Role of genetic introgression during the evolution of cultivated rice (Oryza sativa L.)

Peter Civáň and Terence A. Brown*

Abstract

Background: Models for the origins of cultivated rice currently fall into two groups: ones that identify independent domestications of the indica, japonica and possibly also the aus types, and others that propose that the domestication phenotype was initially acquired by japonica, the underlying alleles then transferred by introgression to other pre-domesticated populations, giving the indica and aus varieties. Identifying the impact of past gene flow on cultivated rice genomes is therefore crucial to distinguishing between these models and understanding the domestication history of rice. To this end, we used population-scale polymorphism data to identify the progenitor gene pools of indica, japonica and aus. Variation shared among the cultivated groups but absent from at least one progenitor population was identified, and genomic blocks putatively transferred by gene flow among cultivated groups mapped.

Results: Introgression signals were absent at the major domestication loci (Prog1, Rc, qSH1, qSH3, Sh4) of indica and aus, indicating that these loci were unaffected by gene flow from japonica. Other domestication-related loci (Ghd7, LABA1, Kala4, LG1) show signals of introgression from japonica or indica to aus. There is a strong signal for LABA1 in japonica, possibly indicating introgression from indica. The indica genome is the least affected by gene flow, with just a few short regions with allelic frequencies slightly altered by introgression from japonica.

Conclusion: Introgression has occurred during the evolution of cultivated rice, but was not responsible for transfer of the key domestication alleles between the cultivated groups. The results are therefore consistent with models in which japonica, indica and aus were domesticated independently, with each of these cultivated groups acquiring the domestication alleles from standing variation in wild rice, without a significant contribution from inter-group gene flow.

Keywords: Allele frequency spectra, Domestication alleles, Gene flow, Introgression, Oryza sativa, Rice

Background

Cultivated Asian rice (Oryza sativa L.) is one of the oldest and most important staple crops worldwide. It is well established that O. sativa originated from a wild progenitor species Oryza rufipogon Griff., although the number of domestication events and the nature of the evolutionary processes that gave rise to the modern crop remain controversial [1–8]. Based on ecology, genetics and culinary properties, O. sativa can be divided into five groups – japonica (subdivided into tropical and temperate japonica), indica, aus, and aromatic rice [5, 9, 10]. Within the japonica subspecies, typically giving sticky rice after cooking, the temperate and tropical groups are adapted to distinct climatic conditions. Indica and aus rice are long-grained, with the latter consisting of drought-tolerant, early maturing cultivars. Aromatic rice includes cultivars with specific flavours popular in Pakistan and northern India (basmati) and Iran (sadri).

Diverse reproductive barriers have been described between indica and japonica cultivars, though the fertility of the hybrids differs from one cross to another [11, 12]. Low hybrid fertility has also been reported in crosses of either indica or japonica with aus [13] and aromatic rice [14]. The incomplete reproductive separation among the groups of cultivated and wild rice permits inter-population gene flow, which has been well documented in the O. sativa→O. rufipogon direction due to concerns of transgene escape from genetically modified rice [15–18].

Traditionally, studies of rice domestication have asked whether indica and japonica – the two largest groups grown in modern times – have common or independent origins. It has been shown that the evolutionary divergence of the indica and japonica genomes pre-dates
their domestication by 200,000–400,000 years [19–21], which argues against the two types originating from a single domestication. Moreover, genetic structure differentiating the indica, japonica and aus populations has been detected by analysis of microsatellites [9], gene haplotypes [22] and genome-wide single nucleotide polymorphisms (SNPs) [3, 10]. However, the implication that the cultivated groups have independent origins is contradicted by analyses of several of the genes controlling rice domestication traits, which have revealed striking allelic uniformity across O. sativa [23–25]. Such observations have stimulated new hypotheses about the origin of the rice groups, proposing that some crucial domestication genes emerged in O. sativa only once and were subsequently transferred across other pre-domesticated populations by introgressive hybridization [3, 26, 27]. Modelling of nucleotide variation patterns have similarly led to the conclusion that either gene flow or strong artificial selection have been important demographic forces during the domestication of rice [28].

Domestication models favouring separate origins of japonica and indica followed by inter-group gene flow have also been supported by two genome-wide studies [1, 4], which showed that although the major fractions of the indica and japonica genomes are similar to distinct wild genotypes, a minor low-diversity genomic fraction groups indica and japonica together. Later analyses of rice genes apparently targeted by the domestication process have interpreted allelic uniformity as further evidence for the gene flow hypothesis [29, 30]. Most recently, the same domestication model (multiple origins plus gene flow) was expanded to aus by coalescent modelling of genome-wide data from several Oryza individuals [31]. However, none of the previous studies have demonstrated that the genomic segments supposedly involved in gene flow could not have descended vertically from the ancestral wild populations. This is either because the examined wild sample was too small to account for standing variation of the progenitors (e.g. refs. 4 and 31 analysed only two wild accessions; ref. 24 did not examine wild genotypes at all) or, more profoundly, because the ancestral wild gene pools of indica and aus have not been convincingly identified.

