The Rice Paradox: Multiple Origins but Single Domestication in Asian Rice

Jae Young Choi,[1] Adrian E. Platts,[1] Dorian Q. Fuller,[2] Yue-Ie Hsing (邢禹依),[3] Rod A. Wing,[4] and Michael D. Purugganan*[1,5]

[1]Department of Biology, Center for Genomics and Systems Biology, New York University, New York, NY

[2]Institute of Archaeology, University College London, London, United Kingdom

[3]Institute of Plant and Microbial Biology, Academica Sinica, Nankang, Taipei, Taiwan, Republic of China

[4]Arizona Genomics Institute, University of Arizona, Tucson, AZ

[5]Center for Genomics and Systems Biology, New York University Abu Dhabi, Saadiyat Island, Abu Dhabi, United Arab Emirates

***Corresponding author:** E-mail: mp132@nyu.edu.

Associate editor: Yuseob Kim

Abstract

The origin of domesticated Asian rice (*Oryza sativa*) has been a contentious topic, with conflicting evidence for either single or multiple domestication of this key crop species. We examined the evolutionary history of domesticated rice by analyzing de novo assembled genomes from domesticated rice and its wild progenitors. Our results indicate multiple origins, where each domesticated rice subpopulation (*japonica*, *indica*, and *aus*) arose separately from progenitor *O. rufipogon* and/or *O. nivara*. Coalescence-based modeling of demographic parameters estimate that the first domesticated rice population to split off from *O. rufipogon* was *O. sativa* ssp. *japonica*, occurring at ~13.1–24.1 ka, which is an order of magnitude older then the earliest archeological date of domestication. This date is consistent, however, with the expansion of *O. rufipogon* populations after the Last Glacial Maximum ~18 ka and archeological evidence for early wild rice management in China. We also show that there is significant gene flow from *japonica* to both *indica* (~17%) and *aus* (~15%), which led to the transfer of domestication alleles from early-domesticated *japonica* to proto-*indica* and proto-*aus* populations. Our results provide support for a model in which different rice subspecies had separate origins, but that de novo domestication occurred only once, in *O. sativa* ssp. *japonica*, and introgressive hybridization from early *japonica* to proto-*indica* and proto-*aus* led to domesticated *indica* and *aus* rice.

Key words: **crop species, adaptation, introgressive hybridization, gene flow.**

Introduction

The Asian rice *Oryza sativa* is the world's most important food crop, and is a staple for more than one-third of the world's population (Gnanamanickam 2009). *Oryza sativa* is genetically differentiated into several groups, the main ones being *japonica* and *indica*, which have been considered as subspecies/subpopulations with distinct morphological and physiological characteristics (Matsuo et al. 1997). While these two represent the major groups, large-scale studies indicate that *O. sativa* is better described as comprising five major subpopulations (Garris et al. 2005): *aus*, *indica*, temperate *japonica*, tropical *japonica*, and aromatic rice. Temperate and tropical *japonica* are closely related and group with aromatic rice, while *indica* and *aus* show closer genetic affinities (Garris et al. 2005). There is incipient intraspecific reproductive isolation within *O. sativa*, at least between *japonica* and *indica* (Chen et al. 2008), and these two are sometimes considered subspecies. *Oryza rufipogon* is commonly accepted as the wild progenitor of *O. sativa* (Khush 1997), although *O. nivara*, often considered as the annual form of *O. rufipogon*, has also been proposed as the ancestor (Yamanaka et al. 2003).

The evolutionary origins of the domesticated Asian rice have been enigmatic. Proponents have advocated either single or multiple origins of *O. sativa*, and different lines of genetic evidence support conflicting hypotheses on the origin of this domesticated species (Sang and Ge 2007; Gross and Zhao 2014). The single-origin hypothesis suggests *O. sativa* was domesticated once and differentiated into various subpopulations (Vaughan et al. 2008). Genes controlling critical domestication traits, such as seed shattering (Li et al. 2006) and pericarp color (Sweeney et al. 2006), for example, indicate that domesticated alleles are shared between *indica* and *japonica* (Sang and Ge 2007; Sweeney et al. 2007; Zhang et al. 2009; Huang et al. 2012a). Moreover, demographic modeling using polymorphism data provide support for a single domestication (Molina et al. 2011).

The two-origin hypothesis, on the other hand, posits that *japonica* and *indica* have independent origins from distinct *O. rufipogon* subpopulations (Oka 1988). This would explain the deep genetic differentiation between *japonica* and *indica*, where divergence times between these subspecies are much earlier (Ma and Bennetzen 2004; Zhu and Ge 2005) than the earliest archeological evidence of domesticated rice ~8–9

Article

Open Access

thousand years ago [ka] (Fuller et al. 2010). In addition, genome-wide data shows *japonica* and *indica* clustering with different *O. rufipogon* accessions and not with each other (Second 1982; Cheng et al. 2003; Londo et al. 2006; Huang et al. 2012a; Yang et al. 2012). A recent study (Civáň et al. 2015) even suggests a multiple-origin model hypothesizing *japonica*, *indica*, and *aus* having three separate origins with independent domestications [but see Huang and Han (2015)].

How can one resolve these conflicting lines of evidence that point to disparate evolutionary models for the origin of rice? To address this question, we conducted phylogenomic analyses and demographic modeling using high-quality whole genome sequence data from various subspecies of *O. sativa* and its wild progenitors *O. rufipogon* and *O. nivara*. Whole genome sequences allow for population genomic inference even from a single representative of each species/subspecies, since unlinked genomic regions have independent coalescent histories; therefore, genome-wide analysis of single genomes can provide species-wide population genetic information (Gronau et al. 2011).

We show that rice subspecies *japonica* and *indica*, as well as *aus*, are derived from genetically distinct lineages of *O. rufipogon* and/or *O. nivara*, originating prior to rice domestication. We also show significant gene flow from *japonica* to *indica* and *aus* lineages, consistent with previous studies that show the transfer of domestication alleles into *indica* from *japonica* (Huang et al. 2012a). Our results suggest that modern Asian rice subspecies or subpopulations originated from multiple and distinct wild rice populations. However, de novo domestication occurred only once, in *japonica*, and the domestication of *indica* and *aus* arose from gene flow from this early *japonica* domestication that introduced domestication alleles into a proto-*indica* population.

Results

We generated a multi-genome alignment of Asian rice (*japonica*, *indica*, and *aus*), the wild progenitors (*O. rufipogon* and *O. nivara*), and an outgroup species (*O. punctata*); the *japonica* genome was used as a reference. For most pairwise genome alignments, an average 74% of a genomes' sequence aligned with *japonica* (see supplementary table S1, Supplementary Material online). *Oryza punctata*, however, had 37% of its sequence aligned to the *japonica* genome. We focused our analysis on orthologous regions from the six key species/subspecies (*japonica*, *aus*, *indica*, *O. rufipogon*, *O. nivara*, and *O. punctata*). For demography analysis, we excluded *O. punctata* due to its evolutionary divergence. For gene topology analysis, ~19,000 genes were analyzed depending on the cultivar genome that was being compared (see supplementary table S2, Supplementary Material online). For demographic modeling, there were 1,183 regions with sizes of 1-kb that matched our neutral criteria.

Estimating the Species Tree

Initially, we inferred the species phylogeny for domesticated and wild rice by reconstructing the genome-wide phylogenetic tree. Using the *O. punctata* genome as an outgroup a maximum-likelihood based phylogenetic tree was reconstructed using 4,477,815 4-fold degenerate sites (fig. 1). Bootstrap support for all internal nodes were 100%, and *japonica* was monophyletic with *O. rufipogon* while *aus* and *indica* formed a monophyletic group with *O. nivara*.

These results are concordant with the major population relationships determined using genome-wide polymorphism datasets (Garris et al. 2005; McNally et al. 2009; Xu et al. 2011). Interestingly, analysis of *aus*, *indica*, and *O. nivara* indicated that *O. nivara* was more closely related to *aus* than to *indica*. This suggested that *aus* evolved from *O. nivara*, while *indica* originated earlier from either *O. nivara*, or a separate, genetically distinct *O. rufipogon* or *O. nivara* population. It is also possible that *aus* and *indica* have a common origin but *aus* was extensively backcrossed to an ancestral *O. nivara*. It should be noted that while the wild rice genome sequences used in this study are representative of each of the wild rice species, they might not necessarily represent the direct wild progenitor populations for domesticated rice. Nevertheless, our proposed species topology for the Asian rice complex suggests all three domesticated rice originated from two or three independent populations.

