle due to the long awns present on the lower immature seeds, potentially making these plants more attractive to gatherers. By choosing rice plants with the SPRO3 genetic mutation, gatherers would have increased their collection rate, particularly if the plants with closed panicles also exhibited suppressed seed shattering. In addition, selection of the closed panicle formation also brought about increased self-pollination rates. Therefore, this trait may have potentially contributed to the accumulation and fixation of multiple favorable mutations, leading to the evolution of non-seed-shattering plants thereafter.

In this study, we conducted a genetic analysis of qSH3, a locus involved in the loss of seed shattering together with sh4, and identified the causal mutation selected during rice domestication. The frequency of the qSH3 alleles in rice cultivars and their effects on the loss of seed shattering were also evaluated. We further conducted seed-gathering experiments, which showed how slight genetic changes can significantly impact yields. Finally, we assessed the potential role of closed panicle formation on reducing seed shattering and the relationship between these two distinct traits, seed shattering and closed panicle formation, based on structural mechanical analysis to better understand the selection process toward a loss of seed shattering in rice.

Results and Discussion

Identification of the Causal Mutation at qSH3, a Locus Involved in the Loss of Seed Shattering During Rice Domestication. To identify additional genomic regions involved in reducing seed shattering, we previously produced an IL with chromosomal segments of O. sativa Nipponbare at both sh4 and qSH1 in the genetic background of wild rice O. rufipogon W630 (8) (SI Appendix, Fig. S1). IL(qSH1-N, sh4-N), which contains small chromosomal segments from O. sativa Nipponbare at both qSH1 and sh4 loci, was crossed with Nipponbare, and the derived F2 population was subjected to QTL analysis to determine the degree of seed shattering, and the locus qSH3 was identified (8, 9). High-resolution linkage analysis using a mapping population from a cross between IL(qSH1-N, sh4-N) and Nipponbare (10) (SI Appendix, Fig. S1) identified part of a previously known seed-shattering gene, OsSh1 (Os03g0650000), a homolog of Sh1 controlling abscission layer formation in sorghum (11) (SI Appendix, Figs. S2–S4 and Table S1). The function of OsSh1 in seed shattering was studied using artificially induced rice materials showing that the null mutations caused a complete loss of seed shattering (11, 12), but the causal mutation selected during rice domestication was not determined. No significant difference was detected in qSH3 expression levels in the spikelet base between Nipponbare and W630 (SI Appendix, Fig. S4), suggesting that differences in the degree of seed shattering were not caused by changes in qSH3 expression levels. Among the seven polymorphisms in the region (SI Appendix, Fig. S4 and Table S2), only SNP-70, with a SNP of C in W630 and T in Nipponbare, was located in the coding region (exon 1) of qSH3. This change caused an amino acid substitution from leucine in W630 to phenylalanine in Nipponbare (8) (SI Appendix, Fig. S5 and Table S1).

To test whether SNP-70 is associated with degree of seed shattering, we transformed Nipponbare using two constructs that differed only at the SNP-70 position. In the preliminary experiment using Nipponbare ILs without transformation (Fig. 1A), IL(qSH3-W), carrying the W630 allele at qSH3 in the Nipponbare genetic background, showed a non-seed-shattering behavior similar to that of Nipponbare, whereas IL(qSH1-W, qSH3-W) displayed a significantly higher seed-shattering degree than that of IL(qSH1-W) based on the breaking tensile strength (BTS) value (Fig. 1B and SI Appendix, Fig. S6). These findings indicated that the seed-shattering effect of qSH3 was considerably enhanced by a functional allele at qSH1 (SI Appendix, Fig. S7).

Therefore, we introduced two types of constructs carrying the qSH3 complementary DNA sequence of W630 (qSH3\textsuperscript{W630}) and Nipponbare (qSH3\textsuperscript{Npb}) into IL(qSH1-W) (Fig. 1C). The transgenic plants were expected to express both endogenous qSH3 of Nipponbare in IL(qSH1-W) and qSH3\textsuperscript{W630} or qSH3\textsuperscript{Npb} as the transgene (SI Appendix, Fig. S7). Therefore, we screened transformants expressing transgenes using the derived cleaved amplified polymorphic sequences assay targeting a SNP at the 5’ untranslated region, encoded by the W630 promoter region (SI Appendix, Figs. S7–S9). As a result, transformants with wild-type qSH3\textsuperscript{W630} showed enhanced seed shattering, compared with the control IL(qSH1-W), whereas those with domesticated-type qSH3\textsuperscript{Npb} did not shatter (Fig. 1D and SI Appendix, Fig. S7), confirming that SNP-70 is the causal mutation for reduced seed shattering.