In a previous study, we provided evidence for independent domestications of indica, japonica and aus rice [5], based on examination of genomic regions that have been under selection in each of the three groups. We found that distinct sequence types had been selected in the large majority of these co-located low diversity regions (CLDGRs). This is the opposite of what would be expected if the indica, japonica and aus groups have a shared domestication history, as the latter should result in most CLDGRs appearing monophyletic. It has also been shown that some important domestication alleles thought to have spread across the O. sativa groups by introgressive hybridization are in fact widespread in O. rufipogon (e.g. sh4 [32]; rc, laba1 [8]) and these alleles do not always confer the domestication phenotype in wild and hybrid rice [8, 32–34]. Such observations raise the possibility that identical domestication alleles could have been selected multiple times from standing variation in independent domestication events. If this were the case, then inter-group gene flow need not be invoked as an explanation for the presence of identical domestication alleles in the different types of rice.

Understanding the extent and direction of gene flow during the evolutionary histories of indica, japonica and aus is therefore one of the keys to understanding the origins and domestication history of cultivated rice. We believe that a population approach with extensive sampling of wild diversity is necessary for an accurate evaluation of gene flow, and that such an analysis is lacking for rice. Prior knowledge of the progenitor gene pools is also necessary, because sharing of any genomic region among cultivated groups implies gene flow only if that genomic region is absent from at least one progenitor population. This point is particularly important in the study of crop origins, where domestication is known to have had convergent effects (e.g. all cereal crops are characterized by a lack of seed dormancy, non-shattering ear and increased kernel weight) and identical variants beneficial for cultivation and widespread in wild populations are likely to be selected multiple times. We have therefore assessed the role of gene flow by analysis of previously published genome-wide and population-scale polymorphism data [3]. We identify the progenitor gene pools of indica, japonica and aus by analysis of unshared ancestral variants and confirm the results using a phylogeographic approach. Subsequently, we summarize the variation shared among the cultivated groups but absent from at least one progenitor population, and then integrate our results into a comprehensive scheme of genetic ancestry that identifies genomic blocks likely to have been transferred by gene flow among the O. sativa groups.

Methods
Progenitor populations of the O. sativa groups
The complete genotype dataset for 1529 wild and cultivated rice accessions consisting of ~ 8 million SNPs from all 12 rice chromosomes [3] was downloaded from the Rice Haplotype Map Project database (http://202.127.18.221/RiceHap3/). Accessions with intermediate phenotypes (44) and aromatic rice (5) were discarded (Additional file 1: Table S1). The reduced dataset was split according to the group membership — 520 indica, 484 japonica, 30 aus and 446 wild rice accessions (using the bash command cut) — and the group SNP matrices converted into a table of allelic frequencies (using basic
operations and the function COUNTIF in Libre Calc). Subsequently, only SNP positions with at least one third of non-missing data points in each analysed group were retained. A total of 705,124 variable positions passed this filter. The data filtering and analysis pipeline is schematically summarized in Additional file 2: Figure S1.

In order to identify the progenitor populations of the cultivated groups, we analysed the wild distributions of non-shared ancestral variants, by which we mean variants that are common in wild rice and one cultivated group (allelic frequency ≥ 0.05) but absent or insignificant in the remaining two cultivated groups (< 0.01). For each cultivated group, the SNP positions meeting these criteria were extracted from the SNP matrix. Subsequently, each wild accession was assessed for the presence of japonica-specific, indica-specific and aus-specific ancestral variants and the proportions of sites with these variants were calculated. The forty wild accessions with the highest proportions were identified for each cultivated group, yielding three non-overlapping groups of 40 wild accessions that we regard as the progenitor populations of indica, japonica and aus. Geographic distributions of the identified progenitors are shown on ArcGIS maps (ArcGIS software by Esri).

The robustness of the identified relationships was evaluated by a phylogenetic analysis and a principal component analysis (PCA). All format conversions and data extractions were done using bash command line utilities. For the phylogenetic reconstruction, a subset of the 705,124 SNP dataset was prepared by selecting 150 accessions with the least amount of missing data were chosen), yielding an alignment with 358,218 variable positions and 30.3% missing data points. A maximum likelihood (ML) tree was computed with RAxML [35] using a joint allele frequency spectrum (AFS) constructed in Libre Calc (using basic operations, functions FREQUENCY and LOG10, and conditional formatting). Three additional datasets and AFSs were prepared in order to summarize allele sharing was summarized as a joint allele frequency spectrum (AFS) constructed in Libre Calc (using basic operations, functions FREQUENCY and LOG10, and conditional formatting). Three additional datasets and AFSs were prepared in order to summarize allele sharing was summarized as a joint allele frequency spectrum (AFS) constructed in Libre Calc (using basic operations, functions FREQUENCY and LOG10, and conditional formatting).

The genomic distribution of the shared variants was summarized by an introgression index calculated across the entire genome in 100 kb windows with 50 kb sliding steps, using eq. 1:

\[
I = \frac{1}{n} \sum_{i} p_{iA} p_{iB}
\]

where for every allele \( i \) absent in the selected progenitor, \( p_{iA} \) is the frequency of that allele in one domesticated group, \( p_{iB} \) is the frequency in the other domesticated group, and \( n \) is the number of alleles in a 100 kb window. We used the following assumption: if a genomic window shows an elevated proportion of alleles shared by the two domesticated groups, and \( n \) is the number of alleles in a 100 kb window.

