Deleterious variants in Asian rice and the potential cost of domestication

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ABSTRACT

Many SNPs that are predicted to encode deleterious amino acid variants. These mildly deleterious mutations may provide unique insights into population history, the dynamics of selection, and the genetic bases of phenotypes. This may be especially true for domesticated species, where a history of bottlenecks and selection can contribute to the accumulation of deleterious SNPs (dSNPs). Here we investigate the numbers and frequencies of deleterious variants in Asian rice (*O. sativa*), focusing on two varieties (*japonica* and *indica*) that may have been domesticated independently and their wild relative (*O. rufipogon*). Most dSNPs were lost during domestication, but comparative analyses in two population datasets indicated that the remaining dSNPs shifted in site frequency spectrum (SFS) relative to synonymous SNPs. Moreover, dSNPs were enriched within low recombination regions of the genome and experienced frequency increases similar to synonymous SNPs within regions of putative selective sweeps. A characteristic feature of rice domestication was a shift in mating system from outcrossing to predominantly selfing. Forward simulations suggest that this shift in mating system may have been the dominant factor in shaping extant rice diversity.

INTRODUCTION

Several studies have suggested that there is a "cost of domestication" (Schubert et al., 2014), because domesticated species may accumulate slightly deleterious mutations that reduce their relative fitness (Lu et al., 2006). Under this hypothesis, the small effective population size (N_e) during a domestication bottleneck reduces the efficacy of genome-wide selection (Charlesworth and Willis, 2009), leading to the accumulation of slightly deleterious variants (Lohmueller et al., 2008, Casals et al., 2013). The fate of these variants also relies on linkage, because selection is less effective in genomic regions of low recombination (Hill and Robertson, 1966, Felsenstein and Yokoyama, 1976) and because deleterious variants may hitchhike with alleles that are positively selected for agronomic traits (Fay and Wu, 2000, Hartfield and Otto, 2011, Campos et al., 2014). Overall, the cost of domestication is expected to increase the frequency of deleterious variants in small relative to large populations, in regions of low recombination, and near sites of positive selection.

This hypothesis about the cost of domestication closely parallels the debate regarding the genetic effects of migration-related bottlenecks and demographic expansion in human populations (Lohmueller et al., 2008, Casals et al., 2013, Peischl et al., 2013, Simons et al., 2014). The debate regarding human populations is contentious, perhaps in part because it suggests that some human populations may, on average, carry a greater load of deleterious variants than others. Studies in humans also suggest that subtlety of interpretation is required when considering the relative frequency of deleterious variants in populations; both the effect size and relative dominance of deleterious variants likely play a role in how mutations impact the fitness of populations (Henn et al., 2016). Moreover, deleterious variants in non-equilibrium populations, such as those that have experienced a recent bottleneck, may also return to pre-bottleneck frequencies more rapidly than neutral variants (Brandvain and Wright, 2016). It nonetheless remains an

important task to identify the frequency and genomic distribution of deleterious variants in humans, for the purposes of disentangling evolutionary history and for understanding the association between deleterious variants and disease (Kryukov et al., 2007, Eyre-Walker, 2010, Simons et al., 2014).

In plant crops, the potential for the accumulation of deleterious variants was first examined in Asian rice (*O. sativa*) (Lu et al., 2006). At the time, limited resequencing data were available, so Lu et al (2006) compared two *O. sativa* reference genomes to that of a related wild species (*O. brachyntha*). They found that radical, presumably deleterious amino acid variants were more common within *O. sativa* genomes, suggesting a cost of domestication. A handful of studies have since analyzed deleterious variants in crops based on resequencing data (Gunther and Schmid, 2010, Nabholz et al., 2014, Renaut and Rieseberg, 2015, Kono et al., 2016), and together they suggest that an increased frequency of deleterious variants is a general outcome of domestication. More limited analyses have also shown that deleterious variants are enriched within genes associated with phenotypic traits (Mezmouk and Ross-Ibarra, 2014, Kono et al., 2016), suggesting that the study of deleterious variants is crucial for understanding potentials for crop improvement (Morrell et al., 2011). While a general picture is thus beginning to emerge, most of these studies have suffered from substantial shortcomings, such as small numbers of genes, low numbers of individuals, or the lack of an outgroup to infer ancestral states. Moreover, no study of crops has yet investigated the prevalence of deleterious variants in putative selective sweep regions, which is especially important given that hypotheses that artificial selection has increased the frequency of deleterious mutations (Lu et al., 2006).

In this study, we reanalyze genomic data from hundreds of accessions of Asian rice and its wild relative *O. rufipogon.* Asian rice feeds more than half of the global population (International Rice Genome Sequencing Project, 2005), but the domestication of the two main varieties of Asian rice (ssp. *japonica* and ssp. *indica*) remains enigmatic.

It is unclear whether the two varieties represent independent domestication events (Londo et al., 2006, Civian et al., 2015), a single domestication event with subsequent divergence (Gao and Innan, 2008, Molina et al., 2011), or separate events coupled with substantial homogenizing gene flow of beneficial domestication alleles (Caicedo et al., 2007, Sang and Ge, 2007, Zhang et al., 2009, Huang et al., 2012a, b). It is clear, however, that domestication has included a shift in mating system: from predominantly outcrossing *O. rufipogon* [which has outcrossing rates between 5% and 60%, depending on the population of origin and other factors (Oka and Miroshima, 1967)] to predominantly selfing rice [which has outcrossing rates of ~1% (Oka, 1988)]. This shift in mating system has the potential to affect the population dynamics of deleterious variants, because inbreeding exposes partially recessive variants to selection (Lande and Schemske, 1985), which may in turn facilitate purging of deleterious alleles (Arunkumar et al., 2015).

Commensurate with its agronomic importance, the population genetics of Asian rice have been studied in great detail. Comparative resequencing studies have estimated that nucleotide sequence diversity is ~2 to 3-fold higher in *indica* than in *japonica* varieties (Zhu et al., 2007, Huang et al., 2012b), the latter of which is often separated into temperate and tropical germplasm. Sequence polymorphism data have also shown that the derived site frequency spectrum (SFS) of both varieties exhibits a distinct U-shaped distribution relative to *O. rufipogon*, due either to the genome-wide effects of selection or migration (Caicedo et al., 2007). Surprisingly, however, the population genetics of putatively deleterious variants have not been studied across *O. sativa* genomes.