Inference of Admixture and Incomplete Lineage Sorting

Although the genome-wide species phylogeny supported a single topology (fig. 1), rarely will the entire genome reflect a single topology, as incomplete lineage sorting and gene flow can distort the underlying species tree (Degnan and Rosenberg 2009). To examine the degree of admixture and incomplete lineage sorting, we looked at each gene topology and compared it to the species tree. Proportion of genes that support the minor topologies can estimate the proportion of admixture and incomplete lineage sorting across the genomes (Pollard et al. 2006; see Materials and Method for brief discussion).

Approximately, unbiased (AU) tests (Shimodaira 2002) were conducted for each gene to test for significant support of either the species tree (major topology) or the two minor tree topologies. Results are shown for tests involving *japonica*, $indica_{IR64}$, aus_{DJ123}, *O. nivara*, and *O. rufipogon* (table 1) but results from the full combination of comparisons are listed in the supplementary table S3, Supplementary Material online.

Comparing *O. nivara* and *O. rufipogon* to a domesticated rice sample, most of the genes significant after the AU test supported a major topology that was concordant with the genome-wide species phylogeny (fig. 1). Specifically, more then 80% of the genes supported the topology grouping *japonica* with *O. rufipogon* while more then 55% of the genes supported the topology grouping *O. nivara* with either *aus*/*indica*. For *aus* and *indica*, however, the bootstrap confidence interval for the two minor topologies (minor topology 1 = ([*aus*/*indica*, *O. rufipogon*], *O. nivara*) vs. minor topology 2 = ([*O. rufipogon*, *O. nivara*], *aus*/*indica*)) did not overlap with each other. The topology grouping *aus* or *indica* with *O. rufipogon* had support from significantly greater number of genes (*aus* had 28.6% [27.1–30.1%] of genes supporting minor topology 1 vs. 6.3% [5.5–7.1%] of genes supporting minor

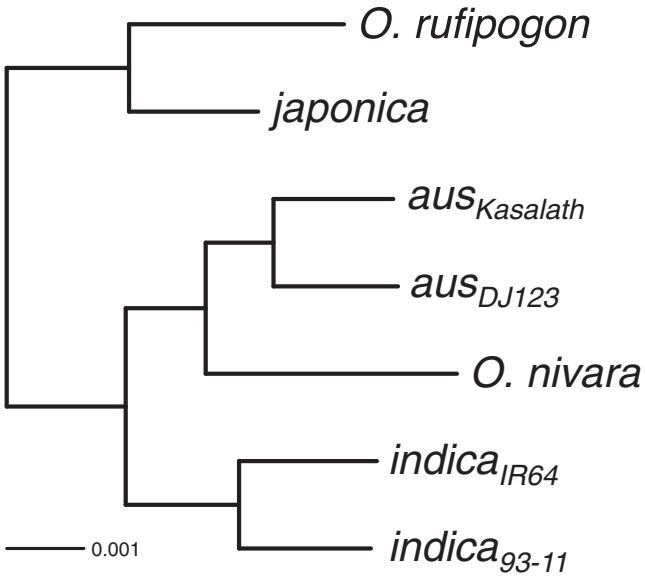


FIG. 1. Species phylogeny for the Asian rice complex. *Oryza punctata* genome was used for rooting the tree but omitted from figure due to its evolutionary distance. Scale bar length represents number of substitutions per site. All nodes had 100% bootstrap support and were thus omitted from labeling. The phylogenetic tree had a log-likelihood (lnL) of −8,171,522.67.

topology 2; and *indica* had 33.0% [31.6–34.3%] of genes supporting minor topology 1 vs. 10.1% [9.2–11.0%] of genes supporting minor topology 2). Although this suggested potential gene flow between *aus*/*indica* with *O. rufipogon* it is more likely due to indirect evolutionary effects from *japonica* and *O. rufipogon* sharing a common ancestor, and the strong *japonica* originating gene flow (see below, ABBA-BABA test, and G-PhoCS results).

Gene topologies involving *indica*, *aus*, and *O. nivara* were consistent with our species topology, where 58.8% of the genes with significant AU test supported monophyly between *aus* and *O. nivara*. Between the two minor topologies, however, a significantly higher number of genes supported the topology grouping *aus* and *indica* together (22.9% [21.7–24.1%] of genes supported ([*aus*, *indica*], *O. nivara*) topology vs. 18.2% [17.1–19.3%] of genes supported ([*indica*, *O. nivara*], *aus*) topology), suggesting potential gene flow between *aus* and *indica*.

Topology tests comparing *japonica* and *aus*/*indica* with a wild rice were then conducted to test possible introgressions involving *japonica*. Consistent with previous results, more then 60% of the genes supported a major topology that was concordant with the species phylogeny. However, for all comparisons the two minor topologies were not represented by an equal number of genes. Specifically, the topology that grouped *aus*/*indica* with *japonica* had significantly greater number of gene support. These results suggested significant gene flow between domesticated *japonica* and *aus*/*indica*.

We then conducted the ABBA-BABA test (Green et al. 2010; Durand et al. 2011; see Materials and Method for brief discussion of method) to differentiate admixture between populations from incomplete lineage sorting. ABBA-BABA tests with Z-scores greater than $|3.9|$ are shown in table 2 (see supplementary table S4, Supplementary Material online, for full results). Consistent with the gene topology results, there was significant evidence of gene flow between *japonica* and either *indica* or *aus*. Also concordant with the topology test results, *O. rufipogon* was the only wild progenitor to show significant evidence of gene flow to both *aus* cultivars and $indica_{93-11}$. However, we note this may be an artifact arising from *japonica* and *O. rufipogon* sharing a common ancestor, and the strong signal of gene flow associated with *japonica*. In contrast to the topology test results, no admixture was detected among *aus*, *indica*, and *O. nivara*.

The methods used so far were able to detect admixture between the domesticated rice genomes, but the direction of introgression could not be determined. To characterize the source and recipient genomes involved in the introgression a five-taxon based phylogenetic test called D_{FOIL} (Pease and Hahn 2015; see Materials and Method for brief discussion of method) was conducted. An extension of the ABBA-BABA test, the D_{FOIL} test analyzes a five-taxon phylogenetic tree with P1, P2, P3, P4, and O lineages (where P1 and P2 are monophyletic with each other; P3 and P4 are monophyletic with each other; and O represents the outgroup). Results from the D_{FOIL} test indicated a complex history of admixture between *japonica* and *aus*/*indica*, where the signs (+, −, 0) from the four D_{FOIL} statistics (table 3 and supplementary table S5, Supplementary Material online) were not compatible with any of the unidirectional gene flow scenarios (see supplementary table S6, Supplementary Material online). However, a recent study by Schumer et al. (2016) have shown that a pattern such as $(++--)$ for the four D_{FOIL} components can occur when there is bidirectional gene flow from two terminal lineages (specifically between P2 and P3; and in our case between *japonica* and *aus*/*indica*). Thus, the admixture history between the domesticated rice samples was most consistent with bidirectional gene flow between *japonica* and *aus*/*indica* genomes.

The Demographic Model for Asian Rice

To model demographic parameters in domesticated and wild Asian rice, we used the Bayesian coalescence-based program G-PhoCS (Gronau et al. 2011). To model gene flow between populations, migration bands were fitted to allow bidirectional gene flow between two current populations. All 20 pairwise migration rate comparisons are shown in the supplementary figure S1, Supplementary Material online. Consistent with genome-wide topology and ABBA-BABA tests, significant evidence of gene flows were detected between *japonica* and *indica*/*aus*. Further, consistent with the D_{FOIL} results all gene flow between *japonica* and *indica*/*aus* were bidirectional. High levels of gene flow were also detected between *indica* and *aus*; however, its 95% HPD overlapped with zero. There was also unexpected evidence of gene flow between *O. nivara* and *japonica*; this estimated migration rate was highest among all significant migration rates, but it also had a large 95% HPD interval.

Table 1. Four-Taxon Topology Test.