Gene flow between domesticated groups of O. sativa

Gene flow among the three domesticated rice groups was examined by quantification of shared alleles and their distribution on a physical map of the rice genome (IRGSP4). First, a table of allelic frequencies in indica, japonica, aus and their respective progenitor populations was prepared for the 8 million SNP dataset. Subsequently, two datasets were extracted for each pair of cultivated groups; one containing variants absent in the progenitor of the first group and another with variants absent in the progenitor of the second group. In order to minimize the risk of false absence (due to small sample size of the progenitor population), only sites with at least 22 data points (out of 40) per progenitor were considered, giving a probability of 1 - 0.922 = 90% of capturing an allele that has a frequency of 0.1. Furthermore, sites with > 75% missing data in the cultivated groups were discarded (i.e. only variable positions with at least 130, 121 and eight data points for indica, japonica and aus, respectively, were retained). To maximize the SNP density, these filters were applied to each of the six datasets independently, yielding 965,497 alleles found in indica and/or japonica while absent in the progenitor of japonica; 612,951 alleles found in indica and/or japonica while absent in the progenitor of indica; 303,652 alleles found in aus and/or japonica while absent in the progenitor of japonica; 276,813 alleles found in aus and/or japonica while absent from the progenitor of aus; 323,665 alleles found in indica and/or aus while absent in the progenitor of aus; and 233,812 alleles found in indica and/or aus while absent in the progenitor of indica. For each of these six datasets, allele sharing was summarized as a joint allele frequency spectrum (AFS) constructed in Libre Calc (using basic operations, functions FREQUENCY and LOG10, and conditional formatting). Three additional datasets and AFSs were prepared in order to summarize sharing of alleles that are absent (≤ 0.01) from the entire wild superpopulation. These datasets consisted of 261,775; 162,523 and 142,381 alleles for indica-japonica, indica-aus and japonica-aus combinations, respectively.

The genomic distribution of the shared variants was summarized by an introgression index calculated across the entire genome in 100 kb windows with 50 kb sliding steps, using eq. 1:
Results and discussion
Progenitor populations of aus, indica and japonica
We conducted a genome-wide search for the ancestral gene pools of indica, japonica and aus by analysing 705,124 variable positions in the nuclear genomes of 1480 wild and cultivated rice accessions, which represent 8.8% of the genome-wide SNPs identified by [3] and roughly 0.16% of the whole genome. Following the rationale that omnipresent characters will not be informative for the resolution of potentially distinct phylogeographic origins, we focused on unshared ancestral variants, i.e. variants that are present in wild rice and one cultivated group but absent in the other two cultivated groups. This strategy does not assume independent origins of the groups a priori – under a single origin scenario there would either be no ancestral alleles specific for one cultivated group, or such alleles would always point to the same wild population. Analysis of unshared ancestral characters also enables the progenitor populations to be identified despite the confounding effects of post-domestication gene flow between the cultivated groups.

We identified 15,549 positions with unshared ancestral variants in japonica; 17,133 in indica and 16,162 in aus. We quantified these variants in wild rice and for each group we selected those 40 O. rufipogon accessions in which the variants are found in the highest proportions (Additional file 1: Table S1). The results (Fig. 1a-c) show that wild rice from which the aus group obtained its ancestral group-specific variants is distributed from the Brahmaputra valley through Bangladesh to the Odisha region in India. Wild accessions carrying the highest proportions of japonica-specific variants are most concentrated in the Yangtze valley and southern China. The indica-specific variants are not particularly concentrated in individual wild accessions; however, the samples with the highest proportions are found in Indochina and the eastern part of the Indian subcontinent.

The geography of the ancestral populations identified here is almost identical to those we previously inferred using a different approach [5], where we examined 38 CLDGRs – genomic regions with low diversity (n) co-located in indica, aus and japonica rice – and marked on maps those wild accessions that clustered most frequently with particular cultivated groups in neighbour-joining CLDGR-trees. In the present study, we analysed tens of thousands of ancestral, group-specific variants and we only used descriptive statistics to identify the progenitor populations. Although both analyses place the ancestral gene pools in identical geographic regions, it should be noted that the analyses may suffer from insufficient sampling. For example, a great diversity of natural wild rice populations was reported from the Gangetic plains (Uttar Pradesh and Bihar states) [37]. Unfortunately, these wild populations are under-represented in the SNP dataset [3], despite the fact that the earliest archaeological evidence of Neolithic rice exploitation on the Indian subcontinent comes from the Gangetic plains [38, 39]. The possible relationships of the wild populations from Uttar Pradesh and Bihar to cultivated indica and aus are therefore yet to be determined.

An ML tree computed from 358,218 variable positions (Fig. 1d) shows 100% bootstrap support for the monophyly of japonica, as well as for its association with the identified progenitor population. The japonica clade is further split into tropical and temperate groups with maximum support. The grouping of aus and its progenitor population is also fully supported, although aus samples do not form a monophyletic group within this clade. This could be a reflection of phylogenetic conflicts introduced by post-domestication gene flow (see the next section), or the origins of aus may indeed be complex. Interestingly, a previous report [40] differentiated two genetic subgroups within 250 aus cultivars, one of which is associated with the term ‘boro’, used to describe the winter growing season in Bangladesh and Assam. It is possible that aus and boro represent two cultivated groups domesticated from closely related wild gene pools. The association of indica and its progenitor population obtained only weak statistical support (76%) and one wild accession that we assigned to the ancestral gene pool of indica (W1959) is consistently resolved as basal to the (japonica, japonica-progenitor) clade (this accession was removed in the subsequent analysis of gene flow). The branching pattern within the (indica, indica-progenitor) group is largely unresolved, but all indica accessions are found in a clade with 85.5% bootstrap support.

