Primer
Rice domestication

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Rice is a staple food crop for more than one-third of the global population (http://www.sustainablerice.org/), of which 90% live at or near the poverty line. Thus, rice genetic improvement is important for global food security and is critical for enhancing socioeconomic benefits and reducing the environmental impacts of agriculture. In continued efforts to address the long-standing problem of food security and sustainable agriculture, scientists are utilizing genes from diverse varieties of rice to improve the resilience of rice to pests, diseases and environmental stress. This Primer describes the history of rice domestication, the importance of wild relatives of rice for crop improvement, and the domestication of wild species of rice not previously planted by farmers — a new approach called neodomestication.

The history of rice domestication
Asian cultivated rice (*Oryza sativa*; Figure 1) is a semi-aquatic annual grass that can be grown across a wide breadth of agricultural ecosystems — from deeply flooded land to dry, hilly slopes. Historically, *O. sativa* has been divided into two major subspecies or varietal groups: *japonica* (or *geng*, 'sticky' rice found in temperate East Asia, upland areas of Southeast Asia, and high elevations in South Asia), and *indica* (or *xian*, 'non-sticky' lowland rice grown throughout tropical Asia). In addition to Asian rice, the genus *Oryza* contains one additional domesticated species — African rice (*Oryza glaberrima*; Figure 1). African rice is now a minor crop grown in both West Africa and Suriname (South America), the latter of which was introduced by Maroons (descendants of escaped African plantation slaves) in the mid-17th and 18th centuries.

Asian cultivated rice was domesticated from the wild rice species *Oryza rufipogon* (Figure 1). The domestication process began around 9,000 years ago in China,

possibly in the Yangtze valley. There, early communities selected for rice plants with beneficial traits, such as enhanced seed/fruit size, better flavor, and resistance to diseases, pests, and environmental stresses. The saving of selected *O. rufipogon* seed and subsequent cultivation led to the evolution of progenitors of *japonica* varieties. These early *japonica* cultivars were subsequently spread southward and eastwards in Asia, and crossed with local populations of *O. rufipogon*. Recurrent rounds of crossing and selection led to the introduction of critical genes that control key domestication traits into the genome of precursors of *indica* varieties. One of the most important of these traits is the loss of the seed shattering habit, which increases the number of seeds that the plant retains, allowing farmers to more easily harvest the grain.

As *O. sativa* spread throughout Asia, another domestication event within the *Oryza* genus was taking place in Africa, where early farmers started cultivating the wild species *Oryza barthii* along the Niger river delta, leading to the evolution of cultivated African rice — *O. glaberrima* (Figure 1). For more than 60 years, scientists have theorized about how the parallel but independent domestication of Asian and African rice

may have occurred. One hypothesis is that neolithic farmers in Asia and Africa selected for a set of similar key traits critical for their domestication. In support of this model, a comparison of a set of known domestication genes in Asian rice (the shattering genes *OsSh1* and *Sh4*) with orthologous (ancestrally derived) genes in Africa rice revealed that loss-of-function mutations could be identified in orthologous pairs of genes, but the mutations were completely different, thereby demonstrating parallel convergent evolution.

The wild relatives of rice provide a source of genetic diversity for rice improvement
In addition to Asian and African rice, the genus *Oryza* contains 25 globally distributed wild species, 16 of which contain two sets of chromosomes (i.e. their genomes are diploid), and 9 of which contain four copies of each chromosome (i.e. their genomes are tetraploid). The domesticated and wild species are classified into 11 distinct and cytologically defined 'genome types'. These genome types differ 3.6 fold in size and span 15 million years of evolutionary history (Figure 2).