Major Topology		Minor Topologies
Topologies Testing the Origin Of Domesticated Rice		
([japonica,*O. rufipogon*],*O. nivara*)	([japonica,*O. nivara*],*O. rufipogon*)	([*O. nivara*,*O. rufipogon*],japonica)
5409 (85.1% [84.3–86.0%])	497 (7.8% [7.2–8.5%])	446 (7.0% [6.4–7.7%])
([aus,*O. nivara*],*O. rufipogon*)	([aus,*O. rufipogon*],*O. nivara*)	([*O. nivara*,*O. rufipogon*],aus)
2214 (65.0% [63.4–66.6%])	975(28.6% [27.1–30.1%])	215 (6.3% [5.5–7.1%])
([indica,*O. nivara*],*O. rufipogon*)	([indica,*O. rufipogon*],*O. nivara*)	([*O. nivara*,*O. rufipogon*],indica)
2499 (56.9% [55.5–58.4%])	1447 (33.0% [31.6–34.3%])	444 (10.1% [9.2–11.0%])
Topology Testing the Relationship Between *aus* and *indica*		
([aus,*O. nivara*],indica)	([aus,indica],*O. nivara*)	([indica,*O. nivara*],aus)
2854 (58.8% [57.4–60.3%])	1113 (22.9% [21.7–24.1%])	883 (18.2% [17.1–19.3%])
Topologies Testing the Relationship Between *aus* and *japonica*		
([japonica,*O. rufipogon*],aus)	([japonica,aus],*O. rufipogon*)	([aus,*O. rufipogon*],japonica)
4887 (77.4% [76.4–78.4%])	972 (15.4% [14.5–16.3%])	451 (7.1% [6.5–7.8%])
([aus,*O. nivara*],japonica)	([japonica,aus],*O. nivara*)	([japonica,*O. nivara*],aus)535
3062 (67.1% [65.8–68.5%])	965 (21.1% [20.0–22.4%])	(11.7% [10.8–12.7%])
Topologies Testing the Relationship Between *indica* and *japonica*		
([japonica,*O. rufipogon*],indica)	([japonica,indica],*O. rufipogon*)	([indica,*O. rufipogon*],japonica)
4932 (77.9% [76.9–79.0%])	947 (15.0% [14.1–15.8%])	450 (7.1% [6.5–7.8%])
([indica,*O. nivara*],japonica)	([japonica,indica],*O. nivara*)	([japonica,*O. nivara*],indica)
2562 (59.9% [58.4–61.3%])	1025 (23.9% [22.7–25.2%])	693 (16.2% [15.1–17.3%])

NOTE.—Total number of gene trees significantly supporting a topology after the Approximately Unbiased (AU) test. Numbers in parenthesis represent percentage with 95% bootstrap confidence interval indicated in square brackets. Oryza punctata was used as outgroup for all topology tests.

Table 2. Significant ABBA-BABA Test for Four Populations (P1, P2, P3, O).

P1	P2	P3	[a]ABBA sites	[a]BABA sites	D (±SE)	[b]Z-score
Gene Flow with *japonica*						
O. nivara	aus_{DJ123}	japonica	65702	27461	0.41 ± 0.07	6.15
O. nivara	$aus_{Kasalath}$	japonica	60243	24190	0.43 ± 0.05	8.28
O. nivara	$indica_{IR64}$	japonica	71212	36520	0.32 ± 0.05	5.86
O. nivara	$indica_{93\text{-}11}$	japonica	79451	32779	0.42 ± 0.05	9.14
Gene Flow with *indica*						
O. rufipogon	japonica	$indica_{IR64}$	55955	31426	0.28 ± 0.05	5.80
O. rufipogon	japonica	$indica_{93\text{-}11}$	58892	30055	0.32 ± 0.04	7.66
Gene Flow with *aus*						
O. rufipogon	japonica	$aus_{Kasalath}$	52744	29107	0.29 ± 0.05	6.38
O. rufipogon	japonica	aus_{DJ123}	55873	31422	0.28 ± 0.05	5.43
Gene Flow with *O. rufipogon*						
O. nivara	aus_{DJ123}	*O. rufipogon*	54327	32606	0.25 ± 0.05	4.71
O. nivara	$aus_{Kasalath}$	*O. rufipogon*	50974	29685	0.26 ± 0.06	4.52
O. nivara	$indica_{93\text{-}11}$	*O. rufipogon*	65547	39474	0.25 ± 0.05	5.00

NOTE.—The ABBA-BABA test examines possible admixture between P3 lineage and P1 or P2 lineage. The *O. punctata* genome was used as the outgroup genome (O).
[a]Number of sites with allele conformations ABBA or BABA for 4 populations in order of P1, P2, P3, and O.
[b]Z-score higher then 3.9 ($P < 0.0001$) are shown.

Using these initial results, we analyzed a multi-migration model that included all significant gene flows. As evidence for gene flow between *indica* and *aus* were not significant, two migration models were examined–with and without the *indica-aus* migration band (see supplementary fig. S2, Supplementary Material online). Results showed that in both models, the *O. nivara* to *japonica* gene flow was no longer significant, while the 95% HPD for gene flow between *indica* and *aus* no longer overlapped with zero. Moreover, gene flows were all unidirectional–from *japonica* to *indica*, *japonica* to *aus*, and *indica* to *aus*. We note, however, G-PhoCS has increased power of detecting migration in cases where the direction of gene flow is from an old to young lineage (Gronau et al. 2011). In our multi-migration model, the direction of gene flow went from an older lineage (*japonica* or *indica*) donor to a younger lineage (*aus* and *indica*) recipient, whereas the gene flows in the opposite direction were no longer significant. As there were different numbers of gene flows detected by the unidirectional vs. bidirectional gene flow models, we also explored if there were any resulting differences in the estimated divergence times. Results indicated that the mis-incorporation of *aus*/*indica* to *japonica* gene flow was not likely to have significantly affected our divergence time estimates and our interpretation of the Asian rice domestication scenario (see supplementary text, Supplementary Material online).

As archeological studies indicate *japonica* as the first domesticated subpopulation (Fuller 2011a) there is interest in

estimating time of split from *O. rufipogon* using genetic data. Five separate replicate G-PhoCS runs were conducted and the replicate with the highest divergence time between *O. rufipogon* and *japonica* (τ_{RJ}) were selected as the upper bound estimate (see supplementary figs. S3 and S4, Supplementary Material online). We note that none of the replicate estimates were significantly different from one another. Estimates for the ancestral population size and divergence times across the Asian rice complex are shown in figure 2.

G-PhoCS estimated current population sizes were inconsistent and had large 95% HPD (see supplementary fig. S3, Supplementary Material online), likely due to weak evolutionary signal in the data for recent demographic events, where G-PhoCS has limited power (Gronau et al. 2011). On the other hand, parameter estimates for the ancestral effective population sizes showed a series of bottlenecks that reduced N_e by ~20- to ~160-fold.

Total rates of gene flow were similar and moderate (median $m_{total} = 11\%$ for *indica* to *aus* gene flow; median $m_{total} = 15\%$ for *japonica* to *aus* gene flow; median $m_{total} = 17\%$ for *japonica* to *indica* gene flow). This is likely an underestimate since neutral sequences were used for the G-PhoCS analysis, and significant introgression between domesticated rice subpopulations are expected to occur at non-neutral domestication genes (Gross and Zhao 2014). Thus, we considered gene flow as a parameter that allows deviations from the standard "lineage splitting and isolation model" while estimating divergence time (τ) along the species phylogeny (Gronau et al. 2011). Results showed mis-incorporation of gene flow led to a significant upward bias in estimated divergence times for the *O. rufipogon*/*japonica* split (τ_{RJ}) and downward bias in estimated divergence time for domesticated and wild rice population (τ_{root}) (see supplementary fig. S4, Supplementary Material online).

Mutation rate estimates from a previous study on grass molecular evolution (Gaut et al. 1996) was used to calibrate the mutation rate scaled τ to absolute divergence time (T) in years. Our analysis indicated the wild and domesticated Asian rice lineages last shared a common ancestor at ~362.4 thousand years ago (ka), comparable to the ~400 ka estimated using molecular clock approaches (Cheng et al. 2003; Ma and Bennetzen 2004; Zhu and Ge 2005). Among the three domesticated rice populations, the absolute divergence time (i.e., mutation calibrated divergence time) of *japonica* lineage (T_{RJ}) occurred earliest at 18.3 ka (with a 95% range of 13.1–24.1 ka). This overlapped with the 95% HPD of *indica* lineage divergence time (12.0 ka with a 95% range of 6.7–17.7 ka). On the other hand, T_{RJ} was significantly earlier than the divergence time of *aus* and *O. nivara* (6.3 ka with a 95% range of 1.7–9.1 ka), indicating that *aus* is a more recently formed rice subpopulation.