The association of the cultivated groups with their identified progenitors was further tested by PCA in which all variable positions (5,759,207) were included (Fig. 2). All cultivated groups are differentiated by the first two eigenvectors with statistical significance, except for aus and indica along the first eigenvector. Each cultivated group is closely associated with a cluster of wild accessions identified as the progenitor population in Fig. 1.

Post-domestication gene flow among the cultivated groups
We found that 73.7% of the 705,124 polymorphic positions carry one allele simultaneously approaching fixation (allelic frequency > 0.95) in japonica, indica and aus. However, all of these alleles are likely to be post-domestication variants, i.e. they are found in the wild population, usually at high frequencies (Additional file 2: Figure S2). Sharing of ancestral variants that are frequent in the wild population cannot be interpreted as evidence of common origin or inter-group gene flow, because such alleles could be obtained independently from the wild gene pool during separate domestimations.
For this reason, we focused on variation that is absent from at least one progenitor population, or absent from *O. rufipogon* altogether and hence a presumed post-domestication variation. Inter-group sharing of variants that are demonstrably absent from one of the groups’ progenitors can only be caused by gene flow (admixture, hybridization) or homoplasy. If the groups are in complete genetic isolation and have independent origins, sharing of such variation can only occur through homoplasy. On the other hand, if the groups shared parts of their genetic history or hybridized extensively, then the proportion of such shared variants to autapomorphies (alleles present in just one domesticated population) would increase. Quantification of the variation shared by the cultivated groups but absent from one progenitor is therefore a means of assessing the direction and magnitude...
of the gene flow that operated during or after the domestication of those groups.

A bona fide summary of genetic variation shared by two groups can be presented in the form of a multi-population AFS (joint distribution of allele frequencies across diallelic variants) [41, 42]. Typically, the alleles that are presented in an AFS are defined as ‘derived’ by reference to an outgroup. Our AFSs (Fig. 3), however, are designed to show the inter-group sharing of domestication or post-domestication variants, and therefore depict alleles that are absent in at least one progenitor population. The rationale is that if an allele is absent from the progenitor of indica, for example, but is shared among indica and japonica, then the implication is that the allele was transferred into indica after domestication, possibly from japonica.

We carried out AFSs of alleles present in indica and japonica but absent in the progenitor population of indica (Fig. 3a) or japonica (Fig. 3b), alleles present in indica and aus but absent in their progenitor populations (Fig. 3d,e) and the same analyses for japonica and aus (Fig. 3g,h). A common feature of all six AFSs is that > 90% of the alleles absent in one progenitor are either unshared or shared below 0.05 frequency by the two cultivated groups. This indicates that the major parts of the indica, aus and japonica genomes have been unaffected by inter-group gene flow. Very few alleles (0.002–0.102%) absent in one of the progenitors are fixed in both cultivated groups, further indicating that only a small fraction of alleles transferred by gene flow was globally subjected to selection. The aus group appears to be the most affected by the gene flow, both from indica (Fig. 3e) and japonica (Fig. 3h), sharing with these two groups 8.352% and 8.673% of alleles absent in the aus progenitor, respectively. Nonetheless, only a minute fraction of these alleles reach fixation. On the other hand, the genome of indica appears to be the least affected by gene flow, sharing only 2.616% and 2.857% of alleles absent in its progenitor with japonica (Fig. 3b) and aus (Fig. 3d), respectively.

The proportions presented on the AFSs are good indicators of the inter-group gene flow if the following three conditions are met: (i) the progenitor populations identified here are a good representation of the actual progenitors at the beginnings of agriculture; (ii) the gene flow operated in the direction crop→crop and not in the direction wild→crop; and (iii) the absence of alleles in the progenitors is correctly determined and does not result from low sample size, missing data or recent allele extinction. The first condition cannot be established, since an exact reconstruction of past demographic processes is not possible. Although we demonstrated significant association of the cultivated groups with their identified progenitors (Figs. 1 and 2), it is possible that the original gene pools were not sampled here (see above), or their diversity was different.

If it was wider, the proportions of shared alleles are overestimated; if it was narrower, the proportions are underestimated. To tackle the possible over-estimation together with the second issue (ii), we constructed AFSs for alleles absent in the entire wild superpopulation (Fig. 4c,f,i). As these AFSs are limited to post-domestication variation (variation that arose in the cultivated groups at any time since the domestication) and exclude wild→crop gene flow, the proportions of shared alleles decrease substantially. Very low levels of post-domestication gene flow between the three cultivated groups are implied, with the strongest signal recorded between japonica and aus (Fig. 3h).