Why is the diversity found in the genus *Oryza* important and how can
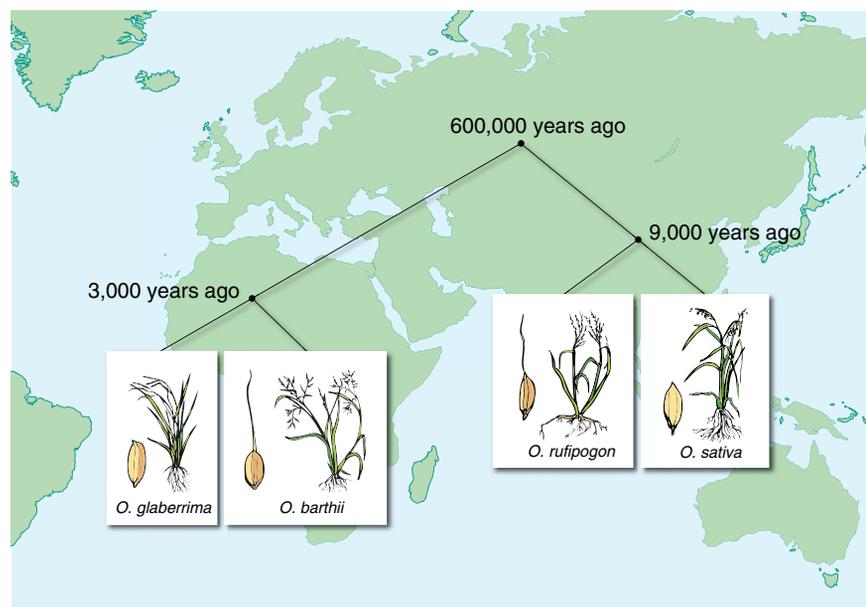
Figure 1. Domestication of two rice species.
Distinct but parallel routes of domestication of *O. sativa* in Asia and *O. glaberrima* in Africa. The former evolved from the wild species *O. rufipogon* starting ~9,000 years ago, the latter from the wild species *O. barthii* starting ~3,000 years ago. Adapted from Purugganan (2014).

it be harnessed to improve cultivated rice? In the field of genetics, variation within and among individuals of a population or species is defined as genetic diversity. Genetic diversity determines the range of inherited traits (phenotypic variation) among individuals, and serves as a mechanism for populations to adapt to changing environmental conditions. With greater genetic diversity, it becomes more likely that some individuals in a population will possess key alleles (specific versions of genes) that are suited for adaptation to a specific environment. The domestication process — through the fixation of beneficial alleles giving superior characteristics and progressive removal of undesired ones — has inevitably reduced the genetic diversity of cultivated crops.

In contrast, the wild relatives of rice contain a vast reservoir of traits important for ecological adaptation that can be used to enhance the genetic diversity of cultivated rice. This type of variation is particularly important with regards to traits critical to sustainable agriculture, such as resistance to biotic stress (e.g. insect pests, fungal and bacterial diseases), tolerance to environmental stress (e.g. extreme temperature, floods, drought, salinity), and enhanced nutritional quality. Rice 'landraces' are local traditional varieties within a species that have been domesticated and adapted to their natural and cultural environments, and harbor higher genetic diversity than many cultivated species. Thus, landraces can also be harnessed as an additional source of diversity, in addition to the wild relatives, for crop improvement.