Causal Mutation at qSH3 Is Conserved in Both japonica and indica But Not in circum-aus Rice Cultivars. Next, we analyzed the distribution of SNP-70 at qSH3 in cultivated rice. First, genotyping at the causal SNPs of sh4, qSH1, and qSH3 was conducted for the three rice cultivars, Nipponbare, IR36, and Kasalath, belonging to japonica, indica, and circum-aus, respectively. All cultivars possessed the causal mutation for nonshattering at sh4, and only Nipponbare had the causal mutation at qSH1. As for qSH3, Nipponbare and IR36 carried the causal mutation, but it was absent in Kasalath (Fig. 2A). We further
found that the Kasalath haplotype is similar to W630 around the qSH3 region (SI Appendix, Table S2). Using the diverse varieties of the World Rice Core Collection (13), we found that 14 lines, all belonging to circum-aus, carried the wild-type functional allele at qSH3 (SI Appendix, Table S3 and Figs. S10 and S11). Furthermore, the Rice 3K genome project data (14) clearly showed that both indica and japonica carried the causal mutation, but almost 90% of circum-aus rice carried a functional allele at qSH3 (Fig. 2B). To further understand the footprint of qSH3 selection, we analyzed nucleotide diversity across the qSH3 genomic region. Using sequences of the Rice 3K genome collection (14), we detected a selective sweep at qSH3 in both indica and japonica but not in the circum-aus lineage (Fig. 2C and SI Appendix, Figs. S12 and S13). These results suggest that circum-aus followed a separate trajectory to evolve reduced seed shattering (15). Thus, the reduction in seed shattering is dependent on lineage-specific variations in the subspecies, which is key to understanding the parallel processes of rice domestication.

Role of qSH3 Causal Mutation in an Initial Loss of Seed Shattering.

We next aimed to understand how the causal SNP at qSH3 contributed to initial rice domestication by reducing seed shattering in japonica and indica. Since the sh4 mutation is conserved in all cultivated rice (SI Appendix, Table S3), we produced IL(sh4-N) and IL(qSH3-N) in the genetic background of wild rice O. rufipogon W630. Evaluation of the seed-shattering mutations in the wild rice genetic background provides clearer morphological information related to the trait in early rice domestication. Complete formation of the abscission layer similar to that of W630 was observed in both ILs (Fig. 3), confirming that the single mutation at each locus was insufficient for phenotypic change in the abscission layer formation (7, 8). However, a slight inhibition of the abscission layer formation around vascular bundles was observed in IL(sh4-N, qSH3-N) (Fig. 3). Furthermore, a slight abscission layer inhibition was also observed in several wild rice accessions of O. rufipogon carrying mutations at both sh4 and qSH3 (SI Appendix, Figs. S14 and S15 and Table S4), although they may have gained these

![Fig. 1. Identification of a causal SNP of qSH3 associated with the degree of seed shattering. (A) Graphical genotypes of O. sativa Nipponbare and ILs for qSH3 and qSH1 in the genetic background of cultivated rice O. sativa Nipponbare. (B) Comparison of seed-shattering degree by BTS values in three ILs, IL(qSH3-W), IL(qSH1-W), and IL(qSH1-W, qSH3-W), in the Nipponbare genetic background. Data are mean ± SD of four plants. n.s. and double asterisk (**) indicate not significant and significant at the 1% level based on unpaired Student's t test, respectively. (C) Two types of constructs carrying qSH3 complementary DNA sequences of W630 and Nipponbare (qSH3W630 and qSH3Npb). (D) The BTS values observed for the transformants with qSH3W630 and qSH3Npb. Black triangles represent the average BTS value of IL(qSH1-W).]

![Fig. 2. Lineage-specific selection at the qSH3 locus in rice. (A) Genotyping at sh4, qSH1, and qSH3 for O. rufipogon W630, O. sativa japonica Nipponbare, indica IR36, and circum-aus Kasalath based on the causal SNPs identified by derived cleaved amplified polymorphic sequence markers. (B) Allele frequency of qSH3 causal SNP (%) in cultivated rice based on the Rice 3K project data. Nipponbare type (T) and W630 type (C) are shown in yellow and green, respectively. (C) Nucleotide diversity (α) observed for domesticated rice in the physical position of 25.0 to 25.3 Mb on chromosome 3. In a flanking region of the qSH3 locus (around 25.2 Mb), α was substantially decreased in japonica and indica cultivars. α was calculated in 10-kb windows using the SNP data of the Rice 3K project (14).]
domestic-type alleles through introgressions from cultivated rice (16). Even if the mutations in the two loci displayed a phenotypic difference in the abscission layer formation adequate to reduce shattering under greenhouse conditions without wind, their effect on seed shattering was much less than expected under field conditions.