The third issue (iii) can be examined by looking at the genomic distribution of the shared alleles. Shared alleles with erroneously established absence in the progenitor are expected to be randomly distributed across the genome. Consequently, clustering of the shared alleles in a particular genomic region points at introgression from a different population. For this purpose, we performed a genomic scan of the introgression signal, using the product of allelic frequencies for a pair of domesticated groups calculated for all sites where the allele is undetected in one of the progenitors (eq. 1; Fig. 4). This introgression index is averaged for 100 kb windows with 50 kb sliding steps, and simultaneously measures allelic similarity in the cultivated pair and their dissimilarity from the selected progenitor. The index value can vary from zero – indicating no introgression (when no shared variants absent in the progenitor are found), to one – indicating total introgression (when all variants absent in the progenitor are fixed in both cultivated groups).
Interestingly, regions containing alleles responsible for the erect growth (Prog1 [25]; Fig. 4g), white pericarp (Rc [24]; Fig. 4g) and non-shattering ear (qSH1, qSH3, Sh4 [23, 33, 43]; Fig. 4a,c,d, respectively) show nil signal of introgression in indica and aus, which implies that the two groups obtained these genomic regions directly from their progenitors. In the case of japonica, weak signal of introgression from indica was detected in the region containing the Rc gene. However, it is not clear whether the Rc gene itself was involved in this putative introgression block, since the signal is detected 23–167 kb downstream of the Rc coding sequence. If the rc allele was indeed introgressed into japonica from indica, the direction of that introgression is the opposite of the one concluded previously [24]. However, the previous conclusion was based on an assumption that the recessive rc allele had evolved from red-grained varieties under cultivation, which we find to be unsubstantiated [8].
Introgression signals in the same direction and with similar magnitude were detected 17–125 kb upstream of the GW2 gene (Fig. 4b), and unambiguously spanning the LABA1 gene (1.8 Mb around the coding sequence; Fig. 4d). The GW2 gene has a modest effect on grain size and shows signs of purifying selection [44]. Since distinct alleles of GW2 are prevalent in indica and japonica [44], introgression of the coding sequence into japonica seems unlikely. The recessive LABA1 allele is responsible for the short and barbless awns in cultivated rice [29]. Since the LABA1 region in the progenitor of japonica is rather dissimilar from the one in japonica, it appears that the locus was introgressed from indica. This direction of introgression is again the opposite of the one suggested previously [29], but we note it is unclear how the direction was established in that study. Interestingly, the laba1 allele is not fixed in temperate japonica which often retains barbed awns [8, 29], indicating that the introgression into japonica was not global. Additionally, a mild signal of introgression into japonica was detected 45–79 kb upstream of the Hd1 gene (Fig. 4f). Variations in the Hd1 gene on chromosome 6 cause changes in photoperiod sensitivity, a trait that can be crucial for cultivation in some areas [45]. Our findings suggest that this locus may have been introgressed into some japonica varieties from aus. The genome of japonica shows additional introgression signals (e.g. on chromosome 7, Fig. 4g), however, these are distant from genes known to be involved in domestication.

Multiple genomic regions were introgressed into the genome of aus, either from japonica or indica. The strongest signal was detected in the direction japonica→aus involving a 3.5 Mb region on chromosome 7 surrounding the Ghd7 locus (Fig. 4g). The Ghd7 gene has a pleiotropic effect on plant height, heading date and yield, and its haplotype structure suggests independent selection during the domestication of indica and japonica [46]. Here we conclude that this locus was subsequently transferred from japonica to aus. Other signals of introgression into aus were detected on chromosome
4, involving the genes Kala4 and LG1 (Fig. 4d). The Kala4 gene regulates expression of several genes responsible for pigment production [47] and the LG1 gene is responsible for the compact panicle architecture in cultivated rice [48]. Our results suggest that both genes were introgressed into aus from japonica. Additional strong signals of introgression into aus from either indica or japonica were also detected on chromosomes 1, 2, 4, 6, 10 and 11 however, these regions do not contain known domestication alleles.

Only two weak introgression peaks were detected in the genome of indica – short regions on chromosomes 4 and 7 that appear to originate from japonica (Fig. 4d,g). To the best of our knowledge, these regions do not contain known domestication-related genes.

Due to the amount of missing data in the original SNP matrix [3], we were only able to examine a fraction of the variable sites observed in wild and cultivated rice. Nonetheless, we analysed data for 96.5% of the genomic windows, and the introgression index was typically calculated from tens to hundreds of sites per window (Additional file 2: Figure S3). Hence, our analyses capture almost the entire genome and the introgression index is robust across most of its length.

