Reloading DNA History in Rice Domestication

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Although crop domestication is a prehistoric event, DNA (or genome) sequences of modern cultivars and the accession lines of wild relatives contain information regarding the history of crop domestication and the breeding process. Accordingly, with plentiful genomic data, many new findings have been obtained concerning the crop domestication process, for which various (some controversial) interpretations exist. Since approximately 20 years ago, dozens of quantitative trait genes (QTGs) related to the domestication process have been cloned from several crops including rice, a global staple food. However, the determination of how and when these QTGs were involved in rice domestication requires a precise understanding of the DNA code. In addition to the identification of domestication-related QTGs, large-scale rice genome analysis based on short-read Illumina data (but with shallow depth) including more than 1,000 rice cultivars and hundreds of wild rice (or *Oryza rufipogon*) lines, along with extensive genome analysis including more than 3,000 cultivars with sufficient Illumina data, has been reported. From these data, the genome-wide changes during rice domestication have been explained. However, these genome-wide changes were not interpreted based on QTG changes for domestication-related traits during rice domestication. In addition, a substantial gap remains between the archeological hypothesis based on ancient relics and findings from DNA variations among current cultivars. Thus, this review reconsiders the present status of rice domestication research from a biologist's perspective.

Keywords: Domestication ● Introgression ● Local adaptation ● Rice ● Seed color ● Seed shattering ● Taste trait

Introduction

Darwin first began scientific explanation on animal and plant domestication (Darwin 1868). In his book entitled 'The variation of animals and plants under domestication', the changes of biological traits in some animals such as doves were extensively documented; prehistoric processes were described as a form of evolution. However, researchers in the fields of molecular phylogenetics and population genetics have hesitated to accept such domestication processes as evolutionary events because they are based on unnatural (or biased) selection by ancient humans; moreover, they are very rapid compared with typical evolutionary trait changes. Instead, archeologists have preferred to study domestication using various relics obtained from prehistoric ruins and precise dating technologies based on radioisotope measurement. Recently, developments in the cloning of quantitative trait genes (QTGs) based on quantitative trait loci analyses and genome-wide association studies and in population genomics analyses using abundant next-generation sequencing data have enabled us to address various questions related to rice domestication. QTGs conferring changes in domestication-related traits such as seed shattering, seed color, plant architecture and seed size have been cloned from rice since 2006 (Izawa 2007, Sweeney and McCouch 2007, Izawa et al. 2009, Civáň and Brown 2017, Chen et al. 2021). Thus far, dozens of QTGs related to rice domestication have been cloned. In addition to these QTGs, several extensive population genomics analyses have been conducted. In 2012, Huang et al. reported a comprehensive map of rice genome variations based on short-read Illumina sequence data for 446 accessions that originated from geographically diverse areas of the wild rice species *Oryza rufipogon*, the ancestral progenitor of cultivated Asian rice, and from 1,083 rice cultivars including both indica and japonica; however, the corresponding depth of Illumina data was very shallow (Huang et al. 2012). Furthermore, Wang et al. (2018) reported genomic variations of 3,010 cultivars of Asian rice with various origins. Although many papers concerning rice domestication have been published based on analyses of these data, the findings are often controversial (Huang et al. 2012, Huang and Han 2015, Choi et al. 2017, Civáň and Brown 2017, 2018, Choi and Purugganan 2018). One major reason for controversy is the lack of information regarding genome variations in wild rice because there are scarce genomic data for *O. rufipogon* (Huang et al. 2012). Thus, regardless of available cultivar information, the data remain insufficient to address a wide range of questions concerning rice domestication. Additionally, there are problems with the setting (or definition) of issues and topics related to rice domestication. For example, although the location where rice domestication occurred was a

major topic in the work by Huang et al. (2012), the places where each line of wild rice accession grows wild today may differ from the locations of rice domestication considering the climatic changes that have occurred in the past 10,000 years. A similar tendency is true for the cultivation areas of cultivars. Thus, in analyses of rice genome data, it is not easy to address the place of origin through rice domestication research, although some associations may exist between current growing locations and the site of the domestication process.

Another challenge in rice domestication research is that there have been no comprehensive integrations of genome-wide variation data with quantitative trait nucleotides (QTNs) that may have been selected by ancient humans. Furthermore, such genomic information should be integrated with archeological evidence as much as possible because determinations of the time and place of domestication are more readily achieved through archeology. This review reconsiders the current status of rice domestication research.

What Are High-Priority Challenges for Rice Domestication Research?

The definition of domestication

When we reported the cloning of a domestication-related QTG designated as *qSW5* in 2008 (Shomura et al. 2008, Liu et al. 2017), we mapped seven QTNs of six domestication-related QTGs (*qSW5*, *Rc*, *qSH1*, *sh4*, *waxy* and *Rd*) among 91 cultivars (mostly land races). We then found that several cultivars belonging to the tropical japonica subgroup of rice possess the original QTNs that were not selected by ancient humans for all seven QTNs tested, suggesting that these old cultivars of tropical japonica are close to the original rice cultivars (Konishi et al. 2008, Shomura et al. 2008, Izawa et al. 2009). Alternatively, the selected QTNs might have been introgressed after the formation of proto-tropical japonica. After this publication, some archeologists have noted that our interpretation does not align well with archeological data from paddy ruins (Fuller and Sato 2008). This claim may be supported when more QTNs from cultivars and wild rice accessions are considered using our methods in the near future. Such interactions between molecular geneticists and archeologists are very welcome. However, this interaction led us to question whether the definition of domestication in archeology is the same as the definition used in molecular genetics; we realized that these definitions may differ.