Discussion

Hypotheses for multiple origins of Asian rice gain support primarily from phylogenetic analyses that indicate different rice subpopulations are more closely related to distinct *O. rufipogon* or *O. nivara* populations, rather than to each other

Table 3. Signs for the D_{FOIL} Test Components.

[a]Compared Genomes (P1,P2,P3,P4)	[b]D_{FO}	[b]D_{IL}	[b]D_{FI}	[b]D_{OL}
O. nivara*, aus$_{DJ123}$, japonica, *O. rufipogon	+	+	−	−
O. nivara*, aus$_{Kasalath}$, japonica, *O. rufipogon	+	+	−	−
O. nivara*, indica$_{IR64}$, japonica, *O. rufipogon	+	+	−	−
O. nivara*, indica$_{93\text{-}11}$, japonica, *O. rufipogon	+	+	−	−

NOTE.—The *O. punctata* genome was used as the outgroup genome (O).
[a]Order of taxa (P1,P2,P3,P4) are arranged so that P1 and P2 are monophyletic and P3 and P4 are monophyletic.
[b]Signs for the four D_{FOIL} test components. D-statistic and chi-square value are listed in the supplementary table S6, Supplementary Material online.

(Garris et al. 2005; Zhu and Ge 2005; Londo et al. 2006; Xu et al. 2007; He et al. 2011; Xu et al. 2011; Huang et al. 2012a). Our genome-wide analysis does show phylogenetic tree topologies consistent with multiple origins for each domesticated rice population. Specifically, the majority shows *japonica* as more closely-related with *O. rufipogon*, and *indica* with *O. nivara*, concordant with previous studies (Zhu and Ge 2005; Londo et al. 2006; Xu et al. 2007; Xu et al. 2011).

Interestingly, we have evidence that *aus* may have separate origins from an *O. nivara* progenitor independent of *indica*. Previous studies of genome-wide polymorphism have clustered *aus* and *indica* together (Garris et al. 2005; McNally et al. 2009; Xu et al. 2011), however, none have specifically tested whether *aus* and *indica* have a single or separate origins. Our results suggest that *japonica*, *indica*, and *aus* have their origins in distinct ancestral populations, consistent with phylogeographic studies that show population structuring and geographic subdivision within *O. rufipogon*, with *O. nivara* as a separate group (Sun et al. 2002; Huang et al. 2012a; Huang et al. 2012b; Liu et al. 2015).

Archeological evidence suggests the domestication of rice occurred at the latest ~6.5 ka (Fuller et al. 2009) or as early as ~8.5 ka (Deng et al. 2015) in the Lower and/or Middle Yangtze River basin (Silva et al. 2015). Our estimated divergence time, on the other hand, for the split of *japonica* and *O. rufipogon* lineages (18.3 ka) is much earlier. This earlier divergence fits with paleoenvironmental and archeological evidence. Wild rice only appeared in the Yangtze Basin after the Last Glacial Maximum ~18 ka, representing an expansion from tropical refugia in the south of China (Fuller et al. 2010). Archeologically recovered wild populations in and north of the Yangtze River, regarded as the most probable source of early domesticated rice (Silva et al. 2015), have since been extirpated by climate change and human impacts (Fuller et al. 2010; Fuller 2011a). Thus, the last common ancestor between extant *O. rufipogon* of southern China and domesticated *japonica* should date to the immediately post-glacial era, that is., ~18 ka. Earliest evidence for human use of wild rice in the Yangtze basin similarly dates to this period (Fuller and Qin 2009; Fuller et al. 2010).

The *indica*/*O. nivara* divergence time (~12.0 ka) similarly coincides with hypothesized geographic expansion of wild rice westwards in India during the terminal Pleistocene (Fuller et al. 2010), and the earliest evidence for rice use in this region. Rice phytoliths associated with vegetation burning suggest management for open habitats suitable for wild

rice in the Ganges valley between ~14.5 and 8 ka (Fuller and Qin 2009). Archeological evidence for reliance on rice in this area dates from ~9 ka, and has been interpreted as early management of a proto-*indica* that, however, did not lead to selection for domestication traits (Fuller and Qin 2009; Fuller 2011a; Fuller 2011b). The importance of proto-*indica* in the evolution of domesticated rice is consistent with this transitional population still experiencing the pre-domestication cultivation phase of crop evolution (Allaby et al. 2008), when early agricultural societies have already begun planting *O. nivara* for food use, but prior to the evolution of domestication traits.

While there is strong genetic evidence for multiple population origins of *O. sativa*, there is also a history of introgressive hybridization during the evolution of this domesticated species. Specifically, we find clear introgression from *japonica* to both *indica* and *aus* in both the ABBA-BABA test, D_{FOIL} test, and Bayesian modeling with G-PhoCS. In full coalescence-based demography modeling, we observe significant gene flow from *japonica* to *indica* (10–28%), and *japonica* to *aus* (8–41%). Parenthetically, we also see introgression from *indica* to *aus* (5–33%). As *aus* have received gene flow from both *japonica* and *indica* it is also possible for some of the *japonica* originating introgression may have indirectly been transferred by *japonica* to *indica* then to *aus*.

Interestingly, previous studies indicate that such introgression resulted in the transfer of domestication alleles from early *japonica* to the *indica* (and now possibly *aus*) lineages. Analysis of key domestication genes *Bh4*, *sh4*, *OsC1*, and *PROG1* (Saitoh et al. 2004; Li et al. 2006; Jin et al. 2008; Tan et al. 2008; Zhu et al. 2011) shows monophyly among domesticated rice subpopulation for these loci. Moreover, genome-wide examination (Huang et al. 2012a) showed phylogenetic trees reconstructed from domestication-associated genomic regions clustered *indica* and *japonica* alleles together, again consistent with gene flow of domestication alleles from *japonica*.

These results validate an alternative model for the origin of domesticated rice that could resolve the various conflicting genetic patterns observed by numerous studies. Aspects of this model have been previously proposed (Molina et al. 2011; Huang et al. 2012a; Huang and Han 2015; Castillo et al. 2016), and our analysis provides more details about the timing and gene flow patterns underpinning this scenario. This model suggests that during the expansion of *O. rufipogon*/*O. nivara* across South and East Asia over the last ~500,000 years, populations of wild rice began to diverge in different parts of the species range. The last major expansion of wild rice, including *O. rufipogon* in East Asia and *O. nivara* in western South Asia took place as glacial conditions retreated from ~18 to ~13.5 ka (Fuller et al. 2010). This maximal range extent of wild rice was in place in the Early Holocene (~11.5 to ~8 ka). Local hunter/gatherer communities and early cultivators began to manage local wild rice populations, possibly in the terminal Pleistocene and certainly in the Early Holocene in India and in China. Differing genetic histories of *indica* and *japonica* together with archeological evidence for early Holocene subsistence in the Yangtze Valley in China and Ganges Valley in India suggests different management strategies (Fuller and Qin 2009; Fuller 2011a), only one of which promoted domestication.

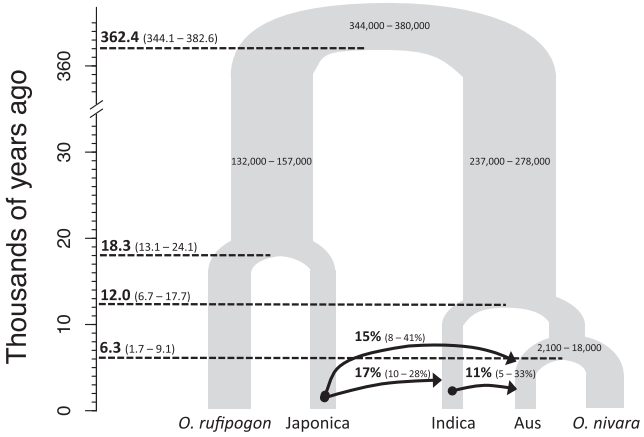


Fig. 2. G-PhoCS estimated demographic model of the Asian rice complex. Each internal node has a median mutation rate calibrated divergence time (T) estimate (ka) with its 95% Highest Posterior Density (HPD) in parenthesis. Only the 95% HPD is shown for each ancestral effective population size (N_e). Arrows indicate the migration band and direction of gene flow. Arrows are labeled with median and 95% HPD for the total migration rate estimates.