Seed-Shattering Behavior Associated with a Slight Inhibition of Abscission Layer Formation Was Mitigated by Closed Panicle Formation. Previously, we reported that the closed panicle trait controlled by OsLG1 had a major effect during rice domestication by facilitating grain harvest (7). In wild rice O. rufipogon, as well as other wild rice species, a spreading panicle is characteristic. The primary branch of wild rice starts against the main rachis and gradually bends out before the flowering stage (Fig. 4 A), reaching nearly a right angle during the seed maturation stage (Fig. 4 B). The open panicle structure is caused by a bump structure of tissue at the basal parts of primary branches, which is not observed in cultivated rice with closed panicle formation (7). We produced wild rice with the closed panicle trait from cultivated rice and found the trait reduced seed shedding by retaining seeds that get trapped by long awns found on seeds in the lower sections of the panicle. Interestingly, the SPR3 (a locus regulating OsLG1) region is under strong selection in indica, japonica, and circums-aus (SI Appendix, Fig. S16), suggesting that the closed panicle trait was selected in the early phase of rice domestication. Thus, closed panicles might have been associated with seed-shattering changes, although this trait, unlike seed shattering, is not visible archaeologically. Therefore, we generated seven wild ILs with different combinations of the three loci (sh4, qSH3, and SPR3) (SI Appendix, Fig. S17) and compared their morphologies with that of the wild rice O. rufipogon W630 (SI Appendix, Fig. S18). The heading date of all ILs was similar to that of W630 (SI Appendix, Fig. S19), but there was a slight difference in inhibition of abscission layer formation around the vascular bundles depending on the double mutations at sh4 and qSH3 (Fig. 3 and SI Appendix, Fig. S20) and in open or closed panicle structure depending on SPR3 mutation (Fig. 4 A and B). Small BTS values were observed only for IL(sh4-N, qSH3-N) and IL(sh4-N, qSH3-N, SPR3-N) due to a slight inhibition of abscission layer formation, while the rest were close to zero, as observed in wild rice (Fig. 4 C). We were interested in assessing the differences in yields brought about by the causal mutations, identifying the combination of alleles that would have provided humans in prehistory with higher yields, and determining whether a closed panicle conferred additional value to humans when gathering wild rice. We therefore subjected the seven ILs and W630 to a seed-gathering experiment in the field (SI Appendix, Fig. S21 and Movies S1 and S2). The seed-gathering rates from three ILs with open panicles, namely IL(sh4-N),
IL(qSH3-N), and IL(sh4-N, qSH3-N), were not significantly different from that of W630, regardless of the presence or absence of abscission layer inhibition (Fig. 4D and SI Appendix, Table S5). However, the three ILs with a closed panicle structure and complete abscission layer formation, namely, IL(SPR3-N), IL(sh4-N, SPR3-N), and IL(qSH3-N, SPR3-N), presented a slightly increased yield (Fig. 4D). In contrast, IL(sh4-N, qSH3-N, SPR3-N), with a combination of a closed panicle structure and slight abscission layer inhibition, showed a significant increase in gathering rate compared with W630 (Fig. 4D and SI Appendix, Table S5). As closed panicles would be easily visible in the field, humans could have targeted this higher-yielding rice (SI Appendix, Fig. S22), but even indiscriminate gathering also would retain larger proportions of grains from closed panicles.

When plants were sown from harvests, closed panicles and reduced seed shattering would increase, owing to the enhanced gathering rate that would occur in the presence of all three domesticated-type alleles. As these alleles increased in frequency and became fixed, yields would have increased, which would have encouraged further investment in rice cultivation (17). The selection for closed panicles instigates self-pollination behavior owing to the long awns, which disturb the free exposure of anthers and stigmas (stigmas and closed panicles) (7). Thus, a closed panicle may also be advantageous in mitigating natural variations in seed-shattering loci by reducing outcrossing. Although awns are undesirable in modern cultivated rice, gathering of rice in the early stages of rice domestication might have benefited from the presence of awns in plants with closed panicles, as these would have increased yield and self-pollination rates.