The date and place estimates of when the genome was selected by ancient humans according to key trait changes due to genetic effects of QTNs could provide key information of domestication for molecular geneticists; in contrast, the excavation of samples from ruins provides key evidence used by archeologists. In order for any remains of ancient paddy fields and related relics to exist, the presence of a human community and quite large-scale cultivation may be prerequisites. Thus, archeological estimates of the time and place

of domestication must be delayed relative to the estimates obtained from genomic information. In addition, the resolution of dating differs between phylogenetic analysis using nucleotide sequences and archeological analysis using radioisotopes (Fuller et al. 2009, Choi et al. 2017). With these considerations, the data obtained from genome and QTN selection are expected to extensively overlap with archeological evidence. A similar tendency is true among molecular geneticists, who engage in endless controversy regarding the number of occurrences and locations of rice domestication. Therefore, we must clearly define domestication to address this question in a scientific manner.

Classification based on genome diversity and bottleneck selection

In a comprehensive map of genome variations among more than 3,000 rice cultivars (Wang et al. 2018), at least eight sub-groups [XI-1, XI-2, XI-3, Aus, Bas, GJ-Subtrp, GJ-tmp (or temperate japonica) and GJ-trp (or tropical japonica)] of rice cultivars are defined. These eight subgroups can be categorized into four major groups [XI (or indica), Aus, Bas and GJ (or japonica)]. This categorization suggests that at least four major bottleneck selection events, as well as a succession of minor bottleneck selection events, occurred to form the eight subgroups (or subspecies) during the domestication process or early breeding of rice. To precisely determine how the wild species of rice, *O. rufipogon*, contributed to these ancient selection events, more accurate genomic information is needed from diverse accessions of wild rice. Without such information, the domestication process would be simply an irritating logic problem. Nevertheless, many hypotheses have been proposed regarding the number of rice domestication events; one major subgroup, japonica (or GJ), diversified around 400,000 years ago from a common ancestor with another major subgroup of rice, known as indica (or XI). Apparently, this date was considerably earlier than the expected date of rice domestication. This finding was originally based on the evolutionary speed of long terminal repeat sequences of retrotransposons in the rice genome (Vitte et al. 2004); it has been supported by genome-wide assessments of japonica and indica (Choi et al. 2017). Because rice domestication presumably occurred fewer than 10,000 years ago (Fuller et al. 2009), some believe that sufficient genomic information is available at this moment from japonica (GJ) and indica (XI) cultivars to elucidate the rice domestication process. Thus, it remains controversial among rice molecular geneticists whether rice domestication occurred one, two or more times.

Basic selection processes during rice domestication

Let us imagine the beginning of human rice cultivation around 10,000 years ago. Until then, ancient humans gathered rice seeds from wild rice, which grew naturally in waterside areas, and then brought them home to utilize as food. Some of these gathered seeds grew into plants after they had been dropped at sites near the homes of those humans. A very strong genetic bottleneck
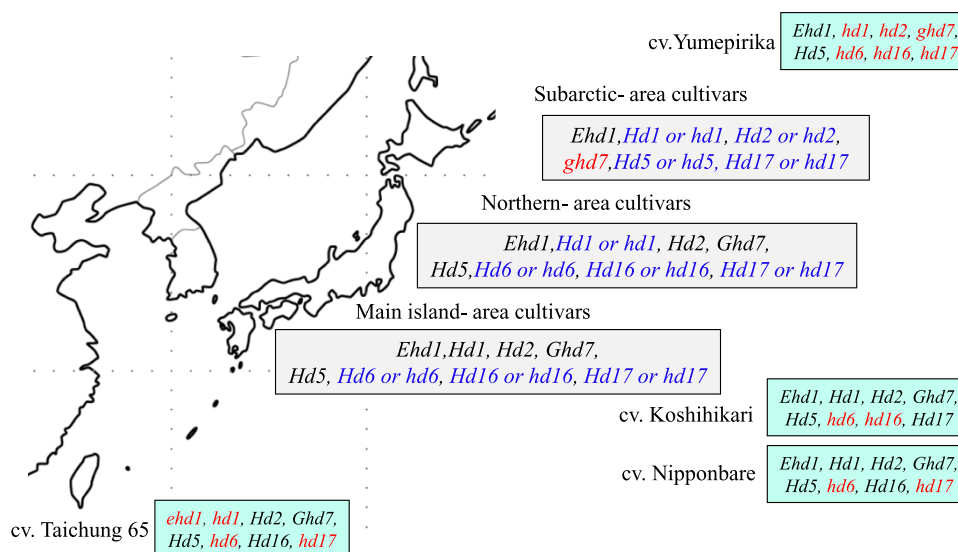
Fig. 1 Local adaptation of flowering-time genes in rice. Natural variations of at least eight heading-date (or flowering-time) genes are involved in local adaptation in Japan. The defective mutation in *Ghd7* is required for cultivation in a northern part of Japan (Hokkaido Island). Yumepirika is a cultivar adapted to the northern island of Japan, while Koshihikari and Nipponbare are cultivars adapted to the main island of Japan. Taichung 65 is a cultivar found in Taiwan. Genes with only lowercase letters have the selected QTNs. Cultivars having either functional (starting with uppercase letters) alleles or selected QTNs (starting with lowercase letters) are often found in the local area.