In this study, we reanalyze genomic data from hundreds of *indica*, *japonica,* and *O. rufipogon* accessions to focus on the population frequencies of putatively deleterious genetic variants. To assess the robustness of our results, we have utilized two *O. sativa* datasets: one with many accessions (n = 766) but low sequencing coverage (1-2x), the other with fewer individuals (n = 45) but enhanced coverage. For both datasets, we have

re-mapped raw reads and then applied independent computational pipelines for SNP variant detection. We have also used two different approaches – PROVEAN (Choi et al., 2012) and SIFT (Kumar et al., 2009)- to predict deleterious variants from nonsynonymous SNPs. Armed with results from multiple datasets and different methodological approaches, we address four questions. First, what has been the fate of deleterious mutations during domestication, and does this fate reflect a 'cost of domestication' in Asian rice? Second, does the diversity of deleterious variants vary with recombination rate, suggesting a pervasive effect of linkage? Third, is the frequency of deleterious variants altered in genomic regions that may have experienced a selective sweep? Finally, can we garner insights into the relative contributions of demography, linkage, positive selection, and inbreeding on the population dynamics of deleterious variants?

RESULTS

Data sets

To investigate the population dynamics of deleterious variants, we collated two rice datasets. The first was based on the genomic data of 1,212 accessions reported in Huang et al. (2012b) (Table S1). This dataset, which we call the 'BH' data after the senior author, contains raw reads from 766 individuals of Asian rice, including 436 *indica* accessions and 330 *japonica* accessions. The BH dataset also included 446 accessions representing three populations of *O. rufipogon,* the wild ancestor of cultivated rice (Table 1). Huang et al. (2012b) determined that their *O rufipogon* accessions represented three different wild populations, which we denote W_I, W_{II} and W_{III}. They also inferred that W_I was ancestral to *indica* rice and that W_{III} was ancestral to *japonica* rice. Accordingly, we based our cultivated-to-wild comparisons on *indica* vs. W_I and *japonica* vs. W_{III} for the BH data, but when appropriate we also included comparisons to the complete set of wild accessions (W_{all}). For these BH data, we remapped sequencing reads to the *japonica* reference sequence (Goff et al., 2002), then used ANGSD (Korneliussen et al., 2014) to apply cut-offs for quality and coverage and to estimate the SFS (see Materials and Methods).

The second dataset, which we call the '3K' data (Li et al., 2014), consisted of 15 cultivated, high-coverage (>12x) accessions for each of *indica*, tropical *japonica,* and temperate *japonica* (Table S2). We also included data from 15 wild *O. rufipogon* individuals, which we denote W_{15}; this sample includes the 15 individuals from Huang et al. (2012) with the highest (>5x) coverage (Table S1). For this dataset, reads were again mapped to the *japonica* reference, but SNPs were called using tools from GATK and SAMtools (see Materials and Methods). The 3K dataset was used to assess the robustness of results based on the larger, lower coverage dataset, but the greater read depth and quality of these data permitted additional analyses.

The number and diversity of deleterious variants

Based on reads in the BH dataset, we identified between >230,000 SNPs from the cultivated samples and >1.6M SNPs from each of W_I, W_{II} and W_{III} wild populations (Table 1). Once identified, we annotated SNPs as either non-coding (ncSNPs), synonymous (sSNPs), Loss of Function (LoF) or nonsynonymous. LoF SNPs were those that contribute to apparent splicing variation, the gain of a stop codon or the loss of a stop codon. Nonsynonymous SNPs were predicted to be tolerant (tSNPs) or deleterious (dSNPs) based on PROVEAN (Choi et al., 2012) or SIFT (Ng and Henikoff, 2003).

In the *japonica* and *indica* BH samples, we identified hundreds of LoF mutations and predicted 4,530 and 7,506 dSNPs using PROVEAN (Table 1). Table 1 reports mean pairwise diversity, averaged across polymorphic sites ($\bar{\pi}$). This diversity value tended to be higher in cultivated samples, regardless of SNP class. For example, $\bar{\pi}$ was higher for *indica* compared to W_I for both dSNPs (0.1848 vs. 0.1523) and sSNPs (0.2014 vs. 0.1717; Table 1), and *japonica* rice had higher diversity compared to W_{III} (dSNPs: 0.1517 vs. 0.1170; sSNPs: 0.1641 vs. 0.1391). Despite fewer accessions, we identified more SNPs within the 3K data, owing to higher sequence coverage (Table 1). The 3K data indicated increased mean pairwise diversity for polymorphic sites in *indica* and tropical *japonica*, but not temperate *japonica*, relative to the species-wide W_{15} sample (Table 1).

Site frequency spectra

To better test whether the frequency distribution of dSNPs shifted during domestication, we defined SNPs as either ancestral or derived based on comparison to 93 *O. barthii* accessions (Table S3) and then plotted the SFS for different SNP categories. For the BH data, we reduced the sample size to 70 for each population, based on sampling and coverage criteria (Materials and Methods). The resulting SFS had a U-shape for all SNP categories in cultivated rice, as observed previously (Caicedo et al., 2007), but not for ancestral *O. rufipogon* (Figures 1 & S1). The SFS differed significantly

between wild and domesticated samples for all SNP categories (Kolmogorov-Smirnoff tests; $p<0.001$; Figures 1 & S1). The SNPs in Table 1 were based on detecting polymorphisms within each taxon separately, which limited the ability to infer the zero and fixed classes of the SFS. To include these classes, we followed the precedent of Simons *et al.* (2014) and identified SNPs within the entire $n=60$ sample of the 3K dataset, focusing only on sites without missing data and on sites that segregate within the combined sample. This subset of the 3K data included 2,266,987 SNPs, including 22,377 dSNPs, 65,594 tSNPs, 81,648 sSNPs and 4,102 LoF variants. With this subset of SNPs, most comparisons between the wild and cultivated SFS were not significant (Kolmogorov-Smirnoff; $p>0.05$), but the resulting SFS were similar to the BH data in exhibiting hints of a U-shaped SFS for all three cultivated taxa and for most site categories (Figures 2 & S2). This U-shape included enhanced frequencies of fixed and high frequency (>12) derived variants and a dearth of low frequency (<3) variants in domesticates compared to the W_{15} sample (Figures 2 & S2). Importantly, these comparisons also illustrate that the zero class was greatly enhanced in domesticated taxa, which is indicative of the loss of rare, low frequency variants.