**Complementary Interaction of a Slight Inhibition of Abscission Layer and Closed Panicle Formation Synergistically Contributed to Structural Stability of the Panicle Increasing Yield.** To better understand how the closed panicle, caused by SPR3, and the inhibition of abscission layer formation, caused by sh4 and qSH3, contributed to the initial loss of seed shattering, we analyzed their roles by performing structural mechanics analysis. The awns of wild rice play a pivotal role in seed dispersal in spreading panicles (Fig. 5A). We measured the lengths and weights of awns and grains in wild rice *O. rufipogon* W630 (Fig. 5B), and using these values, we calculated the sectional force exerted on the spikelet base depending on panicle angles (SI Appendix, Fig. S23). The axial and shear forces were slightly increased in a closed panicle compared to an open panicle. However, the bending moment, which is the predominant factor affecting seed dispersal in an open panicle, was considerably reduced in a closed panicle (SI Appendix, Fig. S23). A slight inhibition of the abscission layer formation by sh4 and qSH3 led to an increase in the length of abscission layer inhibition (Fig. 3). Therefore, we measured the length of the abscission layer and central vascular bundle in *O. rufipogon* W630 by scanning microscopy (Fig. 5 C and D). We also calculated the moment of inertia of the area, a property that describes the torque required to break the abscission layer and so disarticulate the grain, for the disrupted abscission layer (SI Appendix, Fig. S23). The value increased exponentially with increasing length of abscission layer inhibition. A reduction in the bending moment and an increase in the moment of inertia of the area act synergistically to reduce bending stress (Fig. 5E), contributing to the structural stability of spikelets without shattering. Thus, the interaction between a closed panicle and abscission layer inhibition acted complementarily to increase yield.

**Conclusions**

In this study, we identified key functional genes that contributed to early rice domestication by increasing harvest yields. We identified the causal SNP at qSH3 involved in reduced seed shattering and demonstrated that the previously proposed sh4 mutation alone could not trigger nonshattering morphology in rice (6, 7, 16). We also explained how early selection of a closed panicle and the resulting mechanics of spikelet retention would have increased yields and facilitated selection for nonshattering. Our results showed that the initial step in rice
domestication was more complex than previously thought. Based on information from our studies, changes are needed from the long-held perspective that the process of rice domestication can be explained by a single domestication allele model to one where synergistic effects of several domestication genes are involved.

The origin and spread of domesticated rice subspecies based on population genetic analysis have been the subject of numerous discussions (15, 18–21). However, most of these studies have been conducted using the genome information of modern cultivars and wild rice, without considering the importance of visible phenotypic changes that could have been targeted by humans. The lineage-specific variations associated with quantitative traits are a key to deepening our understanding of the process of rice domestication.

Based on our work, we propose a stepwise route for rice domestication. In wild rice, any of the natural variations in the loci for seed shattering and closed panicle formation alone had little effect on increasing yield. In combination, however, they established an archaic rice that could have been visibly recognized by ancient gatherers as advantageous to increasing yields. The change in harvesting efficiency along with the use of harvesting tools further promoted the selection of natural variants in domestication-related traits in rice, a crop which now supports billions of people worldwide.

Materials and Methods

Details regarding plant materials, QTL mapping, fine mapping, transformation, population genetic analysis, histological analysis, seed-gathering experiment, and structural mechanics analysis are provided in the SI Appendix. Primers used for genetic mapping, gene expression analysis, production of transgenic plants, and genotyping are shown in SI Appendix, Tables S6–S8.

Data Availability. All study data are included in the article and/or SI Appendix.

ACKNOWLEDGMENTS. The wild rice accessions of O. rufipogon were provided by the National Institute of Genetics supported by the National Bioresource Project, Ministry of Education, Culture, Sports, Science, and Technology, Japan. We thank R. Morita and H. Fukayama, Kobe University, for their support in producing transgenic plants and H. Furumi, National Institute of Genetics, for growing wild rice accessions. This work was partly supported by Grants-in-Aid from the Japan Society for the Promotion of Science 15KK0280 and 18KK05994 (R.I.), JSPS overseas research program (C.C.C.), JSPS Bilateral Open Partnership Joint Research Projects JPJSPB120189948 and JPJSPB12019922 (R.I., D.Q.F.), Nikki Saneyoshi and Kinoshita foundations (R.I.), and National Institute of Genetics Joint Research 82A 2016-2018 (K.-I.N., T.I.).

Author affiliations: Laborat°ry of Plant Breeding, Graduate School of Agricultural Sciences, Kobe University, Kobe 657-8501, Japan; Institute of Archaeology, University College London, London WC1H 0PY, United Kingdom; Laboratory of Hydraulic Structures and Geo-Environmental Engineering, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan; Laboratory of Plant Cytogenetics, National Institute of Genetics, Mishima 411-8540, Japan; Department of Genetics, School of Life Science, The Graduate University for Advanced Studies (SOKENDAI), Mishima 411-8540, Japan; School of Life Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom; and School of Cultural Heritage, Northwest University, Shaanxi 710069, China.


13. N. Tanaka et al., Whole-genome sequencing of the NARO World Rice Core Collection (WRC) as the basis for diversity and association studies. Plant Cell Physiol. 61, 922-932 (2020).