event may have occurred then for the selected rice plants; rice is a self-fertilizing plant and natural crossing may not occur when the plants grow in distant areas. Thus, several cycles of isolated cultivation of selected rice plants could have led to the genetic fixation of large parts of the rice genome (or bottleneck events). In this context, it may be impossible to identify the key genes selected during domestication via DNA polymorphism analysis since the entire genome itself was selected. However, substantial parts of the rice genome have a few haplotypes even within a subgroup, suggesting that both genome fixation and novel haplotype formation occurred immediately after the bottleneck selection events. These processes may have occurred because of natural outcrossing among a mixture of plants derived from several selection events at distinct times and adjunct sites. During the genome fixation and haplotype formation, strong selection affected key loci, which may result in selective sweeps. Local adaptation of rice cultivation provides a strong motive force for such haplotype formation within a subgroup. For example, during modern breeding, selection for defective alleles of the *Ghd7* heading-date gene (Xue et al. 2008, Lu et al. 2012), which led to dramatically earlier flowering with acceptable yields, was a key event that supported rice cultivation in subarctic areas of Japan (**Fig. 1**) (Fujino et al. 2022). As another example, selection for defective alleles of both the *Hd1* and *Ehd1* heading-date genes, which confers prolonged growth until flowering and results in high yields, was preferred in rice cultivars in southern areas of Asia, such as Taiwan (**Fig. 1**) (Doi et al. 2004, Wei et al. 2016). Since these exemplify the use of recent natural mutations, they did not contribute to any haplotype formation. However, such functional variations related to local propagation may provide a motive force for the haplotype formation within a subgroup

during rice domestication (Izawa 2007, Ogiso-Tanaka et al. 2013, Itoh et al. 2018, Cui et al. 2020). Accordingly, the selection of key alleles of such local adaptation genes may introduce variations, even within a subgroup. Such haplotype selection mainly derives from selection for standing variations with substantial genetic distances among *O. rufipogon*. Meanwhile, selection for de novo mutations can also contribute to subgroup formation. Thus, the domestication process of rice can be explained as basic selection processes of genetic bottleneck, genome fixation and haplotype formation, selection of standing variations and selection of de novo mutations (**Fig. 2**).

Critical introgressions between subgroups during rice domestication

In addition to these processes, substantial directional introgression, such as introgression events with *Rc* and *Kala4* seed color genes, should also be considered when establishing a comprehensive model of rice domestication (Civáň and Brown 2018) (**Fig. 2**). First of all, consider *Kala4*, a black seed color gene (Oikawa et al. 2015), which is a distinct QTG to *Rc*, a well-known domestication gene that resulted in white color rice seed traits (Sweeney et al. 2006). Molecular analysis shows that the *Kala4* mutation occurred after rice domestication because wild rice accessions with black seed color are very rare (and to my knowledge none have been reliably published). Old studies state that the origin of black rice occurred at least 1,000 years ago because it was recorded as an imperial food in ancient China (Oikawa et al. 2015). When *Kala4* was cloned, black rice lines were found in various subgroups of rice, including both japonica and indica. Then, detailed sequencing data for *Kala4* genes from various rice
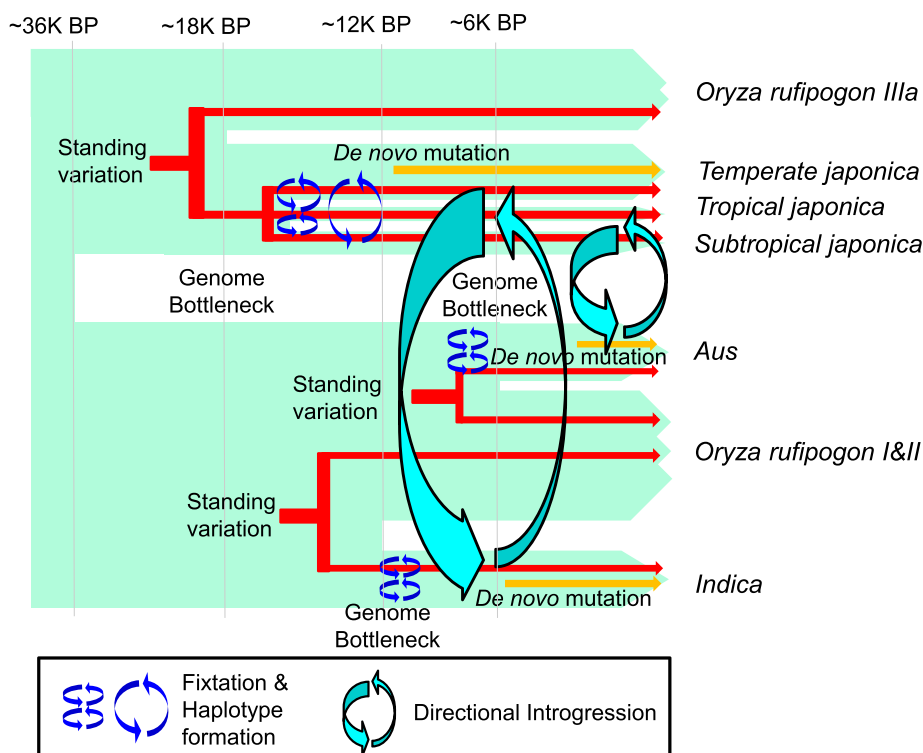
Fig. 2 Current domestication model for rice. This is a revised version of the model described by Choi et al. (2017). Five key events explaining rice domestication have been added to this model: (1) genetic bottleneck, (2) fixation and haplotype formation, (3) directional introgression, (4) selection for traits because of standing variation and (5) selection for traits because of de novo mutation.

cultivars revealed that black rice may have originated from the ancient tropical japonica subgroup; however, black rice with an indica genetic background is most prevalent in China. This de novo insertion mutation in *Kala4*, conferring black seed color, was presumably introgressed into indica subgroups. The introgressed locus of *Kala4* might have been selected repeatedly over a few thousand years, resulting in the introgression of genome fragments of just a few hundred kb from tropical japonica to indica subgroups. The size of the introgressed fragments varies among indica black rice cultivars. Modern breeding using artificial crossing technologies in rice has enabled the replication of such selection. Thus, some black seed cultivars now exist in a temperate japonica background. In this context, the introgressed *Kala4* fragments have been further introgressed into temperate japonica modern cultivars. This process raises questions of how to explain *Kala4* in domestication research; namely, when and where was the *Kala4* gene involved in rice domestication or early rice breeding processes? Indeed, a similar process may have affected the *Rc* gene, a white seed color gene, because of the QTN selection of a 14-bp deletion; however, major cultivars of rice frequently possess this mutation (Sweeney et al. 2007), in contrast to the *Kala4* mutation. From such data, we can only estimate when such mutations first occurred; we cannot date the bottleneck selection event.