Overall, both 3K and BH data showed that the derived variants that remained after domestication were shifted to higher frequency, as is expected following a bottleneck (Simons et al., 2014). These inferred shifts in the SFS were robust to: *i*) dataset, because the 3K and BH datasets yielded similar results, *ii*) SNP calling approaches, because different methods were applied to the 3K and BH datasets, *iii*) the composition of the wild sample, because similar patterns were observed when the BH *japonica* and *indica* samples were compared to W_{all} ($p \leq 1.93e^{-08}$ for all comparisons in both varieties) (Figure S3), *iv*) variation in sample sizes (n) among taxa, because the BH data did not have the same number of individuals per taxon, while the 3K data did (Table 1), and *v*) the prediction approach used to identify dSNPs (i.e., PROVEAN or SIFT; Figures S4 & S5).

Effects on dSNPs

A primary question is whether frequency shifts affected dSNP differentially. To investigate this question, we plotted the ratio of the number of derived dSNPs vs. derived sSNPs for each frequency category of the SFS. Figure 3 shows that both *indica* and *japonica* have enhanced numbers of derived dSNPs to sSNPs across the entire frequency range for the BH data (Wilcoxon rank sum: *indic*a vs.W_I, $p = 4.98e^{-16}$; *japonica* vs. W_{III}, $p < 2.20e^{-16}$; Fig 3). The 3K dataset exhibited similar properties throughout most of the frequency range, with the exception of the zero class, but the distributions remained significantly different overall (Wilcoxon rank sum: *indica* 3K vs. W_{15}, $p = 0.029$; tropical *japonica* 3K vs. W_{15}, $p = 0.017$; Figure 3).

We also calculated $R_{(A/B)}$, a measure that compares the frequency and abundance of dSNPs vs. sSNPs in one population (A) relative to another (B) (Xue et al., 2015). When $R_{(A/B)}$ is > 1.0, it reflects an overabundance of derived dSNPs (or LoF variants) relative to sSNPs in one population over another across the entire frequency range. As expected from SFS analyses, we found that $R_{(A/B)}$ was > 1.0 for LoF variants and for dSNPs in *indica* relative to the W_I population ($p \leq 2.30e^{-139}$ for all three comparisons; Figure 3) and in *japonica* relative to W_{III} ($p \sim 0.000$ for the three comparisons; Figure 3). The subset of 3K data, which included both the zero and fixed classes of variants, yielded similar results ($p \sim 0.000$ for all six comparisons; Figure 3). Hence, all cultivated samples contained increased proportions of derived dSNPs to derived sSNPs compared to wild samples.

Diversity as a function of recombination rate

Theory predicts that diversity should be lower in low recombination regions (Begun and Aquadro, 1992, Charlesworth, 1994) and also that fate of dSNPs relative to sSNPs may differ between high and low recombination regions due to interference (Felsenstein, 1974b). To test these predictions, we used a genetic map to calculate recombination rate

in windows across rice chromosomes, and then estimated diversity using mean pairwise diversity averaged separately across dSNPs and sSNPs within each window. Owing to different numbers of SNPs, we used larger (3MB) windows for the BH data than the 3K data (2MB). We first found that the diversity of both sSNPs and dSNPs were significantly positively correlated with recombination rate (Table 2; Figure 4), indicating reduced diversity in low recombination regions (Begun and Aquadro, 1992, Charlesworth, 1994).

We then investigated diversity of dSNPs relative to sSNPs in each window, based on the ratio of $\bar{\pi}$ for dSNPs and sSNPs. For the BH dataset, the ratio was negatively correlated with recombination, but not significant for either *indica* or *japonica* (Figure 4 and Table 2). However, the correlation was significantly negative for all three cultivated samples of the 3K data (Figure S6 and Table 2). These negative correlations are similar observations within other plant genomes (Lu et al., 2006a, Renaut and Rieseberg, 2015, Rodgers-Melnick et al., 2015, Kono et al., 2016) and consistent with less efficacious selection against dSNPs in low recombination regions.

dSNPs in regions of putative selective sweeps

Regions linked to selective sweeps (SS) may have increased frequencies of derived mutations (Fay and Wu, 2000), including dSNPs (Hartfield and Otto, 2011). Consistent with this expectation, a previous study of domesticated dogs has shown that the frequency of both dSNPs and sSNPs are inflated within SS regions (Marsden et al., 2016). Prompted by these observations, we investigated the distribution of deleterious and synonymous variants in putative SS regions, to test two hypotheses. The first was that SS regions have increased frequencies of derived SNPs relative to the remainder of the genome. The second was that SS regions alone explain the accumulation of high frequency derived dSNPs in Asian rice.

To test our hypotheses, we made use of previously identified SS regions. Huang et al.

(2012c) defined SS regions based on the relative difference in π between wild and domesticated populations (Huang et al., 2012c). That is, the regions were based on π_d/π_w, where π is measured per base pair, and the subscripts refer to domesticated and wild samples. We also inferred selective sweeps using two additional approaches: SweeD (Pavlidis et al., 2013) and XP-CLR (Chen et al., 2010). SweeD identifies regions of skewed SFS relative to background levels for a single population (i.e., the rice sample). In contrast, XP-CLR searches for genomic regions for which the change in allele frequency between two populations (cultivated vs. wild samples) occurred too quickly at a locus, relative to the size of the region, to be caused by genetic drift. Both SweeD and XP-CLR were applied with a 5% cutoff. Because XP-CLR requires explicit genotypes, we used the 3K datasets for all of the SS analyses (Methods).

Focusing first on the *indica* 3K dataset, the three approaches identified different numbers, locations and sizes of selective sweeps (Table 3). For example, Huang et al. (2012c) defined 84 SS regions that encompassed 9.98% of the genome. In contrast, SweeD identified 485 SS regions, and XP-CLR distinguished an intermediate number of 161 SS regions. Consistent with the 5% cutoff, SweeD and XP-CLR identified 4.61% and 5.02% of the genome, respectively, as having been under selection (Table 3). To see if the same genes were identified with different SS identification methods, we calculated the degree of overlap across methods, focusing on the percentage of genes that two methods identified in common (see Methods). The overlap was surprisingly low (Figure 5 & S7-S17). Across the entire genome, the putative SS regions defined by SweeD and Huang et al (2012c) shared 6.24% of genes. Similarly, the regions defined by XP-CLR shared 8.51% and 8.69% of genes with Huang et al. (2012c) and SweeD, respectively.