Recently, the ABBA-BABA test and coalescent modelling on several Oryza individuals with de novo assembled genomes have been used to infer ancestral population sizes, divergence times and gene flow rates [31]. The authors estimated that indica obtained 17% of its genome from japonica, and aus obtained 15% and 11% of its genome from japonica and indica, respectively. Although our analyses do not yield exact quantification of the genomic fractions subjected to introgression, the conclusions of Choi et al. [31] do not appear consistent with our results, particularly in case of indica. We believe that there are several potential sources of bias in the approach used by Choi et al. [31]. First, since the authors did not analyse populations, the individuals used for the coalescent modelling may not be representative of the gene pool compositions in the japonica, indica and aus groups. Particularly, the indica cultivar IR64 used for their coalescent modelling has a complex pedigree with several japonica parents [49] and the second indica cultivar used by Choi et al. [31], 93–11, is also known to have a japonica cultivar in its pedigree [50]. Such recent hybrid history could seriously affect the gene flow estimate, which will therefore not be representative of the indica group as whole. Since modern cultivars are genetically altered by breeding efforts, traditional landraces are the preferred material for crop domestication studies. The domesticated dataset utilized in our analyses [3], consists almost entirely of landraces. Moreover, both coalescent reconstruction and the ABBA-BABA test are models of neutral evolution that cannot account for selection. Accordingly, the authors [31] have chosen and analysed genomic regions void of signs of selection. On one hand, this is the correct application of the methods, but on the other hand it means that coalescent modelling and the ABBA-BABA test cannot inform us about the fraction of the rice genome that was under selection during domestication, and therefore cannot answer the questions about the origin of domestication alleles.

Conclusions
Considerations of gene flow among O. sativa groups need to recognize the existence of the partial reproductive barrier between indica and japonica [11, 12], their different ecological requirements, and the unusually high pairwise FST values (Additional file 1: Table S2), all of which argue against there having been high levels of genetic interaction. Nonetheless, gene flow between indica and japonica is a popular concept, because it offers a simple explanation for the conundrum of why some genetic loci appear identical in these two groups despite their generally distinct genetic architectures. Several models of rice domestication have hypothesized that gene flow occurred in the early stages of rice cultivation [26, 27] and was crucial for the emergence of the non-japonica groups [3, 31]. These hypotheses assume that some of the domestication alleles were acquired from local standing variation or emerged under cultivation (through unique mutations), were targeted by artificial selection and – because of their superior phenotypes – were introgressed into other proto-domesticated populations where they became fixed and helped to establish the domestication phenotype.

One genetic expectation of such a model is the existence of alleles shared by the cultivated groups at high frequencies while absent from the progenitor of the recipient group. Here we tested this expectation and examined clustering of such alleles, which we interpret as a signal of introgression (Fig. 4). We did not detect any introgression signal at the major domestication loci Prog1, Rc, qSH1, qSH3 and Sh4 in indica and aus, which implies that gene flow from japonica was not involved in the establishment of crucial domestication characteristics in aus and indica. Other domestication-related loci in the genome of aus, namely Gh7, Kala4 and LG1, show signals of introgression from japonica. The genome of japonica also shows signals of introgression, some of which are associated with domestication-related loci (unambiguously with LABA1, tentatively with GW2, Hdi1 and Rc). Interestingly, the indica genome appears to be the least affected by gene flow, with a couple of introgression peaks on chromosomes 4 and 7, although distant from known domestication-related genes. Another important observation is that among 261,775 post-domestication variants occurring in indica and/or japonica (Fig. 3c) there
is not a single allele simultaneously fixed (> 0.95) in both groups, while only seven alleles are shared at frequencies > 0.5. All of these observations, together with our genome-wide phylogenetic and population diversity analysis (Fig. 1, Fig. 2) are consistent with our previous proposal [5] that there were three independent origins for japonica, indica and aus, with each of these cultivated groups acquiring the crucial domestication characteristics without a contribution from gene flow. Our results suggest that most domestication alleles were present in the wild progenitor populations of all three groups, and selection from standing variation was the major force in rice domestication. Signatures of gene flow are apparent in all three cultivated gene pools and several domestication-related loci were exchanged between the groups, particularly in case of aus. Nonetheless, it appears that the introgressions into the genomes of indica and japonica occurred at later stages of rice cultivation, perhaps quite recently, and that transcontinental cultural contacts – a prerequisite of such gene flow – were not necessary for the emergence of agriculture in different parts of Asia.

Additional files

Additional file 1: Supporting tables. (Tables S1 and S2). (XLS 190 kb)

Additional file 2: Supporting figures. Figure S1. Schematic summary of the data processing and analysis pipeline. Figure S2. Variants shared and fixed in cultivated groups. Alleles that are simultaneously fixed in indica, japonico and aus (counts shown on y axis) are always found in wild populations, usually with high allelic frequencies (x axis). Figure S3. Histogram of SNP densities. Histograms show the number of sites per 100 kb window used for the calculation of the introgression index shown on Fig. 4. Mean number of sites per window is shown for each dataset in corresponding colours. (PDF 119 kb)

Abbreviations

AFS: Allele frequency spectrum; CLDGR: Co-located low diversity region; ML: Maximum likelihood; PCA: Principal component analysis; SNP: Single nucleotide polymorphism

Acknowledgements

We thank Hayley Craig, Cymon J. Cox and Sandra Kennedy for technical assistance.

Funding

This work was supported by European Research Council grant 339941 awarded to TAB.