Notably, trait selection due to standing variations would have begun upon the emergence of trait changes because the presence of both original allele and selected alleles in wild rice would arise due to the weak effects of the mutations. It is well known that all of the tested cultivars contain the weak *sh4* QTN and some wild rice also possesses it (Li et al. 2006, Zhang et al. 2009, Zhu et al. 2012). In addition, the effects of the *sh4* QTN were masked in some genetic backgrounds of wild rice (Ishikawa et al. 2017). Thus, ancient plants possessing the weak-standing *sh4* QTN for good agronomical traits would have become targets for ancient human selection during the early domestication process. Because there are large genetic distances among the rice subgroups, the weak *sh4* QTN may have been introgressed into other subgroups of rice from the original subgroup line. Notably, the dating of directional introgressions through the comparison of introgressed lines with recurrent parent lines is very difficult; this difficulty becomes apparent when trying to estimate the dates of introgressions using nearly isogenic lines. However, population genomics analysis may provide insights into such introgression events too (Choi et al. 2017). Since another seed-shattering QTN in *qSH1* was only found in some temperate japonica cultivars, the selection for the weak *sh4* QTN occurred prior to the *qSH1* selection (Konishi et al. 2006). Accordingly, the identification of QTNs that affect agronomic traits and the mapping of these QTNs to diverse genomes of cultivars and wild rice accessions provide clues to estimate the timing of allele propagation among distinct QTNs to elucidate rice domestication in terms of molecular genetics.
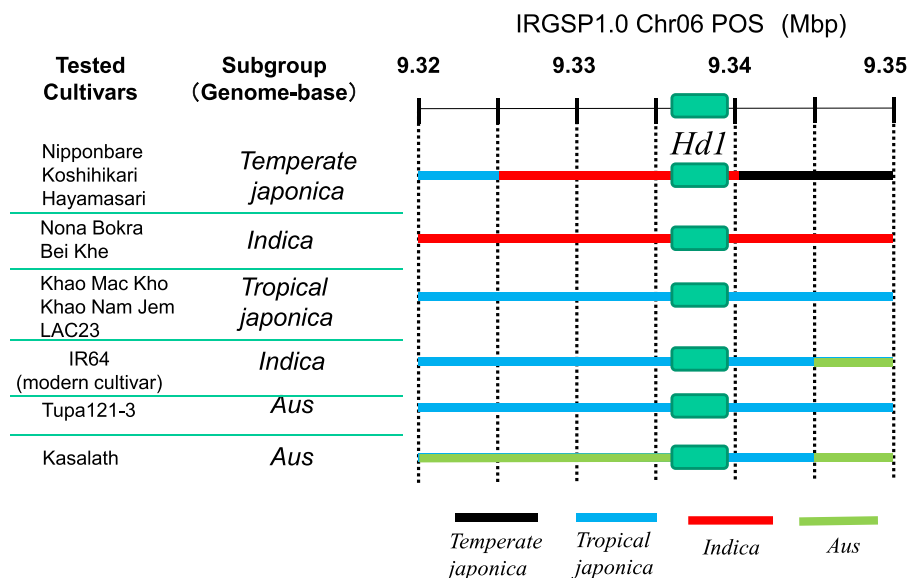
Fig. 3 Example of complex introgression in rice. This is a revised version of Fig. 4D in the work by Itoh et al. (2018). Complex introgression of the *Hd1* gene in rice cultivars is summarized.

Potentials of QTN mapping in diverse rice lines

When gene sequences of certain QTGs in wild rice are compared with those in cultivars, various outcomes will be possible. In other words, obtaining an integrated history of each gene's evolution during rice domestication is difficult without such sufficient gene sequence information from diverse wild rice accessions. In case that a gene group of cultivars differs from those of the wild rice accessions with certain genetic distances, such a gene may have a critical role in rice domestication, although some members of the group have resulted from absolute directional introgression. In other scenarios, in case that a group of genes map to two haplotype groups, one group overlaps only with japonica, while the other group overlaps with both indica and japonica, suggesting the two-origin of domestication in terms of gene evolution. This pattern would be representative of the rice domestication and subsequent breeding processes based on current knowledge.

DNA sequence variations of certain key genes in various rice cultivars have been analyzed (Sweeney et al. 2006, 2007, Fujino et al. 2010, Asano et al. 2011, Jiang et al. 2012, Shao et al. 2013, Hori et al. 2015, Civáň and Brown 2017, Choi and Purugganan 2018, Itoh et al. 2018, Liu et al. 2018a, b, Wu et al. 2018, 2020, Huang et al. 2020, Li et al. 2021, Zhang et al. 2019, 2021, Wang et al. 2022). For example, variations in the *Heading date 1* (*Hd1*), a heading-date gene, or one of the rice orthologs of the *Arabidopsis thaliana CONSTANS* gene, were extensively analyzed; the findings demonstrated that a large part of the temperate japonica (GJ-tmp) group is close to the representative haplotypes of indica (XI), presumably because of *Hd1* introgression during temperate japonica subgroup formation (**Fig. 3**) (Yano et al. 2000, Fujino et al. 2010, Itoh et al. 2018, Wu et al. 2020). It is intriguing to speculate whether the introgression region of *Hd1* arose from ancient domesticated indica or ancient predomesticated wild rice species, but insufficient genome data