Domestication thus occurred first with *japonica* in the Yangtze Valley in China (Fuller et al. 2009; Fuller and Qin 2009; Deng et al. 2015), associated with fixation of alleles for non-shattering, color, and other domestication traits. Subsequent spread of domesticated *O. sativa* ssp. *japonica* to India led to hybridization with wild *O. nivara* or a cultivated proto-*indica*, resulting in the incorporation of domestication alleles from *japonica* that resulted in the origin of *indica* and intensification of rice agriculture and its expansion in South/Southeast Asia. Archeobotanically, there is no strong evidence for proto-*indica* having any domestication traits until the 2nd millennium BC, which is substantially later then the domestication time for *japonica* (Fuller et al. 2010; Fuller 2011a; Bates et al. 2016). We, therefore, have a case of multiple origins of the various Asian rice subspecies/subpopulations (in China and India) but only one original de novo domestication (in China).

This multiple origin/single domestication model is consistent with archeological evidence (Fuller and Qin 2009; Fuller 2011a; Fuller 2011b). Archeobotanical samples have suggested the existence of a possible proto-*indica* population genetically divergent from the progenitor of *japonica* (Fuller 2011b). Ancient DNA from rice >2 ka of age in India shows both *O. nivara*/*indica* and *japonica* chloroplast haplotypes, which indicates mixed populations are indeed ancient (Castillo et al. 2016). Proto-*indica* may have been cultivated in India without being domesticated for millennia until introduction of domestication alleles from *japonica* (Fuller and Qin 2009; Fuller et al. 2010; Fuller 2011b). Archeological finds of rice in northwest India dating 4–4.5 ka, for example, are morphologically wild, with <10% non-shattering spikelet bases (Bates et al. 2016). Modeling of archeological data suggests that *japonica* rice moved westward out of China and into South Asia via Central Asia along a precursor of the historical Silk Route (the so-called Hexi corridor) perhaps shortly after

~4 ka (Stevens et al. 2016). *Aus* may have had a similar evolutionary trajectory with a proto-*aus* origin and millennia of pre-domestication cultivation, until it also received domestication alleles from *japonica* or *indica*.

Consistent with McNally et al. (2009), our evidence of gene flow was not strictly unidirectional (from *japonica* to *aus*/*indica*) as there were some evidence of gene flow from *aus*/*indica* to *japonica* as well. The introgression from *aus*/*indica* to *japonica*, however, may have occurred during the diversification phase of rice, as historical and archeological evidence suggests the contact between domesticated *indica* and *japonica* were more recent then the initial time of their domestication (Fuller et al. 2010). Here, estimating the dates of gene flow between domesticated rice from both genetic and archeological data would be an important area for future research. If consistent with our proposed domestication scenario, the gene flow originating from *japonica* would be older then gene flows originating either from *aus* or *indica*.

In the end, our analysis calls into question how we conceptualize our definition of domestication. Domestication is an evolutionary process that results in the genetic transformation of a wild species into one dependent on humans for reproduction and dispersal, as a consequence of its adaptive evolution to meet human needs. At the genetic level, however, domesticated species can be operationally defined as species that carry the suite of alleles that have been positively selected for human association, adaptation to cultivation environments, and to meet human needs. As humans translocated domesticated populations during species dispersal, these populations come into contact and hybridize with distinct regional wild populations, creating regional affinities in domesticated varieties. Such processes, dubbed introgressive capture, have been widely documented in domesticated animals (Larson and Fuller 2014), and there is now increasing evidence of a similar process operating in some crop plants (e.g., in apples, Cornille et al. 2014). The introgressive transfer of domestication alleles from *japonica* to other populations through gene flow is what led to the genetic merging of distinct populations into what we now consider one domesticated crop species, *O. sativa* (Molina et al. 2011; Huang et al. 2012a). Indeed, *O. sativa* may be considered a complex of between 2–5 genetically distinct and (to greater or lesser degrees) reproductively isolated subspecies, bound together as a single crop species by shared presence of domestication alleles. As we identify various domestication genes in *O. sativa*, we can precisely identify the entire suite of loci that were selected and which characterize this one crop species, and model the precise evolutionary sequence that gave rise to this crucial domesticated crop species.

Materials and Methods

Oryza Genomes Analyzed

For consistency in the analyzed dataset *Oryza* species that have de novo assembled genome sequences were only analyzed. These include genome sequences that were obtained from EnsemblPlants release 30 (ftp://ftp.ensemblgenomes.org/pub/plants/; last accessed January 14, 2017) for cv. Nipponbare (International Rice Genome Sequencing Project 2005), cv. 93–11 ($indica_{93-11}$) (Yu et al. 2002), cv. Kasalath ($aus_{Kasalath}$) (Sakai et al. 2014), cv. IR64 ($indica_{IR64}$), and cv. DJ123 (aus_{DJ123}) (Schatz et al. 2014). In addition, genome assembly from the International *Oryza* Map Alignment Project (Jacquemin et al. 2013) were obtained and includes genome assemblies for *O. nivara*, *O. rufipogon*, *O. punctata*, *O. glaberrima*, *O. barthii*, *O. brachyantha*, *O. longistaminata*, *O. glumaepatula*, and *O. meridionalis*.

Whole Genome Alignment of *Oryza* Genomes

Repetitive regions were identified and masked using Repeatmodeler ver. 1.0.8 (http://www.repeatmasker.org/RepeatModeler.html; last accessed January 14, 2017) and Repeatmasker ver. 4.0.5 (http://www.repeatmasker.org/RMDownload.html; last accessed January 14, 2017). The *japonica* genome was used as reference and other genomes were aligned using LASTZ ver 1.03.73 (Harris 2007; http://www.bx.psu.edu/~rsharris/lastz/; last accessed January 14, 2017). Alignment blocks less then 100 kb apart were then chained together (Kent et al. 2003) using the UCSC Kent utilities suite (http://hgdownload.cse.ucsc.edu/admin/exe/linux.x86_64.v287/; last accessed January 14, 2017). To avoid evolutionary differences arising between ortholog and paralogs (Koonin 2005), a single chain with the highest score was selected to represent each orthologous region of the reference genome. To generate a multi-genome alignment, the aligner MULTIZ (Blanchette et al. 2004) was used to combine the target non-reference genome alignments.

Estimating the Species Phylogeny

Genome annotations for the *japonica* genome were downloaded from EnsemblPlants release 30. Four-fold degenerate (4D) sites were extracted for each gene using the msa_view program from the phast package ver. 1.3 (Hubisz et al. 2011; http://compgen.cshl.edu/phast/; last accessed January 14, 2017). All 4D sites were then concatenated into a single supergene alignment which was then used by RAxML ver. 8.2.5 (Stamatakis 2014) to estimate the maximum-likelihood based phylogenetic tree. We assumed a general time reversible model of DNA substitution with gamma distributed rate variation. Confidence of the phylogeny was determined after 1,000 bootstrap replicates.

Testing Gene Tree Topologies

Using the *japonica* genome annotation, genes with overlapping genome coordinates were excluded from analysis. The program msa_split from the phast package was used to parse out *japonica* CDS and its orthologous sequences from the multi-genome alignment. To control artifacts arising from missing data and alignment gaps, we omitted a gene if the proportion of missing sequence and gaps exceeded 20%.

RAxML estimated site-likelihood values were calculated for each non-overlapping *japonica* gene and its orthologs from *aus*, *indica*, *O. rufipogon*, and *O. nivara*. Significance of the phylogenetic relationships were determined using the Approximately Unbiased (AU) test (Shimodaira 2002) using the program CONSEL ver 0.20 (Shimodaira and Hasegawa

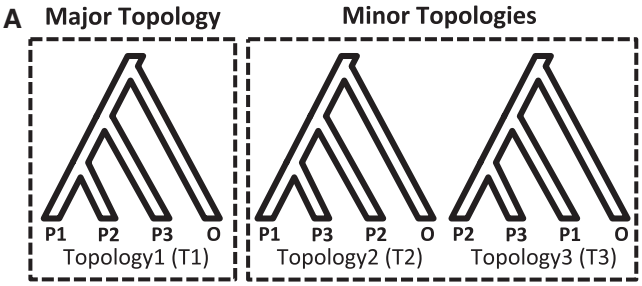
2001). AU test results for genes with likelihood difference of zero between topologies were excluded. For each gene, the topology with AU test *P* value greater then 0.95 was selected as the best representative topology (Schumer et al. 2016).