To determine if SS regions have increased frequencies of derived dSNPs, we contrasted the SFS between SS and non-SS regions for derived segregating and fixed sSNPs and dSNPs (Marsden et al., 2016). The SFS were skewed for SS regions relative to non-SS regions for both SNP classes, independent of the method used to detect

selective sweeps (Figure 5A). We summarized the shift in frequencies by counting the number of derived alleles (DAC) per SNP (Figure 5B) (Marsden et al., 2016), which showed that SS regions also contained higher DACs (Figure 5B). Note that these results were not completely unexpected, because all of the methods used to define SS regions rely, in part, on identifying a skewed SFS relative to the genomic background (see Discussion).

Did sweeps affect dSNPs more or less than sSNPs? To investigate this question, we calculated the ratio of the mean DAC for SS and non-SS regions. There was some variation among SS methods. For example, the SS regions exhibited a 1.27-fold enrichment for dSNPs vs. a slightly smaller 1.21-fold enrichment for sSNPs when SS regions were based on SweeD (Table S4). Similarly, the SS regions defined by Huang et al. (2012c) included a 1.17- and 1.13-fold enrichment for dSNPs and sSNPs, respectively. SS regions defined by XP-CLR showed the reverse: slightly higher enrichment for sSNPs (1.32) than for dSNPs (1.30). Altogether, the extent to which hitchhiking drove dSNPs and sSNPs to higher frequency seems to roughly equivalent.

Enhanced SNP frequencies in SS regions raise the possibility that selective sweeps alone explain the shifted SFS of *indica* rice relative to *O. rufipogon*. To examine this second hypothesis, we removed all SS regions (as defined by SweeD, XP-CLR and π_d/π_w) from the *indica* 3K dataset and recalculated the SFS for non-SS regions. Even with SS regions removed, the SFS for wild and cultivated samples remained significantly different for sSNPs and dSNPs ($p \leq 0.0067$). These results imply either that positive selection is not the only cause of the U-shaped SFS in *indica* rice (Caicedo et al., 2007) or, alternatively that linked selection has affected more of the genome than is encompassed within the identified SS regions.

We performed these analyses of SS regions for the 3K temperate and tropical *japonica* datasets (Table 3), with similar results. First, although a greater extent of the genome tended to be identified as SS regions in *japonica* (Table 3), the overlap among

SS regions identified by different methods was again low (< 9%). Second, for both *japonica* datasets, derived sSNPs and dSNPS were generally at higher frequencies in putative SS regions, although the effect was not as apparent for sweeps identified with SweeD (Figure S18). Third, like *indica* rice, the SS regions alone did not account for the difference in SFS between wild and tropical or temperate *japonica* ($p \leq 0.0049$ for both comparisons).

Factors affecting the distribution of variants

Finally, we sought to gain insights into the relative effects of processes that have to the distribution of genetic variation in Asian rice. To do so, we first measured the 'mean derived allele frequency' (MDAF) for the subset of the 3K data, for which derived counts are comparable across populations (Simons et al., 2014). The MDAF was calculated as the average number of derived sites per individual, divided by twice the number of sites containing derived variants within that taxon (Methods). Similar to Simons et al. (2014), we calculated the MDAF separately for different site classes. The empirical results indicate that the MDAF was higher for all three rice groups than for the W_{15} *O. rufipogon* sample, regardless of SNP type (Figure 7A).

Rice has a complex history that includes a population bottleneck, positive selection and a shift in mating system. We were curious about the relative effect of these evolutionary forces on genetic diversity, as summarized by the MDAF, and so employed forward simulations to model these varied forces. We simulated models with and without a domestication bottleneck, with and without positive selection, and with and without inbreeding (Methods). To investigate relative effects on different classes of sites, all simulations included both neutral (synonymous) variants and deleterious variants.

Figure 7B presents simulation results for six models: an outcrossing population (out), an outcrossing population with a bottleneck (out+bot), an outcrossing population with a bottleneck and positively selected alleles (out+bot+pos) and three analogous models that

included complete selfing that co-occurs with the bottleneck (inb, inb+bot, inb+bot+pos). Focusing first on simulations for outcrossing populations, the MDAF was higher for synonymous compared to deleterious sites, as was found in the empirical data (Figure 7A). The MDAF of both site classes increased under a bottleneck (out+bot) and yet again with positive selection (out+bot+pos), indicating that both processes drive surviving variants to higher frequency. As the models progressed from out to out+bot to out+bot+pos, the difference in mean MDAF between synonymous and deleterious variants became larger (from 0.035 to 0.083 to 0.088, respectively).

The inclusion of selfing (inb) had a more substantive effect on the shift of the MDAF than the inclusion of either a bottleneck or positive selection (Figure 7B). Under inbreeding models, the inclusion of a population bottleneck (inb+bot) and positive selection (inb+bot+pos) had no effect on the mean MDAF of synonymous sites (t-tests, $p>0.55$). However, the addition of a bottleneck did increase the mean MDAF of deleterious sites (t-test, $p<0.05$), such that the difference in mean MDAF between synonymous and deleterious variants became less pronounced from inb (mean difference = 0.067) to inb+bot (0.058). In other words, the MDAF of dSNPs was enriched relative to sSNPs as our models progressed from inb ➔ inb+bot ➔ inb+bot+pos.

DISCUSSION

Recent focus on the population genetics of dSNPs in humans (Henn et al., 2015, 2016), plants (Lu et al., 2006b, Gunther and Schmid, 2010, Mezmouk and Ross-Ibarra, 2014, Nabholz et al., 2014, Renaut and Rieseberg, 2015, Rodgers-Melnick et al., 2015, Kono et al., 2016) and animals (Schubert et al., 2014, Marsden et al., 2016, Robinson et al., 2016) reflect an emerging recognition that dSNPs may provide unique clues into population history, the dynamics of selection and the genetic bases of phenotypes. This is especially true for the case of domesticated species, where the increased frequency of deleterious variants reflect a potential "cost of domestication" (Schubert et al., 2014).