for wild rice accessions are available to accurately assess this introgression at this moment. This *Hd1* gene variation may have contributed to local adaptation to a temperate climate during the expansion of rice cultivation from tropical areas. If fragments of the wild rice genome contributed to this adaptation, there is additional difficulty in identifying the number of domestication events. Thus, the number of domestication events in this species may be impossible to determine without a clear and refined definition of domestication, despite widespread debate. Furthermore, the 2-bp deletion mutation in the same *Hd1* gene was originally found as a QTN between Nipponbare (a temperate japonica cultivar) and Kasalath (an aus cultivar) (Yano et al. 2000). Thus, this mutation has been thought to be characteristic of the aus subgroup due to the heading-date trait characteristic of aus. However, with the 3K data in the population genomics analysis of rice (Wang et al. 2018), we know that the 2-bp deletion might have originated from the tropical japonica subgroup (**Fig. 4A–C**), suggesting a directional introgression from tropical japonica to aus. An accurate estimation of the process underlying the establishment of each subgroup based on DNA history would be a major development in this field. Thus, an integrated map or timeline of the establishment of major rice subgroups and a detailed introgression history based on comprehensive genomic and QTG information are major goals of this research field.

Current Model of Rice Domestication

Using a large dataset of genome-wide information for wild rice accessions with shallow depth data (Huang et al. 2012), it has been demonstrated that the japonica (or GJ) subgroup genome is close to a group of wild rice accessions designated as *OrIIIa* (**Fig. 2**). Thus, a strong bottleneck selection must have occurred to establish the japonica (or GJ) group. Although

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Gene	Frame-shift site	Cultivar Num.
RFT1	Ser3	2
RFT1	His119	3
RFT1	Trp139	35
RFT1	Asn147	3
Hd1	Glu19	1
Hd1	Glu230	1
Hd1	Phe279	303
Hd1	Lys352	1

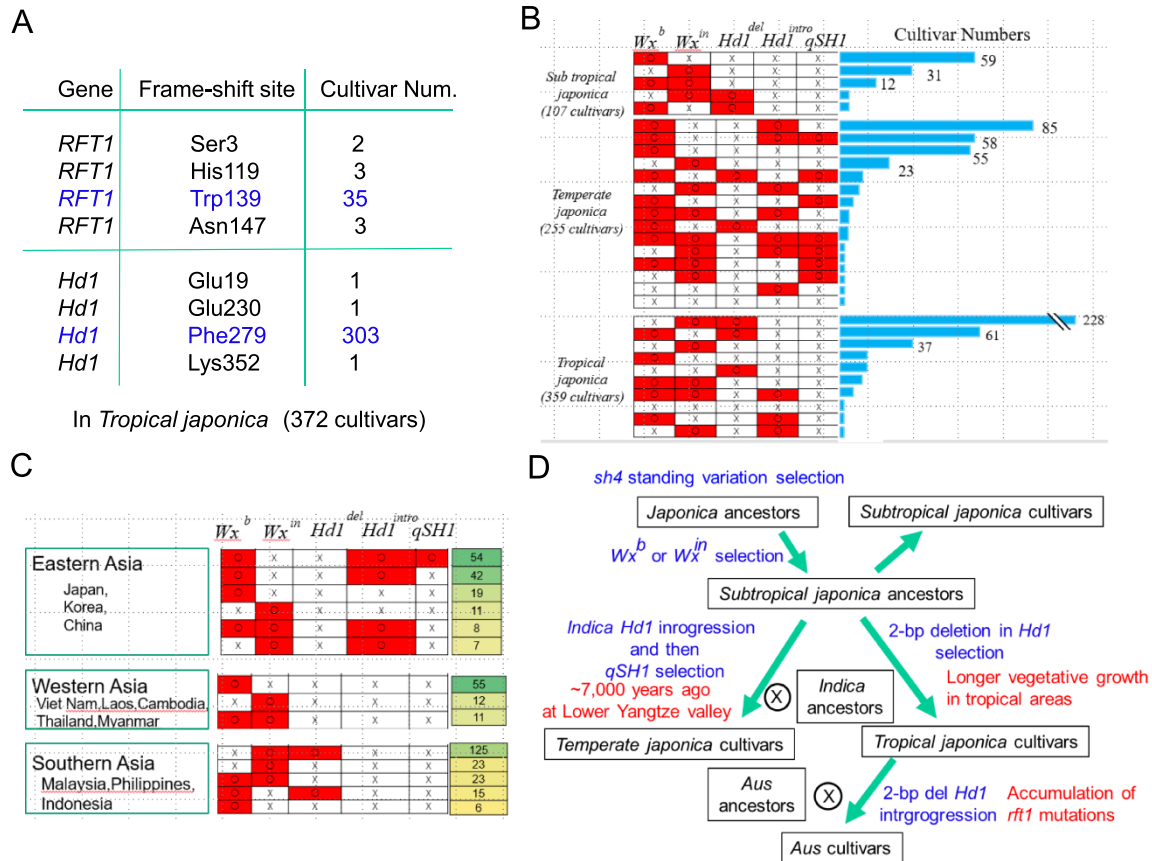In *Tropical japonica* (372 cultivars)

Fig. 4 Some trials for the reconsideration of QTNs to explain the rice domestication process. (A) Frame-shift mutations in *RFT1* and *Hd1*, obtained from public 3K genomic information (Wang et al. 2018). (B) Cultivar numbers with listed QTNs in *japonica* subgroups. (C) Cultivar numbers of *japonica* with listed QTNs in local areas of Asia. (D) Model of the establishment of *japonica* subgroups. Selection for the *qSH1* QTN (Konishi et al. 2006) might have occurred in the Lower Yangtze valley around 7,000 years ago (Zheng et al. 2016). QTN information; Wx^b (Isshiki et al. 1998), Wx^{in} (Zhang et al. 2019), $Hd1^{del}$ (Yano et al. 2000), $Hd1^{intro}$ (Itoh et al. 2018) and *qSH1* (Konishi et al. 2006).