The collection of gene tree topologies can then be used to infer the evolutionary history of a species complex (fig. 3A). For example, in a rooted four-taxon, involving three species (P1, P2, and P3) with an outgroup (O), there are three topologies that can be supported for each gene. The major topology would represent a single topology with support from majority of the genes, and its overrepresentation is likely to be due to its concordance with the underlying species phylogeny. The remaining two minor topologies will also be observed for some genes and are predicted to occur at almost equal frequencies due to incomplete lineage sorting (Pamilo and Nei 1988). However, admixture between one of the two closely related sister species (P1 or P2 in Topology 1 of fig. 3A) with the third ingroup species (P3 in Topology 1 of fig. 3A) would generate an excess of one of the two minor topologies. Thus, quantification of gene tree topologies can be used to infer the underlying species phylogeny and admixture between taxa (Cui et al. 2013; Martin et al. 2013; Schumer et al. 2016).

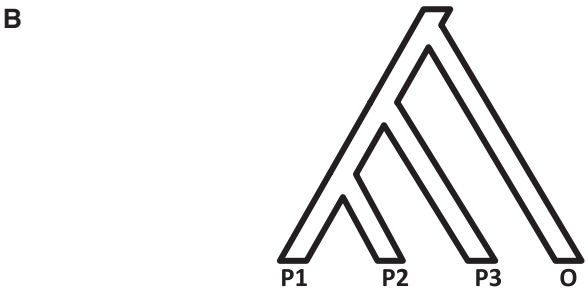
ABBA-BABA and D_{FOIL} Test for Detecting Evidence of Admixture

To detect evidence of admixture between and within the domesticated and wild rice ABBA-BABA test were conducted (Green et al. 2010; Durand et al. 2011). Similar to the topology test, the ABBA-BABA test also involves the same four-taxon phylogeny while examining sites with biallelic conformations (fig. 3B; where "A" corresponds to the ancestral allele and "B" corresponds to the derived allele state). Specifically, the test is restricted to sites where the outgroup has an "A" allele and the P3 taxon has a "B" allele, while the two most closely related taxa P1 and P2 differ in its allele state. Hence, with a taxa ordered as {P1,P2,P3,O} the possible allele conformations for each biallelic sites are "ABBA" and "BABA." Sites with ABBA or BABA conformation are then counted to determine whether there has been admixture between P1 and P3 or P2 and P3 taxa. As incomplete lineage sorting leads to an almost equal number of biallelic sites with the ABBA or BABA conformation, gene flow between P2 and P3 or P1 and P3 is expected to generate an excess of ABBA or BABA sites, respectively. Using the ABBA and BABA site counts a D-statistic (Green et al. 2010; Durand et al. 2011) can then be calculated where a positive D value indicates an excess of ABBA sites while a negative D value indicates an excess of BABA sites.

The *O. punctata* reference genome was used as the outgroup population (O) for all ABBA-BABA tests. Standard errors for the D-statistic were determined using jackknife resampling method by dividing the genome into 1 Mb blocks. Significance of D-statistic was determined using a Z-test and ABBA-BABA test with Z-score greater then $|3.9|$ were considered significant. We note that this does not correct for multiple hypothesis testing, however, our cutoff ($P < 0.0001$) is higher than previous studies that have used this method (Green et al. 2010; Freedman et al. 2014; Li et al. 2016).



Topology Counts			Scenario
		T2 ≈ T3	No gene flow
T1	>	T2 > T3	Gene flow between P1 & P3
		T2 < T3	Gene flow between P2 & P3



Scenario	Conformation Counts	D statistic
No Gene Flow	$N_{ABBA} \approx N_{BABA}$	$D \approx 0$
Gene Flow between P2 & P3	$N_{ABBA} > N_{BABA}$	$D > 0$
Gene Flow between P1 & P3	$N_{ABBA} < N_{BABA}$	$D < 0$

Fig. 3. Phylogenomic methods for examining species relationship and admixture. The methods involve a rooted four-taxon phylogeny, specifically with three species (P1, P2, and P3) and an outgroup (O). P1 and P2 are most closely related to each other while P3 is an in-group taxon that is equally distant to both P1 and P2. (*A*) Gene tree topology test. T1, T2, and T3 represent total number of genes supporting Topology 1, 2, and 3, respectively. In this hypothetical example, Topology 1 is assumed to be the major topology. (*B*) ABBA-BABA test. N_{ABBA} and N_{BABA} represent total number of sites with the "ABBA" and "BABA" conformation, respectively.

Although the ABBA-BABA test detects evidence of admixture it is not able to determine the direction of the introgression. To determine the direction of introgression the D_{FOIL} test (Pease and Hahn 2015) was conducted. This method is an extension of the four-taxon ABBA-BABA test by analyzing biallelic sites from a five-taxon phylogeny. Specifically, the test requires that the underlying species phylogeny for the five-taxon consists of two pairs of sister species in a symmetrical conformation: (((P1,P2),(P3,P4,)),O) (see supplementary fig. S6, Supplementary Material online). The ABBA-BABA test D-statistic essentially measures the relatedness between two sister species and a third ingroup species from a four-taxon

phylogeny. With a four-taxon there is only one possible D-statistic but in a five-taxon with a symmetrical conformation there are four possible D-statistic values (referred as D_{FO}, D_{IL}, D_{FI}, and D_{OL} by Pease and Hahn [2015]; see supplementary fig. S6, Supplementary Material online). Unidirectional introgression involving a species from one monophyletic group to another (i.e., P1→P3) would lead to a paraphyletic relationship, clustering the recipient species with the donor group: that is, ((((P1,P2),P3),P4),O). Here, the recipient species not only clusters with the donor species, but also with the donors' sister species leading to distinct signs (+, −, or 0) for the four D-statistics. Thus, the combination of signs from each four D-statistic holds information on the direction of introgression (see supplementary table S6, Supplementary Material online).

Each D_{FOIL} statistic was calculated using the dfoil script (https://github.com/jbpease/dfoil; last accessed January 14, 2017) and significance for each D-statistic was assessed using a chi-squared goodness-of-fit test with 1 degrees of freedom (Pease and Hahn 2015). Similar to the ABBA-BABA test cutoff, we chose D_{FOIL} test with chi-squared values greater than 15.14 ($P < 0.0001$) as significant.

Estimating Demographic Parameters

The software Generalized Phylogenetic Coalescent Sampler (G-PhoCS) ver 1.2.3 was used to estimate the demography parameters ancestral population sizes, divergence times, and migration rates using loci close to neutrality (Gronau et al. 2011). Each locus length was chosen as 1 kb while between loci at least 50 kb apart. Sites without insertions/deletions were only counted towards the final size of a locus.

Neutral loci were selected in a similar manner to Gronau et al. (2011). Three non-neutral regions were of interest: i) genic regions, ii) repetitive regions, and iii) conserved noncoding sequences (CNSs): Using the *japonica* genome as the reference, site-wise phastcons scores (Siepel et al. 2005) were calculated using 4-fold degenerate site as the neutral model. Sites with phastcons scores greater than 0.8 were considered candidate CNSs. Any CNSs separated by 10 bps were merged and 100 bps flanking each CNS were considered to be functional.

For every demography scenario, we ran G-PhoCS five times to ensure convergence. Each MCMC run had 3,000,000 iterations and 10–25% of the iterations were discarded burn-in runs. Priors were modeled using a gamma distribution ($\alpha = 1$ and $\beta = 10{,}000$ for population size and divergence time; $\alpha = 0.002$ and $\beta = 0.00001$ for migration rates). To estimate gene flow, we took an approach similar to Freedman et al. (2014). Initially, migration bands were fitted between two terminal branch lineages where gene flow was hypothesized to occur in both directions. Then we ran a model incorporating all significant migration bands and estimated population sizes and divergence times. Outputs were analyzed with program Tracer ver 1.6 (http://tree.bio.ed.ac.uk/software/tracer/; last accessed January 14, 2017) to determine the burn-in cutoff and 95% HPD for each parameter.