Access to natural genetic diversity (both physically in seed banks, and virtually in data banks) for both cultivated and wild *Oryza* species is essential for continued advancement in crop improvement. The T.T. Chang Genetic Resources Center (TTCGRC) is a well-known and internationally accessible germplasm bank, located in the Philippines at the International Rice Research Institute (IRRI). The TTCGRC stores and maintains over 130,000 accessions (i.e. distinct and uniquely identifiable plant material representing a cultivar, breeding line or population) of *Oryza* that includes genetic stocks (plants or populations crossed and/or
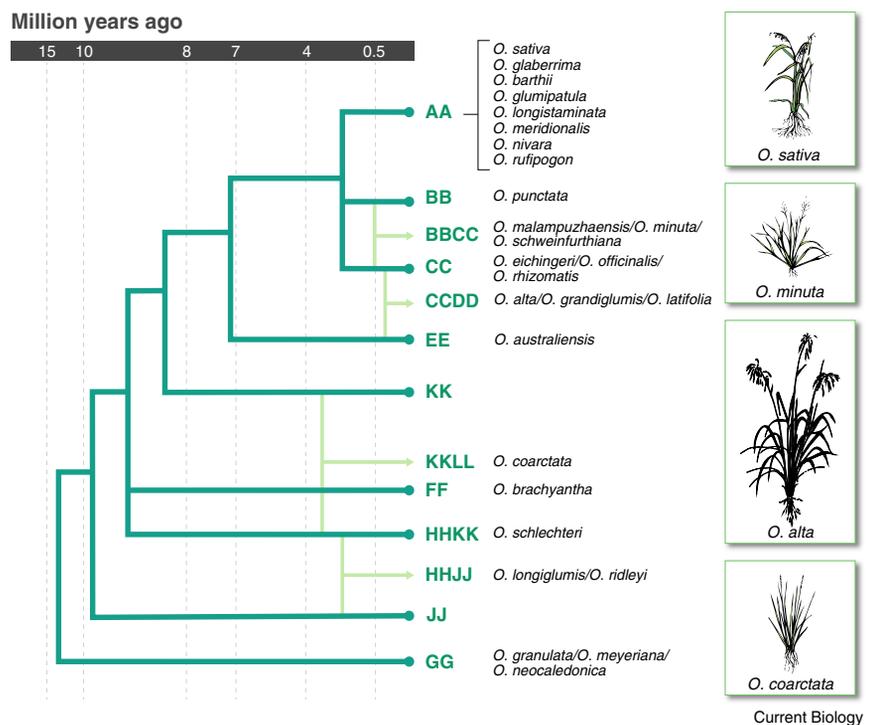

Figure 2. Genome types and phylogenetic relationship between the major rice species within the genus *Oryza*.
(Insets) Examples of species with different genome type showing wide range of trait variation. Genome types are described with letters, where AA defines the primary gene pool including also, but not exclusively, the domesticated rice species (i.e. *O. sativa* and *O. glaberrima*); wild diploid and tetraploid species are divided into AA, BB, CC, EE, FF, GG and BBCC, CCDD, HHJJ, HHKK, KKLL genome types, respectively. Adapted from Solis *et al.* (2020).

selected for genetic studies), landraces and wild relatives. An increasing number of these genetic resources have been sequenced, thanks to the rapid advancement and decreasing costs of sequencing technologies. This genomic information is crucial to define genes and their functions, and to compare genomes from different individuals and species to assess important biological questions, such as tolerance to stress.

The importance of seed banks, and the landraces and wild species stored therein, is reflected by the numerous agronomically important genes that have been introgressed into wild species (Table 1). Introgression is the process of incorporating novel genes from a donor plant (landrace or wild species) into a domesticated species. Introgression is achieved when hybrid progeny derived from a cross-pollination between a domesticated and a wild species is back-crossed for several generations to the domesticated parent. Key examples

of genes introduced through breeding include genes that confer resistance or tolerance to both biotic (*Bph* and *Xa21*) and abiotic (*Sub1A*) stresses.

Brown planthopper (BPH) is a damaging insect pest of rice found across Asia. The use of insecticides to control this pest can be harmful to humans and the environment, and can select for pests that are resistant to insecticides. For these reasons, host-plant resistance is the preferred strategy for pest control. Four such resistance genes — *Bph1*, *bph2*, *Bph3* and *bph4* — have been introgressed into domesticated Asian rice and released to the market.

Bacterial leaf blight (BLB), a devastating rice disease caused by the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is the cause of dramatic yield loss in rice production. Forty-six resistance genes against *Xanthomonas* (*Xa* genes) have been identified from cultivated and wild rice species, including *Xa21*, which was introgressed from the wild species

Table 1. Traits introgression between cultivated rice *O. glaberrima* or a wild *Oryza* species as the donor parent, and an *O. sativa* cultivar as the recurrent parent.