Our analyses have provided a snapshot of the fate of deleterious variants during rice domestication. First, dSNPs are typically found at low frequency in wild populations (Figures 1 and 2) and at lower average diversity than corresponding sSNPs (Table 1). Second, many of these low frequency SNPs were lost during domestication, probably due to increased rates of genetic drift during the domestication bottleneck and/or due to inbreeding. The phenomenon of loss is reflected in the large zero class in the SFS of domesticated vs. wild germplasm (Figure 2). Third, the surviving dSNPs shifted toward higher frequency (Figures 1 and 2). Both of these processes – i.e., the loss of rare variants and a shifted SFS – also apply to sSNPs, but our data suggest a differential effect on dSNPs vs. sSNPs. This differential effect is evident in the higher proportion of derived dSNPs to sSNPs in domesticated rice than wild rice across most frequency classes and in significant $R_{(A/B)}$ measures (>1.0) for dSNPs (Figure 3). For all of these measures, the results were largely consistent between different types of presumably deleterious variants (i.e., dSNPs vs. LoF variants; Figure 3), different methods to predict deleterious SNPs (PROVEAN vs SIFT; Figs. S4 and S5), and rice datasets (BH data vs. 3K data).

What do these results imply about the "cost of domestication"? In their landmark study of human populations, Simons et al (2014) have argued that demographic processes have little impact on the individual burden of deleterious mutations. Indeed, we find that the average number of dSNPs per individual is nearly identical among the four groups in the subset of the 3K data based on the combined population (W_{15} *O. rufipogon*: 3184.1 dSNPs per individual, on average; *indica*: 3192.3; tropical *japonica*: 3158.8; temperate *japonica*: 3149.3). This result mimics that of Simons et al. (2014) and suggests that the loss of dSNPs through domestication perfectly balances the increase of surviving deleterious variants. If the number of deleterious mutations per individual is equivalent to genetic load, then there has been no increase in genetic load during domestication by this measure. However, as noted by Lohmueller (2014), genetic load may not be proportional to the number of deleterious alleles within an individual, because load is also a function

of the way "…those alleles are distributed into heterozygous and homozygous genotypes". For all practical purposes, selfing in Asian rice eliminates heterozygosity and the possibility that heterozygous deleterious alleles are hidden by recessive effects. Thus, load is likely higher in rice compared to heterozygous *O. rufipogon,* assuming that some deleterious alleles in *O. rufipo*gon are not dominant.

Processes that contribute to frequency enrichment of dSNPs

Our principle finding is that the frequency of derived SNPs has shifted from wild *O. rufipogon* to domesticated Asian rice and that this shift is more pronounced for dSNPs than sSNPs (Figures 1-3). At least four major evolutionary factors could influence this trajectory: *i*) population size, particularly bottlenecks associated with domestication (Caicedo et al., 2007, Zhu et al., 2007), *ii*) linkage effects, especially to selective sweeps (Hartfield and Otto, 2011, Marsden et al., 2016), *iii*) selfing, because the domestication of rice included a shift in mating system and, finally, *iv*) relaxed selection on wild traits that are no longer important under cultivation (Renaut and Rieseberg, 2015).

Evidence about linkage effects is accumulating. The enrichment of dSNPs in low recombination regions appears to be a general phenomenon, based on studies in *Drosophila* (Campos et al., 2014), humans (Hussin et al., 2015), sunflower (Renaut and Rieseberg, 2015), soybean (Kono et al., 2016) and rice (Lu et al., 2006; Figure 4). Note that we detect this effect despite the fact that selfing should reduce the strength of this relationship (Marais et al., 2004). It remains unclear whether differences between high and low recombination regions of the genome are driven by lower N_e in regions of low recombination (Hill and Robertson, 1966, Felsenstein, 1974a, Charlesworth et al., 1993) or by linkage effects to positively selected variants (Begun and Aquadro, 1992).

Another aspect of linkage is the enrichment of dSNP frequencies near genes that have experienced selective sweeps (SS). In domesticated dogs, Marsden et al. (2016) document that the average DAC of dSNPs is significantly elevated within SS regions and

also that dSNPs experienced the same increase in frequency as sSNPs due to hitchhiking. We find similar effects in rice – i.e., roughly equivalent increases in DACs for dSNPs and sSNPs due to hitchhiking (Fig. 4B). This suggests that alleles within selected genes, which are presumably of phenotypic importance, may be more often associated with mildly deleterious variants. One must nonetheless be cautious about our approach, because methods that detect SS regions, including π_d/π_w, rely to some extent on a skew of the SFS. This skew should manifest itself as elevated DACs. It is therefore difficult to separate potential methodological artifacts from true signal, but we take some comfort from the fact that the signal is consistent among SS methods (Figure 5).

Finally, we address the concomitant shift in population size and mating system in rice. It is generally thought that a shift to selfing offers advantages for an incipient crop, such as reproductive assurance, reduced opportunities for gene flow between an incipient crop and its wild ancestor (Dempewolf et al., 2012), and the creation of lines that "breed true" for agronomically advantageous traits (Allard, 1999). This shift may also affect the accumulation of deleterious mutations, but the effect can be difficult to predict, because of antagonistic effects (Arunkumar et al., 2015). On one hand, inbreeding increases homozygosity, exposing recessive deleterious mutations to natural selection (Lande and Schemske, 1985) and potentially leading to the purging of deleterious alleles (Charlesworth and Willis, 2009). On the other hand, inbreeding reduces both population size and effective recombination rates (Nordborg, 2000), thereby reducing the efficiency of selection and contributing to the retention and possible fixation of deleterious variants (Takebayashi and Morrell, 2001).

We have used forward simulations to examine the interplay between inbreeding and demographic (bottleneck) effects under parameters thought to be similar to those of *O. sativa* domestication. These simulations are unlikely to precisely mimic rice genome history, but they do offer some insight into the relative effects of evolutionary forces that may have shaped segregating variation in rice. Under our outcrossing model, a bottleneck

increases the MDAF, and positive selection increases it even further for both deleterious and synonymous variants (Figure 7B). However, the MDAF became progressively more different between site classes (Figure 7B). Under the selfing model, the MDAF of synonymous sites increased dramatically. The addition of a bottleneck and positive selection enriched the MDAF of deleterious variants, but not synonymous variants, such that the MDAF of synonymous and deleterious variants became more similar (Figure 7B).