Estimated parameters are scaled by the mutation rate (μ) (population size $\theta = 4N_e\mu$ where N_e is effective population size; divergence time $\tau = T\mu/g$ where T is absolute divergence time in years and g is average generation time in years). To estimate calibrated population size (N_e) and divergence time (T), μ was estimated at 6.5×10^{-9} mutations per site per generation (= year) from grass data (Gaut et al. 1996). Migration rate (m) is also scaled by μ and estimates the proportion of individuals in the target population that have origins in the source population. To estimate migration independent of μ, total migration rate (m_{total}) was calculated by scaling against the total time of the migration event ($m_{total} = m \cdot \tau$). Here, m_{total} represents the probability a target population received gene flow from the source population.

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

Acknowledgments

We would like to thank Bin Han and Nori Kurata for providing early access to the *O. rufipogon* genome assembly used in this study. We thank New York University - High Performance Computing for providing computational resources and support. This work was funded in part by grants from the National Science Foundation Plant Genome Research Program (IOS-1126971 and IOS-1546218), the Zegar Family Foundation (A16-0051) and the NYU Abu Dhabi Research Institute (G1205) to M.D.P., and from the UK Natural Environment Research Council (NERC) to D.Q.F. (NE/N010957/1).

References

Allaby RG, Fuller DQ, Brown TA. 2008. The genetic expectations of a protracted model for the origins of domesticated crops. *Proc Natl Acad Sci*. 105:13982–13986.

Bates J, Petrie CA, Singh RN. 2016. Approaching rice domestication in South Asia: new evidence from Indus settlements in northern India. *J Archaeol Sci*. Advance Access published November 21, 2016, doi: http://dx.doi.org/10.1016/j.jas.2016.04.018.

Blanchette M, Kent WJ, Riemer C, Elnitski L, Smit AFA, Roskin KM, Baertsch R, Rosenbloom K, Clawson H, Green ED, et al. 2004. Aligning multiple genomic sequences with the threaded blockset aligner. *Gen Res*. 14:708–715.

Castillo CC, Tanaka K, Sato Y-I, Ishikawa R, Bellina B, Higham C, Chang N, Mohanty R, Kajale M, Fuller DQ. 2016. Archaeogenetic study of prehistoric rice remains from Thailand and India: evidence of early japonica in South and Southeast Asia. *Archaeol Anthropol Sci*. 8:523–543.

Chen J, Ding J, Ouyang Y, Du H, Yang J, Cheng K, Zhao J, Qiu S, Zhang X, Yao J, et al. 2008. A triallelic system of S5 is a major regulator of the reproductive barrier and compatibility of indica-japonica hybrids in rice. *Proc Natl Acad Sci*. 105:11436–11441.

Cheng C, Motohashi R, Tsuchimoto S, Fukuta Y, Ohtsubo H, Ohtsubo E. 2003. Polyphyletic origin of cultivated rice: based on the interspersion pattern of SINEs. *Mol Biol Evol*. 20:67–75.

Civáň P, Craig H, Cox CJ, Brown TA, Fuller DQ, Molina J, Huang X, Gross BL, Zhao Z, Goff SA, et al. 2015. Three geographically separate domestications of Asian rice. *Nat Plants*. 1:15164.

Cornille A, Giraud T, Smulders MJM, Roldán-Ruiz I, Gladieux P. 2014. The domestication and evolutionary ecology of apples. *Trends Genet*. 30(2):57–65.

Cui R, Schumer M, Kruesi K, Walter R, Andolfatto P, Rosenthal GG. 2013. Phylogenomics reveals extensive reticulate evolution in Xiphophorus fishes. *Evolution* 67(8):2166–2179.

Degnan JH, Rosenberg NA. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends Ecol Evol*. 24:332–340.

Deng Z, Qin L, Gao Y, Weisskopf AR, Zhang C, Fuller DQ. 2015. From early domesticated rice of the middle yangtze basin to millet, rice and wheat agriculture: archaeobotanical macro-remains from Baligang, Nanyang Basin, Central China (6700-500 BC). *PLoS One* 10:e0139885.

Durand EY, Patterson N, Reich D, Slatkin M. 2011. Testing for ancient admixture between closely related populations. *Mol Biol Evol*. 28:2239–2252.

Freedman AH, Gronau I, Schweizer RM, Ortega-Del Vecchyo D, Han E, Silva PM, Galaverni M, Fan Z, Marx P, Lorente-Galdos B, et al. 2014. Genome sequencing highlights the dynamic early history of dogs. *PLoS Genet*. 10:e1004016.

Fuller DQ. 2011a. Pathways to Asian civilizations: tracing the origins and spread of rice and rice cultures. *Rice* 4:78–92.

Fuller DQ. 2011b. Finding plant domestication in the Indian subcontinent. *Curr Anthropol*. 52:S347–S362.

Fuller DQ, Qin L. 2009. Water management and labour in the origins and dispersal of Asian rice. *World Archaeol*. 41:88–111.

Fuller DQ, Qin L, Zheng Y, Zhao Z, Chen X, Hosoya LA, Sun G-P. 2009. The domestication process and domestication rate in rice: spikelet bases from the Lower Yangtze. *Science* 323:1607–1610.

Fuller DQ, Sato Y-I, Castillo C, Qin L, Weisskopf AR, Kingwell-Banham EJ, Song J, Ahn S-M, van Etten J. 2010. Consilience of genetics and archaeobotany in the entangled history of rice. *Archaeol Anthropol Sci*. 2:115–131.

Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. 2005. Genetic structure and diversity in Oryza sativa L. *Genetics* 169:1631–1638.

Gaut BS, Morton BR, McCaig BC, Clegg MT. 1996. Substitution rate comparisons between grasses and palms: synonymous rate differences at the nuclear gene Adh parallel rate differences at the plastid gene rbcL. *Proc Natl Acad Sci USA*. 93:10274–10279.

Gnanamanickam SS. 2009. Rice and its importance to human life. In: Biological control of rice diseases. Dordrecht: Springer Netherlands. p. 1–11.

Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson N, Li H, Zhai W, Fritz MH-Y, et al. 2010. A draft sequence of the Neandertal genome. *Science* 328:710–722.

Gronau I, Hubisz MJ, Gulko B, Danko CG, Siepel A. 2011. Bayesian inference of ancient human demography from individual genome sequences. *Nat Genet*. 43:1031–1034.

Gross BL, Zhao Z. 2014. Archaeological and genetic insights into the origins of domesticated rice. *Proc Natl Acad Sci*. 111:6190–6197.

Harris RS. 2007. Improved pairwise alignment of genomic dna. Ph.D. Thesis, Pennsylvania State Univ.

He Z, Zhai W, Wen H, Tang T, Wang Y, Lu X, Greenberg AJ, Hudson RR, Wu C-I, Shi S. 2011. Two evolutionary histories in the genome of rice: the roles of domestication genes. Mauricio R, editor. *PLoS Genet* 7:e1002100.

Huang X, Kurata N, Wei X, Wang Z-X, Wang A, Zhao Q, Zhao Y, Liu K, Lu H, Li W, et al. 2012a. A map of rice genome variation reveals the origin of cultivated rice. *Nature* 490:497–501.

Huang P, Molina J, Flowers JM, Rubinstein S, Jackson SA, Purugganan MD, Schaal BA. 2012b. Phylogeography of Asian wild rice, Oryza rufipogon: a genome-wide view. *Mol Ecol*. 21:4593–4604.

Huang X, Han B. 2015. Rice domestication occurred through single origin and multiple introgressions. *Nat Plants*. 2:15207.

Hubisz MJ, Pollard KS, Siepel A. 2011. PHAST and RPHAST: phylogenetic analysis with space/time models. *Brief Bioinform*. 12:41–51.

International Rice Genome Sequencing Project. 2005. The map-based sequence of the rice genome. *Nature* 436:793–800.

Jacquemin J, Bhatia D, Singh K, Wing RA. 2013. The International Oryza Map Alignment Project: development of a genus-wide comparative genomics platform to help solve the 9 billion-people question. *Curr Opin Plant Biol*. 16:147–156.

Jin J, Huang W, Gao J-P, Yang J, Shi M, Zhu M-Z, Luo D, Lin H-X. 2008. Genetic control of rice plant architecture under domestication. *Nat Genet*. 40:1365–1369.