additional big data analysis of diverse rice cultivars (Wang et al. 2018) revealed further categorization within the japonica group, including subtropical japonica (GJ-sbtrp), tropical japonica (GJ-trp) and temperate japonica (GJ-tmp), the contribution of wild rice to this categorization remains unknown (**Fig. 2**). In contrast, indica and aus were integrated into a large category containing wild accessions, thus indicating close relationships among the Or-I group, indica and aus (Huang et al. 2012). The ability to distinguish DNA sequences in indica and aus from DNA sequences of wild rice accessions depends on the contribution of each gene during the domestication and subsequent breeding of rice.

With these data, either single or multiple origins are possible for cultivated Asian rice, *O. sativa*, largely depending on the definition of rice domestication. Several conflicting models to explain the origin of this domesticated crop have been developed thus far (Huang et al. 2012, Huang and Han 2015, Civán et al. 2016, Choi et al. 2017, Civáň and Brown 2017, 2018, Choi and Purugganan 2018). Some QTGs (or QTNs) involved in critical key domestication traits such as seed shattering (due to *sh4* QTN) and pericarp color (due to *Rc* QTN) are shared among major subgroups, strongly supporting a single origin (Li et al. 2006, Sweeney et al. 2006, 2007, Sweeney and McCouch 2007). This hypothesis is supported by demographic modeling using DNA polymorphism information (Choi et al. 2017). In contrast, the two-origin model, in which major subgroups such as indica and japonica have independent origins from distinct *O. rufipogon* subpopulations, explains the genetic differences (or distances) identified in the genome-wide comparison of japonica and indica (Vitte et al. 2004, Civán et al. 2016). The earliest archeological evidence of domesticated rice is from approximately 8,000–9,000 years ago, while the japonica and indica genomes are separated by a mean genetic distance of around 400,000 years (Vitte et al. 2004, Fuller et al. 2009, Choi et al. 2017). The distinct genome clustering of japonica, indica and *O. rufipogon* suggests a complex origin story for cultivated Asian rice. Even based on the same genome-wide data from diverse accessions, conflicting models of both single and multiple origins have been proposed (Civáň and Brown 2017; Huang and Han 2015). In addition to the differing definitions of rice domestication, this controversy may arise from the limited accuracy of available genomic information

for both cultivars and wild rice accessions (Huang et al. 2012). Accurate information based on diverse accessions is restricted to cultivars; such information is lacking for wild rice accessions. Huang and Han (2015) proposed a reasonable model that explains both the key QTN selection events described above and the large genetic distances among subgroups of rice cultivars, which included a single origin and multiple introgressions. However, important questions remain unanswered, such as the number of key QTNs and the number of *O. rufipogon* accession groups involved in rice domestication. Thus, Purugganan and colleagues proposed a similar domestication model that uses de novo–assembled genomic information of five representative subgroup cultivars (one japonica, two indica, two aus and two wild rice accessions) in collaboration with genome biologists and archeologists, although genome information from the small amount of rice lines may bias the model and reduce accuracy (Choi et al. 2017). However, it is of note that the portions of genomes that are introgressed into the target genome have been estimated using both phylogenetic and coalescence-based modeling of demographic parameters in this work. Accordingly, around 17% and 15% of the genome were estimated to have introgressed from japonica into indica and aus, respectively. Furthermore, the times of bottleneck selection for indica and aus formation were estimated separately. From an archeobotanical perspective, no strong evidence has been found for proto-indica domestication traits until around 4,000 years ago, which is much later than the timing of japonica domestication. Reliable archeological evidence in the Ganges valley dates from approximately 9,000 years ago (Choi et al. 2017). This archeological evidence has been interpreted to indicate the early management of proto-indica rice, with this management (early cultivation) preceding the selection of domestication alleles of key QTGs. This explanation is often given by archeologists; it may arise from the differing definitions of domestication between archeology and molecular biology. For example, because there are subtle phenotypic differences in seed shattering deriving from the QTN in *sh4*, one of the first domestication genes, their effects may be difficult to identify using archeological methods (Li et al. 2006, Zhu et al. 2012). Thus, archeologically defined pre-domestication management may have involved some QTN selection (or the bottleneck events described above). The application of genome data from diverse accessions allows estimation using traces of bottleneck selection (such as genome sweeps) in the genome with associated selection of standing variations and new useful de novo mutations, although the effects of directional introgressions after repeated natural crossings should be considered together. Here, traces of bottleneck selection can be readily identified through population genomics analysis (Huang et al. 2012, Wang et al. 2018). After numerous selection events, the local cultivation scale of rice increased alongside human community formation, which might explain the excavation of ruins indicating rice cultivation. Thus, an explanation gap must have existed between proto-domestication and archeological domestication. Some gaps between molecular and archeological lines

of evidence are logically acceptable when a causal relationship can be established. Based on the hypothesis that various introgression events occurred during rice domestication and the early rice breeding process because of repeated natural crossings and subsequent selection processes, the definition of domestication origins cannot be determined in a simple manner.

Even ignoring clear introgression cases such as *Kala4*, representative sequences of each gene in a subgroup can be categorized into a single or a few group(s) containing a distinct haplotype group, mainly because of haplotype formation following bottleneck selection events. Using this basic information, several key directional introgression events can be considered; the story of rice domestication from an integrated genomic perspective will become more complex.