Donor parent (accession)	Recurrent parent (cultivar)	Trait introgressed/mapped	Reference
O. glaberrima (IRGC103544)	Tropical *japonica* (Caiapo)	Rice stripe necrosis virus resistance	Gutiérrez *et al.* (2010). BMC Plant Biol. *10*, 6
O. glaberrima (IRGC103544)	*Japonica* (WAB56-104)	Drought resistance, early vigor	Ndjiondjop *et al.* (2010). Plant Sci. *179*, 364–373
O. glaberrima (IRGC103544)	*Indica* (Milyang 23)	Yield and yield components	Kang *et al.* (2008). Kor. J. Breed Sci. *40*, 243–249
O. nivara (IRGC 105444)	*Japonica* (Taichung 65)	Pollen sterility gene	Win *et al.* (2011). Theor. Appl. Genet. *122*, 385–394
O. nivara (IRGC 105444)	*Japonica* (Koshihikari)	Hybrid breakdown locus	Miura *et al.* (2008). Breed Sci. *5*, 99–105
O. rufipogon (IRGC105491)	Tropical *japonica* (Jefferson)	Early flowering	Maas *et al.* (2010). Theor. Appl. Genet. *120*, 895–908
O. rufipogon (IRGC105491)	*Indica* (IR64)	Yield and yield components	Cheema *et al.* (2008). Euphytica *160*, 401–409
O. rufipogon (IRGC105491)	*Japonica* (Hwaseongbyeo)	Yield components	Xie *et al.* (2008). Theor. Appl. Genet. *116*, 613–622
O. minuta (IRGC101141)	*Indica* (IR31917-45-3-2)	Brown planthopper (BPH) resistance	Ram *et al.* (2010). Rice Genet. Newsl. *25*, 67–68
O. brachyantha (IRGC101232)	*Indica* (IR56)	Bacterial blight (BB) resistance	Ram *et al.* (2010). Rice Genet. Newsl. *25*, 57
O. longistaminata	*Japonica* (Taipei 309)	Bacterial leaf blight (BLB) resistance	Song *et al.* (1995). Science *270*, 1804–1806

Adapted from Jacquemin *et al.* (2013).

Oryza longistaminata into varieties lacking resistance.

Another key challenge for breeders is the introgression of genes that confer resilience to environmental stress. For example, introgression of the *Submergence Tolerance 1A* (*Sub1A*) gene into several farmer-preferred varieties throughout South and Southeast Asia resulted in the development of submergence-tolerant rice. Whereas most rice varieties will die if completely submerged for more than three days, *Sub1* varieties can survive two weeks of complete submergence. Following a flood, *Sub1* varieties confer a 60% yield advantage compared with conventional varieties. A study of 128 villages in the state of Odisha in India revealed that socially disadvantaged farmers, many of whom have been growing rice on flood prone land for generations, disproportionally benefit from the cultivation of *Sub1* rice.

Genetic engineering approaches have provided a complementary strategy to conventional plant breeding. Using genetic engineering, researchers can transfer genes from any species into rice. An excellent example of the use of genetic engineering techniques is the development of a rice cultivar to help address Vitamin A deficiency, the leading cause of childhood blindness and death in many parts of the world. Golden Rice contains high levels of beta-carotene, which can be converted by the human body into Vitamin A. Created two decades ago, Golden Rice was approved for commercial production in the Philippines in July 2021 (http://www.goldenrice.org/).

A new and powerful tool to modify plant genomes is through genome editing, using, for example, the CRISPR–Cas9 system. These techniques allow very precise changes to be made to improve crops. For example, the CRISPR–Cas9 system was used to engineer resistance to bacterial leaf blight disease in two widely grown rice varieties.

Neodomestication of wild tetraploid rice *Oryza alta* and other species

Neodomestication is an innovative approach for crop improvement that enables the generation of a domesticated crop from a non-domesticated species through the manipulation of critically important domestication genes. The successful neodomestication of a plant species was first demonstrated in 'ground-cherry' — a wild *Solanaceae* species related to pepper and tomato. Ground cherry was domesticated through the precise modification of a set of genes that were known to control fruit size and abscission (fruit drop) in tomato. Since this first example, neodomestication has been used in both wild tomato (*Solanum pimpinellifolium*), and the wild relative of rice *Oryza alta*.