To the extent that these are representative models, they suggest that the observed difference in MDAFs between *O. rufipogon* and domesticated rice may be influenced by selfing more than a bottleneck or positive selection. A fitting comparison is dog domestication, which has occurred in two stages: a population bottleneck associated with domestication ~15,000 years ago (Vonholdt et al., 2010) and inbreeding within the last few hundred years to produce modern breeds. In rice, we find that inbreeding may have had the larger effect, but we have also made myriad assumptions in our models. We have, for example, assumed that selfing was coincident with the domestication bottleneck, but we cannot know this with certainly, especially given the lengthy 'pre-domestication' of some crops (Purugganan and Fuller, 2009, Meyer et al., 2016). We have also made assumptions about population sizes, the timing of demographic events, recovery times from those events (Brandvain and Wright, 2016), dominance coefficients (h=5), and patterns of positive selection. In future, it will be important to vary these parameter values more widely to better understand their potential effects on crop diversity and particularly deleterious variants.

Caveats and Assumptions

We close with consideration of the caveats and assumptions of our analyses. While we have tried to overcome potential pitfalls by using multiple approaches (different datasets, SNP calling methods, dSNP predictors, and SS inference metrics), important

limitations remain. One is the potential for a reference bias, because the use of the *japonica* reference is expected to decrease the probability that a *japonica* variant (as opposed to an *indica* variant) returns a low PROVEAN or SIFT score (Lohmueller et al., 2008). We have adjusted for this bias by submitting the ancestral allele -- rather than the reference allele -- to annotation programs (Kono et al., 2016). Without this adjustment, a reference bias was patently obvious, because the SFS of *japonica* dSNPs lacked a high frequency peak, and the U-shape of tSNPs became commensurately more extreme. We cannot know that we have corrected completely for reference bias but do advocate caution when interpreting results from dSNP studies that make no attempt to correct for reference bias. The effect can be substantial.

Our treatment of reference bias requires accurate ancestral inferences. To date, most population genetic studies of Asian rice have relied on outgroup sequences from *O. meridionalis* e.g., (Caicedo et al., 2007, Gunther and Schmid, 2010), a species that diverged from *O. sativa* ~2 million years ago (Zhu and Ge, 2005). When we used *O. meridionalis* as the sole outgroup, we inferred a U-shaped SFS in wild *O. rufipogon*, which is suggestive of consistent parsimony misinference of the ancestral state (Keightley et al., 2016). We instead inferred ancestral states relative to a dataset of 93 accessions of African wild rice (*O. barthii*) (Wang et al., 2014). *O barthii* is closer phylogenetically to *O. sativa* than *O. meridonalis*, but *O. barthii* sequences form clades distinct from *O. sativa* (Zhu and Ge, 2005). Even so, we have found that ~10% of SNPs sites with minor allele frequencies > 5% are shared between African wild rice and Asian rice.

We do not believe that the use of *O. barthii* has distorted our primary inferences, for two reasons. First, systematic misinference of the ancestral state should lead to a U-shaped SFS, which is lacking from *O. rufipogon*. Instead, the U-shaped SFS is unique to *O. sativa* and differentiates wild from domesticated species. Second, we have confirmed our inferences by using *O. meridonalis* and *O. barthii* together as outgroups

(Keightley et al., 2016), considering only the sites where the two agree on the ancestral state. The use of two outgroups decreases the number of SNPs with ancestral states by ~10% and ~15% for the BH and 3K datasets, but all analyses based on these reduced SNP sets were qualitatively identical to those with only an *O. barthii* outgroup (e.g., Figure S19).

Finally, we focus briefly on the locations of SS regions identified by three different methods (Figure 5 and FiguresS7 to 17), which rarely overlapped (Table 3). In other words, the three methods identified almost completely independent regions of the rice genome. The lack of convergence among methods may reflect that different tests are designed to capture different signals of selection. However, the results are also sobering, because overlaps in SS regions have been used by a number of groups to argue for or against independent domestication of *indica* and *japonica* rice (He et al., 2011, Molina et al., 2011). Recently, both Huang et al. (2012b) and Civian et al. (2015) have argued for independent domestication events for *japonica* and *indica* based on the observation that there is little overlap in SS regions between the two taxa. [The Civian *et al*. (2015) analyses also have other critical flaws (Huang and Han, 2015).] The fact that we find little overlap among SS regions identified by different methods mirrors the lack of overlap of SS regions identified across the human genome by different studies (Akey, 2009), between domesticated grasses (Gaut, 2015), and between independent domestication events of common bean (Gaut, 2015). Because the inferred locations of SS regions vary markedly by method, sampling and taxon, they should be interpreted with caution, particularly as markers of independent domestication events.

MATERIALS AND METHODS

Sequence polymorphism data

All of the data used in this study are publicly available. Illumina paired-end reads for the BH and 3K dataset were downloaded from the European Nucleotide Archive (ENA;

http://www.ebi.ac.uk/ena) (see Tables S1 and S2 for accession numbers). The 3K accessions were chosen randomly among the total set of accessions with >12X coverage for an equal representation (*n*=15 for each set) of *indica*, tropical japonica and temperate *japonica* rice accessions. We also downloaded resequencing reads from *O. barthii* to polarize SNPs as either ancestral or derived. Sequencing reads for 93 *O. barthii* accessions (Wang et al., 2014) were obtained from the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/sra/) (see Table S3 for accession numbers). Sequencing reads for another outgroup taxon, *O. meridonalis* were obtained from NCBI (BioProject No: PRJNA264483) (Zhang et al., 2014).

Read alignment and SNP detection

Paired-end reads for *O. sativa* and *O. rufipogon* data were assessed for quality using FastQC V0.11.2, and then preprocessed to filter adapter contamination and low quality bases using Trimmomatic V0.32 (Bolger et al., 2014). The trimmed reads were mapped to the reference genome for *japonica* Nipponbare rice (MSU V7), which was downloaded from the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu). Mapping was performed with the ALN and SAMPE commands implemented in the software Burrows-Wheeler Aligner (BWA) V0.7.8 (Li and Durbin, 2010), using default parameters. All reads with a mapping quality score of < 30 were discarded.