In addition to clear introgression events like *Kala4* and *Rc*, typical selection of de novo mutations in a subgroup must have also occurred during the rice domestication process. In an example from recent temperate japonica breeding efforts, both the original and selected QTNs of three heading-date QTGs—*Hd6*, *Hd16* and *Hd17* (Takahashi et al. 2001, Matsubara et al. 2012, Hori et al. 2013)—have been identified only in Japanese temperate japonica cultivars tested, indicating that these QTNs represent de novo mutations within a subgroup (**Fig. 1**). Each selected QTN affects the heading date of rice cultivated in Japan. The presence of these three QTNs in local varieties differs among cultivars; it is partially associated with the preferred cultivation area in Japan (**Fig. 1**). A similar pattern can be found in some key domestication QTGs. *qSH1*, a seed-shattering gene, is a good example for explaining this situation (Konishi et al. 2006). The selected *qSH1* QTNs were found in some Chinese landraces of rice, but only in the temperate japonica subgroup. This *qSH1* mutation in combination with the defective *sh4* mutation (Li et al. 2006) can lead to complete loss of the abscission layer at the base of each rice floret, which is required for seed shattering. In this case, the *qSH1* mutation was also a de novo mutation selected in Chinese landraces; it was transmitted to primitive Japanese landraces in the temperate japonica subgroup around a few thousand years ago. Apparently, the *qSH1* QTN was selected in China after *sh4* QTN selection but before the occurrence and selection of *Hd6*, *Hd16* and *Hd17* during recent rice breeding efforts in Japan (**Fig. 4D**).

Taken together, most of the genome of each rice subgroup was presumably fixed immediately after the first strong bottleneck selection event by early cultivators (or ancient humans) because of self-fertilization in the cultivated rice. However, many loci within each cultivar subgroup contain several alleles covering multiple haplotype groups, reflecting distinct evolutionary histories for each gene. This allelic diversity may be related to the size of the target population during the first bottleneck selection and subsequent local adaptation processes underlying a subgroup (along with possible natural outcrossing effects), referred to as fixation and haplotype formation in **Fig. 2**. Thus, the presence of fixed alleles in a subgroup locus is an important factor for tracing the history of the subgroup, but

fixation of the locus is not necessarily evidence of selection for biological and agricultural traits.

Perspective Shift from Genome-Wide Means to QTN Selection History in Rice Domestication Research

To leverage the most genomic information for elucidating the rice domestication process, clarification of the QTN selection history in key QTGs would be a better approach than estimating the timing, location and number of events during crop domestication. One important reason for this proposal originates from several studies that describe new QTNs in previously identified genes. A defective allele with a 383-bp deletion in the *Sd1* (*Semi dwarf 1*) gene, encoding a gibberellin biosynthesis enzyme, was selected to develop a series of semi-dwarf cultivars such as IR8 that are tolerant to lodging during the Green Revolution (Asano et al. 2011). This deletion originated from a Taiwanese cultivar named Dee-Geo-Woo-Gen. Recent work elucidating QTGs underlying deepwater rice traits revealed a new QTN in *Sd1* that causes increased enzymatic activity with distinct substrate specificity; it is often found in wild rice accession lines (Kuroha et al. 2018). This finding suggests that selection for the haplotype including the QTN of *Sd1* occurred during domestication because of a bottleneck effect or the selection of new mutations, causing compact stature prior to breeding selection during the Green Revolution. Thus, understanding the entire history of QTN selection for each key gene is a prerequisite; such genes may have undergone selection for a series of QTNs. Similar findings have been obtained for *Wx* (*Waxy*), a gene encoding a granule-bound starch synthase gene and conferring stickiness to cooked rice grains (Zhang et al. 2019) (Fig. 3). In this case, a few distinct alleles conferring moderate *Wx* enzymatic activity, which tends to be preferred by Asian people, were generated and selected repeatedly, resulting in at least six functionally distinct alleles of *Wx* in agricultural use. The identification of each QTN among various rice lines would be required to fully explain the rice domestication process (Fig. 4). Furthermore, *Hd1* has also been extensively analyzed in terms of its polymorphisms among rice cultivars (Fujino et al. 2010, Itoh et al. 2018, Wu et al. 2020). In addition to various defective mutations, complex introgression patterns are characteristic of *Hd1*. Among the introgressions of *Hd1*, an allele from indica has introgressed into a substantial number of temperate japonica cultivars (Itoh et al. 2018, Wang et al. 2021) (Fig. 3). This is a very rare case of directional introgression from indica to temperate japonica, which may have occurred to support this local adaptation (Choi et al. 2017). In addition, as described above, population genomics data regarding a defective allele of *Hd1* with a 2-bp deletion originally found in an aus cultivar (Yano et al. 2000, Itoh et al. 2018) across more than 3,000 cultivars of rice revealed that this mutation might have originated from tropical japonica (Fig. 4D) (Wang et al. 2018). In the assessments of defective mutations among heading-date genes, several such mutations of the *RFT1* gene were found in cultivars of the aus subgroup.

This observation can be explained genetically because the phenotype of the *RFT1* gene is masked in *hd1*-defective mutants (Fig. 4A) (Ogiso-Tanaka et al. 2013).