Requirements for the successful application of neodomestication are: firstly, availability of a high-quality reference genome and transformation system of the plant species to be improved; secondly, knowledge of essential genes for domestication and their function; and thirdly, information on the natural genetic diversity occurring in that species and related ones. A key step for neodomestication
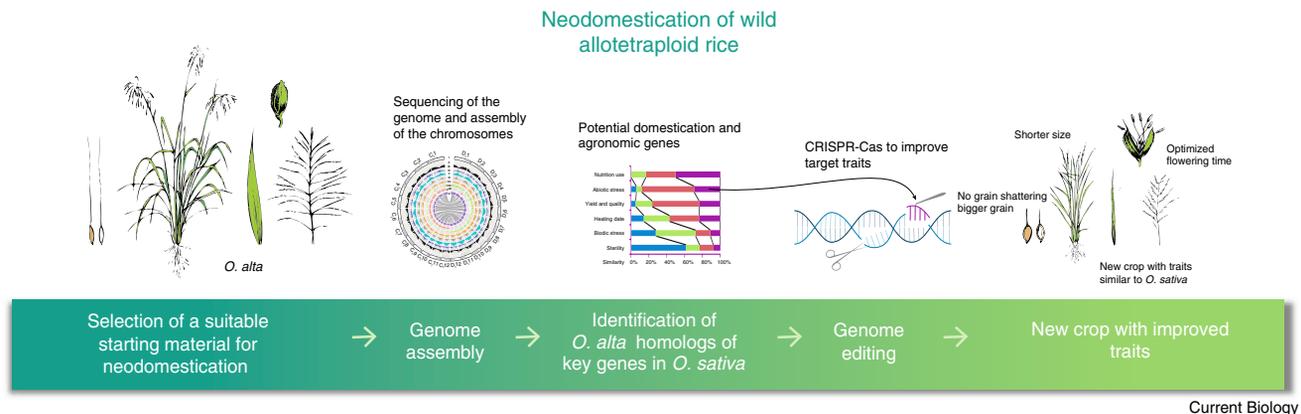
Neodomestication of wild allotetraploid rice

Figure 3. Schematic representation of the major steps required to neodomesticate *O. alta* (leftmost panel) to generate a new crop with improved traits (rightmost panel).
To improve domestication- and agronomic-related traits in *O. alta,* the following steps are required: (1) selection of a suitable starting material; (2) generation of a high-quality genome assembly; (3) genome assembly to identify agronomic and domestication genes; (4) optimization of genome editing system to modify key genes. Adapted from Yu *et al.* (2021).

is the identification of genes in the target species that are homologous to domestication-related genes in the cultivated species by DNA sequence comparison. Domestication-related gene sequences in the target species are then modified using genome editing tools (e.g. CRISPR–CAS) to improve those traits.

The publication in 2021 of the neodomestication of a wild relative of rice represents the first example of a newly domesticated cereal crop with a polyploid genome. Researchers chose the wild species *Oryza alta*, which is tolerant to salinity and resistant to pests compared with many domesticated rice varieties (Figure 3, leftmost panel). The research team developed a high-quality reference genome sequence of *O. alta* and established an efficient genetic transformation protocol to introduce gRNAs targeting genes controlling seed shattering, height, grain size and flowering time (Figure 3, middle panels). The editing of genes controlling these traits led to a shorter plant that does not fall over easily, larger grains, seeds that do not fall off the plant and optimized flowering time (the time from sowing to the emergence of the first panicle). Together, these traits make *O. alta* suitable for grain production under temperate growing conditions (Figure 3, rightmost panel). At the same time, the neodomesticated plant retains useful traits characteristic of the progenitor *O. alta* wild species. This study demonstrated the possibility

of domesticating *de novo* a staple cereal in an unprecedented amount of time using small and highly precise modifications.

Following these initial neodomestications in ground cherry and *O. alta*, scientists are now directing their efforts to other species. For example, African rice cultivars and their wild relatives are known to possess genes that confer environmental stress tolerance (such as *TT1*), regulating response to heat stress, and a gene associated with salt tolerance (*HAK5*). The neodomestication of these wild rice species can lead to genetically improved rice with enhanced thermotolerance and salt tolerance.