The method of SNP calling varied with the dataset. For the BH data, alignment files from BWA mapping were processed further by removing PCR duplicates and by conducting indel realignments using Picard tools and GATK, and then used as input for ANGSD V0.901, which is designed to deal with sequences of low depth (Korneliussen et al., 2014). ANGSD was run with the command line:

```
angsd -b BAMLIST -anc OUTGROUP –out OUTFILE -remove_bads -uniqueOnly 1
-minMapQ 30 -minQ 20 -only_proper_pairs 1 -trim 0 -minInd NUMBER -P
```

```
CPUNUMBERS -setMinDepth 3 -setMaxDepth 15 -GL 1 -doSaf 1 -doMaf 2
-SNP_pval 1e-3 -doMajorMinor 1 -baq 1 –C 50 –ref REFSEQ
```

We considered only SNPs that had between 3X and 15X coverage, with the high-end implemented to avoid regions with copy number variation (Huang et al., 2012b). For SNP calling, we used only uniquely mapping reads, and bases with quality score of < 20 were removed. SNP sites with >50% missing data were discarded.

For the higher coverage '3K' dataset, we used SAMtools V1.2 (Li et al., 2009) and GATK V3.1 (McKenna et al., 2010) to call SNPs. After mapping reads of each accession onto the reference genome, alignments were merged and potential PCR duplications were removed using Picard tools V1.96 (http://sourceforge.net/projects/picard/files/picard-tools/1.96/). Unmapped and non-unique reads were filtered using SAMtools V1.2. We realigned reads near indels by using the IndelRealigner and BaseRecalibrator packages in GATK to minimize the number of mismatched bases. The resulting mapping alignments were used as input for UnifiedGenotyper package in GATK and for SAMtools. SNPs that were identified by both tools, with no missing data and a minimum phred-scaled confidence threshold of 50, were retained. Subsequently, SNP calls were further refined by using the VariantRecalibrator and ApplyRecalibration packages in GATK on the basis of two sets of "known" rice SNPs (9,713,967 and 2,593,842) that were downloaded from the dbSNP and SNP-Seek databases (Alexandrov et al., 2015). These same SNP detection methods were applied to the subset of 29 *O. rufipogon* with >4X coverage that were used as the diversity panel to infer SS regions (Table S1), although no prior variants were available.

Finally, sequence reads for the outgroup dataset were aligned to the reference genome using stampy V1.0.21 (Lunter and Goodson, 2011), and then a pseudo-ancestral genome sequence was created using ANGSD (Korneliussen et al., 2014) with the parameters "-doFasta 2 -doCounts 1". This pseudo-ancestral genome was used to determine the ancestral state of each SNP in *O. sativa* and *O. rufipogon*.

SNP Annotation and Deleterious Mutation Prediction

SNPs were annotated using the latest version of ANNOVAR (Wang et al., 2010) relative to the *japonica* reference genome (MSU v 7.0). SNPs were annotated as synonymous, nonsynonymous, intergenic, splicing, stop-gain and stop-loss related. Throughout the study, we combined SNPs that contribute to splicing variation, stop-gain and stop-loss and called them loss-of-function (LoF) mutations.

To discriminate putatively deleterious nSNPs from tolerant nSNPs, nSNPs were predicted as deleterious or tolerated using PROVEAN V1.1.5 against a search of the NCBI nr protein database (Choi et al., 2012). To reduce the effects of reference bias, predictions of deleterious variants were inferred using the ancestral (rather than the reference) variant. Following previous convention (Renaut and Rieseberg, 2015), we considered an nSNP to be a deleterious dSNP if it had a PROVEAN score ≤ -2.5 and a tolerant tSNP when a PROVEAN score was > -2.5. To assess consistency, we also employed SIFT (Kumar et al., 2009) to predict nSNPs as dSNPs or tSNPs. For these analyses, a nSNP was defined as a dSNP if it had a normalized probability < 0.05, and an nSNP was predicted to be a tSNP with a SIFT score ≥ 0.05.

Calculating site frequency spectra

Following Huang et al. (2012b), we separated the BH dataset of 1,212 accessions into five populations: *indica*, *japonica* (mostly temperate) and three *O. rufipogon* subpopulations (W_I, W_{II}, and W_{III}). The five subpopulations were composed of 436, 330, 155, 121, and 170 individuals, respectively (Table S1).

To calculate the site frequency spectrum (SFS) for BH subpopulations, we initially projected the sample size of all five subpopulations to that smallest W_{II} population of n=121. However, many of the 121 accessions had low sequencing depth and high levels of missing data. We therefore focused on the W_{II} population to find criteria suitable for

inclusion. Ultimately, we sought to retain ≥ 90% of SNP sites within each SNP category, which resulted in a sample size of n = 70 for the W_{II} population. Accordingly, we randomly sampled n = 70 individuals from the remaining four subpopulations, so long as the sample retained ≥ 90% of SNP sites for each category, to mimic the W_{II} sample.

Given a sample of n = 70 for each of the five subpopulations, the SFS for each subpopulation was calculated using the formula proposed by (Nielsen et al., 2005), where the *O. barthii* sequence was used as an outgroup to determine the polarity of the mutations.

$$p_{i,70} = k^{-1} \sum_{j=1}^{k} \frac{\binom{f_j}{i}\binom{n_j - f_j}{70 - i}}{\binom{n_j}{70}} \quad (1)$$

In this formula (1), $p_{i,70}$ represents the probability of the derived allele frequency (DAF) of SNPs found in i individuals in a sample size of 70; k is the total number of SNPs in the dataset; n_j and f_j are the sample size and the number of derived alleles of the jth SNP, respectively. The SFS for the 3K data were calculated by focusing on a common set of SNPs that had no missing data and that were segregating in the total population of n=60 individuals. The SFS for sSNPs, tSNPs, dSNPs and LoF SNPs were compared with the Kolmogorov-Smirnov test, based on proportions of SNPs at different frequencies.

$R_{A/B}$ - A relative measure of dSNPs frequency enhancement

We adopted a metric to assess the accumulation of deleterious variants in either cultivated or wild rice populations (Xue et al., 2015). In this analysis, the statistic $L_{A,B}(C)$ compares two populations (A and B) within a given particular category, C, of SNP sites (e.g., dSNPs). It was calculated by counting the derived alleles found at specific sites in population A rather than B and then normalized by the same metric calculated in synonymous sites (S). The calculation of $L_{A,B}(C)$ was:

$$L_{A,B}(C) = \frac{\sum_{i \in C} f_i^A (1 - f_i^B)}{\sum_{j \in S} f_j^A (1 - f_j^B)} \quad (2)$$

where f_i^A and f_i^B are the observed derived allele frequency at each site i in populations A and B, respectively, and S refers to sSNPs. The ratio $R_{A/B}(C) = L_{A,B}(C) / L_{B,A}(C)$ then measures the relative number of derived alleles that occur more often in population A than that in population B. To obtain the standard errors of $R_{A/B}(C)$ we used the weighted-block jackknife method (HR, 1989), where each of the tested SNP datasets was divided into 50 contiguous blocks and then the $R_{A/B}(C)$ values were recomputed. A P value was assigned by using a Z score assuming a normal distribution (Do et al., 2015).