Similar to previous trials (Konishi et al. 2008), QTN distributions for *Wx*, *Hd1* and *qSH1* were confirmed using new information concerning QTNs based on the 3K data (Fig. 4D). To define both fine subgroups of japonica and the local area where cultivars were bred, in this case, QTNs in the three genes were required. In this process, the presumed propagation of rice cultivation was largely northward based on the analysis of novel defective alleles (Fig. 1). This northward progression required substantial biological reduction of photoperiodic floral induction and floral repression in cold environments (Izawa 2007, Cui et al. 2020, Fujino et al. 2022). After these changes, cultivar performance often worsened when such cultivars were grown in southern areas, indicating that longitudinal change in the growth area required further biological selection even during early rice domestication. Therefore, when constructing a future model of rice domestication, such biological factors should be incorporated. Although popular methods such as phylogenetic tree construction from DNA changes in QTGs provide a good approach to highlight the selection of key QTNs, further developments are needed. A graphical method to integrate subgroup classification, gene evolution and QTN information could be possible for this purpose. Thus, the rice domestication process and early propagation of rice cultivation may be explained in the near future by obtaining abundant data for the QTN histories of key genes. Notably, the resulting models should be evaluated using unbiased DNA polymorphisms. Unbiased DNA polymorphisms have so far guided the construction of a model for rice domestication, but no good method is available for elucidating the hidden history of rice domestication because the biological aspect of target selection is often missing. The use of unbiased DNA polymorphism data alone to explain the domestication process is similar to the extensive usage of a cipher without decoding. Thus, a biological understanding of various QTN selection processes to explain rice domestication is urgently needed, although validations of models with unbiased information are ultimately required (Zheng et al. 2022). Demographic approaches have been published recently (Gutaker et al. 2020, Alam et al. 2021, Spengler et al. 2021), which may lead to novel approaches for rice breeding based on reliable information obtained through population genomics analysis of rice (Qin et al. 2021, Wei et al. 2021).

Possible Causal Link between Archeological Evidence from Ruins and DNA Traces in a Domestication-Related QTG

Ideally, archeological data can be logically explained based on changes in QTNs, which would require comprehensive understanding of the phenotypic changes related to causal QTN selection. One such example can be found in the seed-shattering trait of rice and its changes during the domestication process in rice. Two QTGs associated with seed-shattering traits have

been identified during rice domestication and the early spread of rice cultivation (Konishi et al. 2006, Li et al. 2006). The QTN selection of *sh4* results in panicles with seeds upon maturation, whereas wild rice accessions have panicles devoid of mature seeds because of seed shattering. With respect to plant growth, partial abscission layers may form in cultivars with the selected *sh4* QTN, while complete abscission layers form in wild rice accession lines. In contrast, *qSH1* selection results in complete loss of abscission layers. Thus, the detached surface at the base of the rice seed grain became rough after *qSH1* selection. Because the abscission layer forms during floret formation, the abscission process begins immediately before floret flowering. Thus, phenotypic changes related to seed shattering were very clear for *qSH1* selection but not for *sh4*. In a paper published by Zheng et al. (2016), reduced shattering of archeological rice from the Lower Yangtze valley was extensively examined through morphological and histological comparisons of spikelet bases from four sites with distinct dates using scanning electron microscope imaging; the results revealed that selection for non-shattering seeds proceeded continuously among these sites, confirming a prolonged selection period for the non-shattering trait around 8,000–5,000 years ago in the Lower Yangtze valley (**Fig. 4D**). The authors described a possible QTN selection process for *qSH1*. Thus, earlier selection for *sh4*, which may have caused the first bottleneck in rice domestication, occurred more than 8,000 years ago. Such links between archeological data and phenotypic changes driven by the selection of QTNs are not easily observed; extensive biological analysis may enable more such links to be identified in the future. Notably, some archeological papers missed these botanical changes in seed-shattering traits in rice due to a lack of biological consideration for such genetic effects (Fuller et al. 2009). Yet, while ancient DNA may be isolated from rice remains in the future, to date such samples have not provided any reliable conclusions related to plant domestication. Thus, the link connecting *qSH1* QTN selection with non-shattering seed selection in temperate japonica in the Lower Yangtze valley is a critical step in achieving an integrated view of rice domestication (**Fig. 4D**).

How Domestication Genes and Improvement (or Diversification) Genes Can Be Distinguished in Rice Domestication

As mentioned, both *sh4* and *qSH1* are related to seed shattering in rice, which has been considered as a typical domestication trait in many crops. However, while *sh4* can be termed a domestication gene, *qSH1* may be considered an improvement (or diversification) gene, i.e. a gene associated with post-domestication improvement or diversification. Thus, it may be not a good idea to consider specific traits, such as seed shattering, as criteria by which to distinguish them. In addition, like *Sd1*, *Wx* and *Hd1*, several key QTNs in the one gene are capable of being selected for several times during the domestication

and improvement processes in rice; such a scenario complicates the distinction between domestication and improvement genes. Only after the entire mapping of QTNs among various rice accessions is achieved, will such distinctions become clearer. It is also worth noting that the selection for *qSH1* might have occurred around 7,000 years ago. This was a rather old selection event when considering the entire history of rice domestication and breeding.

A Message from the Past for the Future of Agriculture

Using genome editing technology, desired mutations can be efficiently incorporated into crops (Rodríguez-Leal et al. 2017, Kwon et al. 2020), including possibly for orphan crops (Lemmon et al. 2018). In this context, information regarding crop domestication will be essential to support future breeding. An approach using such information was recently reported to describe the current status of the development of allotetraploid rice cultivars (Yu et al. 2021). During crop domestication, changes in cis-regulatory elements could lead to major QTNs (Lemmon et al. 2014). It is well known that the QTN for *qSH1* selection occurred at a nucleotide in a cis-regulatory element around 12 kb upstream of the region encoding the *qSH1* gene product. With the abundance of genomic information available from diverse cultivars, a novel method for identifying the cis-regulatory elements that underlie phenotypic changes from diverse DNA polymorphisms in cis-regulatory regions of QTGs is now needed.

Data Availability

No new datasets were generated in this study. Source data for figures are provided in the paper, while the data summary was easily obtained using publicly available data.

Disclosures

The authors have no conflicts of interest to declare.

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