Another candidate for neodomestication in the genus *Oryza* is *Oryza coarctata*, the only known wild relative of rice that is considered a tru halophyte (a plant that is adapted to growing under saline conditions). This species grows naturally along coastal regions with brackish water in South Asia, from Pakistan to Myanmar, and is eaten as a local delicacy in Bangladesh. Attempts at introducing salinity stress resistance genes from *O. coarctata* into cultivated rice through conventional methods were unsatisfactory. Challenges to these traditional methods include the limited success of conventional breeding techniques to introgress traits controlled by multiple genes, and reproductive barriers that prevent the successful crossing of *O. coarctata* with cultivated species. The

availability of the genome sequence of *O. coarctata* and the information gained from its genetic diversity are necessary requirements to access its neodomestication potential. The modification of key genes in *O. coarctata* through genome editing could overcome many limitations from previous techniques, and could potentially lead to a new crop that is tolerant to high salinity and that has agronomically useful traits — such as large grain size and good yield. Given that salinity stress affects global rice production by limiting both growth and yield, a neodomesticated *O. coarctata* species could allow rice cultivation in saline/brackish water conditions, thereby reducing the need for a fresh water supply and thus leading to more sustainable rice production and increased food security. The history of cultivated rice has demonstrated that a limited number of genes are required to regulate key domestication traits. This opens up the possibility to neodomesticate other crops that possess resilience and adaptation traits — such as the 'orphan' crops millet and grass pea — thus allowing breeders to broaden the palette of crops that can be grown across the globe.

Conclusions
All the major crops we grow today were domesticated in their centers of origin and were thus well adapted to their local environmental conditions. It was only later through globalization

that domesticated plants were literally uprooted and spread across the globe. There have been many benefits to this globalization — without tomato sauce (tomatoes from the Americas) and wheat noodles (from the Middle East) there would be no Italian spaghetti. Still, there are drawbacks. Many of today's crops lack the genetic diversity of their ancestors and are vulnerable to disease as well as environmental stress. In contrast, the domestication of crops that are already well adapted to local environments would be, by default, more sustainable. As described here, the overall strategy can be approached by first identifying and sequencing plants that already grow well in extreme environments (e.g. dry arid lands, salt marshes), then develop transformation systems, and finally edit critical genes associated with domestication.

As the world's population is set to approach 10 billion by 2050, we must consider new strategies to produce food that will feed the growing population. The examples described here show how genetic diversity can be harnessed as an effective approach for plant domestication with benefits to the environment, food security and local economies. Conservation of wild species and landraces of crop plants is critical to both preserve genetic diversity from being lost, and to facilitate crop improvement. Scientists are now poised to launch new approaches to domestication — using what we have learned from both the history of domestication, and the modern technologies of genetic engineering, genome editing and genomic selection — to generate the crops of the future.

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Evidence of a functional clitoris in dolphins

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In species that copulate during non-conceptive periods, such as humans and bonobos, sexual intercourse is known to be pleasurable for females. Dolphins also copulate throughout the year, largely to establish and maintain social bonds[1]. In dolphins, the clitoris is positioned in the anterior aspect of the vaginal entrance[2], where physical contact and stimulation during copulation is likely. Clitoral stimulation seems to be important during female–female sexual interactions in common bottlenose dolphins (*Tursiops truncatus*), which rub each other's clitorises using snouts, flippers, or flukes[3]. Determining a sexual pleasure response in animals not amenable to neurobehavioral examination is difficult, but investigation of the clitoris may elucidate evidence of functionality. In this study, we assessed macro- and micromorphological features of the clitoris in common bottlenose dolphins to examine functional features, including erectile bodies with lacunae, extensible collagen and/or elastin fibers, and the presence and location of sensory nerves. Our observations suggest the clitoris of dolphins has well-developed erectile spaces, is highly sensitive to tactile stimulation, and is likely functional.

Excised clitorises were collected from naturally deceased post-mortem females of all age classes (n = 11) under a National Marine Fisheries Services parts authorization letter to D.N.O. and P.L.R.B. (Table S1). We examined the presence, shape, and configuration of erectile bodies, and the innervation of the clitoris using gross dissections, diffusible iodine-based contrast-enhanced computed tomography (DiceCT) reconstructions, paraffin histology, and immunohistochemical staining with a peripheral nerve fiber marker (PGP 9.5).

The clitoris of the female dolphin is large, well-developed, and positioned at the entrance of the vaginal opening (Figure 1A). The cranial clitoral body contains the urethral opening that ends