Authors’ contributions

PC and TAB conceived the project. PC carried out the analyses. PC and TAB wrote the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. He Z, Zhai W, Wen H, Tang T, Wang Y, Lu X, Greenberg AJ, Hudson RR, Wu C-J, Shi S. Two evolutionary histories in the genome of rice: the roles of domestication genes. PLoS Genet. 2011;7:e1002100.
2. Molina J, Sikoara M, Garud N, Flowers JM, Rubenstein S, Reynolds A, Huang P, Jackson S, Schaal BA, Basturmane CD, Botko AR, Purugganan MD. Molecular evidence for a single evolutionary origin of domesticated rice. Proc Natl Acad Sci U S A. 2011;108:8351–6.
3. Huang X, Kurata N, Wei X, Wang Z-X, Wang A, Zhao Q, Zhao Y, Liu K, Lu H, Li W, Guo Y, Lu Y, Zhou C, Fan D, Weng Q, Zhu C, Huang T, Zhang L, Wang Y, Feng L, Furumih S, Kubo T, Miyabayashi T, Yuan X, Xu Q, Dong G, Zhan Q, Li C, Fujiiama A, Toyoda A, Lu T, Feng Q, Qian Q, Li J, Han B. A map of rice genome variation reveals the origin of cultivated rice. Nature. 2012;490:497–503.
4. Yang CC, Kawahara Y, Mizuno H, Wu J, Matsumoto T, Ishih T. Independent domestication of Asian rice followed by gene flow from japonica to indica. Mol Biol Evol. 2012;29:1471–9.
5. Civá P, Craig H, Cox CJ, Brown TA. Three geographically separate domestications of Asian rice. Nat Plants. 2015;1:15164.
6. Huang X, Han B. Rice domestication occurred through single origin and multiple introgressions. Nat Plants. 2015;2:15207.
7. Civá P, Craig H, Cox CJ, Brown TA. Multiple domestications of Asian rice. Nat plants. 2016;2:16037.
8. Civá P, Brown TA. Origin of rice (Oryza sativa L.) domestication genes. Genet Resour Crop Evol. 2017;64:1125–32.
9. Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. Genetic structure and diversity in Oryza sativa L. Genetics. 2005;169:1631–8.
10. Zhao K, Tung C-W, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Basturmane CD, McCouch SR. Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nat Commun. 2011;2:467.
11. Harushima Y, Nakagahra M, Yano M, Sasaki T, Kurata N. Diverse variation of reproductive barriers in three intraspecific rice crosses. Genetics. 2002;160:313–22.
12. Yang J, Zhao X, Cheng K, Du H, Ouyang Y, Chen J, Qiu S, Huang J, Jiang Y, Jiang L, Ding J, Wang J, Xu C, Li X, Zhong Q. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. Science. 2012;337:1336–40.
13. Morinaga T. Origin and geographical distribution of Japanese rice. Jpn Agric Res Q. 1968;3:1–5.
14. Engle LM, Chang TT, Ramirez DA. The cyto-genetics of sterility in F1 hybrids of indica×indica and indica×javanica varieties of rice (Oryza sativa L.). Philipp Agric. 1969;53:289–307.
15. Song ZP, Lu B-R, Zhu YG, Chen JK. Gene flow from cultivated rice to the wild species Oryza rufipogon under experimental field conditions. New Phytol. 2003;157:657–65.
16. Chen LJ, Lee DS, Song ZP, Suh HS, Lu B-R. Gene flow from cultivated rice (Oryza sativa) to its weedy and wild relatives. Ann Bot. 2004;93:67–73.
17. Wang F, Yuan Q-H, Shi L, Qian L, Liu W-G, Huang B-G, Zeng D-L, Luo Y-L, Cao B, Jia S-R. A large-scale field study of transgene flow from cultivated rice (Oryza sativa) to common wild rice (O. rufipogon) and barnyard grass (Echinochloa crusgalli). Plant Biotechnol J. 2006;4:667–76.
18. Shivrain VK, Burgos NR, Anders MM, Rajguru SN, Moore J, Sales MA. Gene flow between Clearfield™ rice and red rice. Crop Prot. 2007;26:349–56.
19. Ma J, Bennett NL. Rapid recent growth and divergence of rice nuclear genomes. Proc Natl Acad Sci U S A. 2008;101:12404–10.
20. Vitte C, Ishii T, Lamy F, Brar O, Panaud O. Genomic paleontology provides evidence for two distinct origins of Asian rice (Oryza sativa L.). Mol Gen Genom. 2004;272:504–11.
21. Zhu Q, Ge S. Phylogenetic relationships among A-genome species of the genus Oryza revealed by intron sequences of four nuclear genes. New Phytol. 2005;167:249–55.
22. Londo JP, Chiang Y-C, Hung K-H, Chiang T-Y, Schaal BA. Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestimations of cultivated rice, Oryza sativa. Proc Natl Acad Sci U S A. 2006;103:9578–83.
23. Li C, Zhou A, Sang T. Rice domestication by reducing shattering. Science. 2006;311:1936–9.
24. Sweeney MT, Thomson MJ, Cho YG, Park YJ, Williamson SH, Bustamante CD, McCouch SR. Global dissemination of a single mutation conferring white pericarp in rice. PLoS Genet. 2007;3:e133.

25. Tan L, Li X, Liu F, Sun X, Li C, Zhu Z, Fu Y, Cai H, Wang X, Xie D, Sun C. Control of a key transition from prostrate to erect growth in rice domestication. Nat Genet. 2008;40:1360–4.