Kent WJ, Baertsch R, Hinrichs A, Miller W, Haussler D. 2003. Evolution's cauldron: duplication, deletion, and rearrangement in the mouse and human genomes. *Proc Natl Acad Sci*. 100:11484–11489.

Khush GS. 1997. Origin, dispersal, cultivation and variation of rice. *Plant Mol Biol*. 35:25–34.

Koonin EV. 2005. Orthologs, paralogs, and evolutionary genomics. *Annu Rev Genet*. 39:309–338.

Larson G, Fuller DQ. 2014. The evolution of animal domestication. *Ann Rev Ecol Evol Syst*. 45(1):115–136.

Li C, Zhou A, Sang T. 2006. Rice domestication by reducing shattering. *Science* 311:1936–1939.

Li G, Davis BW, Eizirik E, Murphy WJ. 2016. Phylogenomic evidence for ancient hybridization in the genomes of living cats (Felidae). *Genome Res*. 26:1–11.

Liu R, Zheng X-M, Zhou L, Zhou H-F, Ge S. 2015. Population genetic structure of Oryza rufipogon and Oryza nivara: implications for the origin of O. nivara. *Mol Ecol*. 24:5211–5228.

Londo JP, Chiang Y-C, Hung K-H, Chiang T-Y, Schaal BA. 2006. Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa. *Proc Natl Acad Sci*. 103:9578–9583.

Ma J, Bennetzen JL. 2004. Rapid recent growth and divergence of rice nuclear genomes. *Proc Natl Acad Sci*. 101:12404–12410.

Martin SH, Dasmahapatra KK, Nadeau NJ, Salazar C, Walters JR, Simpson F, Blaxter M, Manica A, Mallet J, Jiggins CD. 2013. Genome-wide evidence for speciation with gene flow in Heliconius butterflies. *Genome Res*. 23:1817–1828.

Matsuo T, Futsuhara Y, Kikuchi F, Yamaguchi H. 1997. Science of the rice plant. Tokyo: Food and Agriculture Policy Research Center.

McNally KL, Childs KL, Bohnert R, Davidson RM, Zhao K, Ulat VJ, Zeller G, Clark RM, Hoen DR, Bureau TE, et al. 2009. Genomewide SNP variation reveals relationships among landraces and modern varieties of rice. *Proc Natl Acad Sci*. 106:12273–12278.

Molina J, Sikora M, Garud N, Flowers JM, Rubinstein S, Reynolds A, Huang P, Jackson S, Schaal BA, Bustamante CD, et al. 2011. Molecular evidence for a single evolutionary origin of domesticated rice. *Proc Natl Acad Sci*. 108:8351–8356.

Oka HI. 1988. Origin of cultivated rice. Japan: Scientific Societies Press.

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. *Mol Biol Evol*. 5:568–583.

Pease JB, Hahn MW. 2015. Detection and polarization of introgression in a five-taxon phylogeny. *Syst Biol*. 64:651–662.

Pollard DA, Iyer VN, Moses AM, Eisen MB. 2006. Widespread discordance of gene trees with species tree in drosophila: evidence for incomplete lineage sorting. *PLoS Genet*. 2:e173.

Saitoh K, Onishi K, Mikami I, Thidar K, Sano Y. 2004. Allelic diversification at the C (OsC1) locus of wild and cultivated rice: nucleotide changes associated with phenotypes. *Genetics* 168:997–1007.

Sakai H, Kanamori H, Arai-Kichise Y, Shibata-Hatta M, Ebana K, Oono Y, Kurita K, Fujisawa H, Katagiri S, Mukai Y, et al. 2014. Construction of pseudomolecule sequences of the aus rice cultivar kasalath for comparative genomics of Asian cultivated rice. *DNA Res*. 21:397–405.

Sang T, Ge S. 2007. Genetics and phylogenetics of rice domestication. *Curr Opin Genet Dev*. 17:533–538.

Schatz MC, Maron LG, Stein JC, Wences AH, Gurtowski J, Biggers E, Lee H, Kramer M, Antoniou E, Ghiban E, et al. 2014. Whole genome de novo assemblies of three divergent strains of rice, Oryza sativa, document novel gene space of aus and indica. *Genome Biol*. 15:506.

Schumer M, Cui R, Powell DL, Rosenthal GG, Andolfatto P. 2016. Ancient hybridization and genomic stabilization in a swordtail fish. *Mol Ecol*. 25:2661–2679.

Second G. 1982. Origin of the genic diversity of cultivated rice (Oryza spp.): study of the polymorphism scored at 40 isozyme loci. *Jpn J Genet*. 57:25–57.

Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst Biol*. 51:492–508.
Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17:1246–1247.
Siepel A, Bejerano G, Pedersen JS, Hinrichs AS, Hou M, Rosenbloom K, Clawson H, Spieth J, Hillier LW, Richards S, et al. 2005. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res*. 15:1034–1050.
Silva F, Stevens CJ, Weisskopf A, Castillo C, Qin L, Bevan A, Fuller DQ. 2015. Modelling the geographical origin of rice cultivation in Asia using the rice archaeological database. *PLoS One*. 10:e0137024.
Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.
Stevens CJ, Murphy C, Roberts R, Lucas L, Silva F, Fuller DQ. 2016. Between China and South Asia : a middle Asian corridor of crop dispersal and agricultural innovation in the Bronze Age. *Holocene* 26:1541–1555.
Sun Q, Wang K, Yoshimura A, Doi K. 2002. Genetic differentiation for nuclear, mitochondrial and chloroplast genomes in common wild rice (Oryza rufipogon Griff.) and cultivated rice (Oryza sativa L.). *Theor Appl Genet*. 104:1335–1345.
Sweeney MT, Thomson MJ, Cho YG, Park YJ, Williamson SH, Bustamante CD, McCouch SR. 2007. Global dissemination of a single mutation conferring white pericarp in rice. *PLoS Genet*. 3:e133.
Sweeney MT, Thomson MJ, Pfeil BE, McCouch S. 2006. Caught red-handed: rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. *Plant Cell*. 18:283–294.
Tan L, Li X, Liu F, Sun X, Li C, Zhu Z, Fu Y, Cai H, Wang X, Xie D, et al. 2008. Control of a key transition from prostrate to erect growth in rice domestication. *Nat Genet*. 40:1360–1364.
Vaughan DA, Lu B-R, Tomooka N. 2008. The evolving story of rice evolution. *Plant Sci*. 174:394–408.
Xu J-H, Cheng C, Tsuchimoto S, Ohtsubo H, Ohtsubo E. 2007. Phylogenetic analysis of Oryza rufipogon strains and their relations to Oryza sativa strains by insertion polymorphism of rice SINEs. *Genes Genet Syst*. 82:217–229.
Xu X, Liu X, Ge S, Jensen JD, Hu F, Li X, Dong Y, Gutenkunst RN, Fang L, Huang L, et al. 2011. Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nat Biotechnol*. 30:105–111.
Yamanaka S, Nakamura I, Nakai H, Sato Y-I. 2003. Dual origin of the cultivated rice based on molecular markers of newly collected annual and perennial strains of wild rice species, Oryza nivara and O. rufipogon. *Genet Resour Crop Evol*. 50:529–538.
Yang C-C, Kawahara Y, Mizuno H, Wu J, Matsumoto T, Itoh T. 2012. Independent domestication of Asian rice followed by gene flow from japonica to indica. *Mol Biol Evol*. 29:1471–1479.
Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, et al. 2002. A draft sequence of the rice genome (Oryza sativa L. ssp. indica). *Science* 296:79–92.
Zhang L-B, Zhu Q, Wu Z-Q, Ross-Ibarra J, Gaut BS, Ge S, Sang T. 2009. Selection on grain shattering genes and rates of rice domestication. *New Phytol*. 184:708–720.
Zhu Q, Ge S. 2005. Phylogenetic relationships among A-genome species of the genus Oryza revealed by intron sequences of four nuclear genes. *New Phytol*. 167:249–265.
Zhu B-F, Si L, Wang Z, Zhou Y, Zhu J, Shangguan Y, Lu D, Fan D, Li C, Lin H, et al. 2011. Genetic control of a transition from black to straw-white seed hull in rice domestication. *Plant Physiol*. 155:1301–1311.