26. Kovach MJ, Sweeney MT, McCouch S. New insights into the history of rice domestication. Trends Genet. 2007;23:78–87.

27. Sang T, Ge S. Genetics and phylogenetics of rice domestication. Curr Opin Genet Dev. 2007;17:533–8.

28. Caicedo AL, Williamson SH, Hernandez RD, Boyko A, Fleeld-Alon A, York TL, Polott NR, Olsen KM, Nielsen R, McCouch SR, Bustamante CD, Purugganan MD. Genome-wide patterns of nucleotide polymorphism in domesticated rice. PLoS Genet. 2007;3:e163.

29. Hua L, Wang DR, Tan L, Fu Y, Xiao L, Zhu Z, Fu Q, Sun X, Gu P, Cai H, McCouch SR, Sun C. LABA1, a domestication gene associated with long, barbed awns in wild rice. Plant Cell. 2015;27:1875–88.

30. Si L, Chen J, Huang X, Gong H, Luo J, Hou Q, Zhou H, Lu T, Zhu J, Shangguan Y, Chen E, Gong C, Zhao Q, Jing Y, Zhao Y, Li Y, Cai L, Fan D, Lu Y, Weng Q, Wang Y, Zhan Q, Liu K, Wei X, An K, An G, Han B. Os3/Ps13 controls grain size in cultivated rice. Nat Genet. 2016;48:447–57.

31. Choi JY, Platts AE, Fuller DJQ, Hsing Y-I, Wing RA, Purugganan MD. The rice paradox: multiple origins but single domestication in Asian rice. Mol Biol Evol. 2017;34:969–79.

32. Zhu Y, Efstathiadis NC, Lu B-R. Sequence polymorphism in wild, weedy, and cultivated rice suggest seed-shattering locus sH4 played a minor role in Asian rice domestication. Ecol Evol. 2012;2:2106–13.

33. Inoue C, Htun TM, Inoue K, Ikeda K-i, Ishii T, Ishikawa R. Estimation of loci involved in non-shattering of seeds in early rice domestication. Genetics. 2017;154:201–7.

34. Ishikawa R, Nishimura A, Htun TM, Nishioka R, Oka Y, Tsujimura Y, Inoue C, Ishii T. Estimation of loci involved in non-shattering of seeds in early rice domestication. Genetics. 2017;154:201–7.

35. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3.

36. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. PLoS Genet. 2006;2:e190.

37. Semwal DP, Pradheep K, Ahlawat SP. Wild rice (Oryza spp.) germplasm collections from gangetic plains and eastern region of India: diversity mapping and habitat prediction using ecocrop model. Vegetos. 2016;29:96–100.

38. Fuller DJQ. Pathways to Asian civilizations: tracing the origins and spread of rice and rice cultures. Rice. 2011;4:78–92.

39. Gross BL, Zhao Z. Archaeological and genetic insights into the origins of domesticated rice. Proc Natl Acad Sci U S A. 2014;111:6190–7.

40. Travis AJ, Norton GJ, Datta S, Sarma R, Dasgupta T, Savio FL, Macaulay M, Thomson MJ, Sweeney MT, Thomson MJ, Bustamante CD, Purugganan MD. New insights into the history of rice domestication. Nat Genet. 2008;40:1360–4.

41. Kovach MJ, Sweeney MT, McCouch S. Global dissemination of a single mutation conferring white pericarp in rice. PLoS Genet. 2007;3:e133.

42. Sousa V, Hey J. Understanding the origin of species with genome-scale data: modelling gene flow. Nat Rev Genet. 2013;14:404–14.

43. Konishi S, Izawa T, Li X, Liu F, Xiao L, Zhu Z, Fu Q, Sun X, Gu P, Cai H, McCouch SR, Sun C. LABA1, a domestication gene associated with long, barbed awns in wild rice. Plant Cell. 2015;27:1875–88.

44. Lu L, Shao D, Qiu X, Sun L, Yan W, Zhou X, Yang L, He Y, Yu S, Xing Y. Natural variation and artificial selection in four genes determine grain shape in rice. New Phytol. 2013;200:1269–80.

45. Yano M, Katayose Y, Ashikari M, Yamamoto K, Umemura Y, Monna L, Fuse T, Baba T, Yamamoto K, Umemura Y, Nagamura Y, Sasaki T. Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell. 2003;15:2473–83.

46. Lu L, Yan W, Xue W, Shao D, Xing Y. Evolution and association analysis of Ghd7 in rice. PLoS One. 2012;7:e36021.

47. Okava T, Maeda H, Opuchi T, Yamaguchi T, Tanabe N, Ebana K, Yano M, Ebitani T, Izawa T. The birth of a black rice gene and its local spread by introgression. Plant Cell. 2015;27:2401–14.

48. Zhu Z, Tan L, Fu Y, Liu F, Cai H, Xie D, Wu F, Wu J, Matsumoto T, Sun C. Genetic control of inflorescence architecture during rice domestication. Nat Commun. 2013;4:2200.

49. Ballini E, Bernuyer R, Morel J-B, Lebrun M-H, Nottéghem J-L, Tharreau D. Modern elite rice varieties of the ‘green revolution’ have retained a large introgression from wild rice around the Pi33 rice blast resistance locus. New Phytol. 2007;175:340–50.

50. Yang CC, Sakai H, Numa H, Itoh T. Gene tree discordance of wild and cultivated Asian rice deciphered by genome-wide sequence comparison. Gene. 2011;477:53–60.