Calculation of recombination rate

The high-density rice genetic map was downloaded from http://rgp.dna.affrc.go.jp/E/publicdata/geneticmap2000/index.html, on which a total of 3,267 EST markers were anchored. We extracted the sequences of these markers from the dbEST database in NCBI, which were used as query to perform a BLAST search against the rice genome sequences (MSU V7) to annotate their physical positions. Finally, we normalized the recombination rate to centiMorgans (cM) per 100kb between different markers, and then calculated the average recombination rate in 3 or 2MB window segments for the BH and 3K datasets.

Identification of selective sweep regions

Both SweeD (Pavlidis et al., 2013) and XP-CLR (Chen et al., 2010) were used for identifying selective sweep (SS) regions separately in *indica* and *japonica* populations. SweeD was used with a sliding window size of 10kb, and the *O. barthii* genome sequence (Zhang et al., 2014) was used as an outgroup to determine whether alleles were ancestral or derived. XP-CLR was applied to the 3K datasets along with a subset of 29 *O.*

rufipogon individuals that had > 4X coverage and for which we could infer explicit genotypes (Table S1). Both packages were applied with 5% cutoffs to define putative sweep regions.

We calculated the percentage of genes overlapping between two sets of SS regions, defined as:

```
Overlap%= number of genes in common/ ((number of genes in the first set of SS regions + number of genes in the second set of SS regions)-number of genes in common))*100
```

Forward simulations and MDAF

We conducted forward simulations using the software SLiM V1.8 (Messer, 2013). SLiM includes both selection and linkage in a Wright–Fisher model with non-overlapping generations. Similar to previous demographic studies of Asian rice domestication (Caicedo et al., 2007), we simulated a population of $N = 10{,}000$ individuals, which were run for $10N$ generations to reach equilibrium. We then introduced a domestication bottleneck of size $N_b/N = 0.01$ at generation $11N$ until generation $15N$, when the population size recovered to size N. For the selfing populations, the population switched from outcrossing to total inbreeding (inbreeding coefficient $F = 1$) at the beginning of the domestication bottleneck.

All simulations assumed a constant mutation rate ($\mu = 6.5 \times 10^{-9}$ substitutions per site per generation) (Gaut et al., 1996) and recombination rate ($\rho = 4 \times 10^{-8}$ recombinants per generation) (Gaut et al., 2007) across a single chromosome of 100 Mb with alternating 400 bp of noncoding and 200 bp of coding DNA. All noncoding and 75% of coding sequences were selectively neutral ($s = 0$). The remaining 25% of coding sequences were under negative selection under an additive model, with s following a gamma distribution with shape parameter 0.3 and mean -0.05. This DFE was taken from another study of plant mating system (Arunkumar et al., 2015), but we also estimated the DFE of *O.*

rufipogon empirically using dfe-alpha-release-2.15 (Eyre-Walker and Keightley, 2009) and the unfolded SFS of the W_{15} sample. The estimated DFE for wild rice was nearly identical to that from Arunkumar et al. (2015), because *s* had an estimated shape parameter of 0.28 (95% CI: 0.25 to 0.31) and a mean of -0.048 (95% CI:-0.055 to -0.043). Given the similarities between the estimated and assumed DFE, we performed simulations using only the DFE from Arunkumar et al. (2015).

For the inbreeding model without a bottleneck, we followed the method of (Arunkumar et al., 2015) to adjust population size after the outcrossing-selfing transition by calculating the reduction in silent genetic diversity ($\theta_w = 4N_e\mu$, where θ_w is genetic diversity, N_e is effective population size and μ is mutation rate). This makes the measures equivalent and the simulations comparable between the inbreeding and outcrossing models that do not include a population bottleneck or positive selection (i.e, out vs. int; Figure 7B).

For the simulations with positive selection, we introduced 20 predetermined mutations with *s* drawn from an exponential distribution of mean 0.05 at the beginning of domestication. Positive selection was applied throughout the entirety of the population simulation, not only during domestication. For all mutations under positive or negative selection, we assumed a dominance coefficient $h = 0.5$ (i.e., an additive model).

The results for each model were summarized over 20 separate runs of SLiM; the SLiM input is available as Supplementary Text. The MDAF was calculated for simulated data sets and the 3K data as the sum of frequency of derived alleles across sites divided by twice the total number of (segregating sites + fixed sites). Note that this definition varies from that of Simons et al (2014) by not including the zero class, but it allows our simulated and empirical results to be compared directly.

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Table 1: The number and category of SNPs identified from different datasets

Sample	*n*	ncSNP[1]	sSNP[1]	dSNP[1]	tSNP[1]	LoF[1]	$\bar{\pi}$(sSNP)[2]	$\bar{\pi}$(dSNP)[2]
BH data								
indica	436	319,679	21,063	7,506	22,295	1,254	0.2014	0.1848
japonica	330	201,829	11,120	4,530	12,928	780	0.1641	0.1517
W_I	155	1,446,653	67,971	20,410	59,793	4,430	0.1717	0.1523
W_{II}	121	3,366,795	110,967	32,352	92,162	6,673	0.1602	0.1191
W_{III}	170	2,911,717	124,325	34,092	101,428	7,214	0.1391	0.1170
3K data								
indica	15	2,958,247	92,045	21,085	80,643	5,319	0.2944	0.2830
japonica temperate	15	2,424,551	74,749	17,660	66,619	4,282	0.2587	0.2537
japonica tropical	15	2,549,598	78,634	18,681	70,570	4,527	0.3323	0.3187
W_{15}	15	1,752,998	69,399	17,434	54,703	3,373	0.2956	0.2459

[1] ncSNP=non-coding; sSNP=synonymous; dSNP=deleterious; tSNP=tolerated; LoF = Loss of Function. dSNPs were predicted with PROVEAN.

[2] $\bar{\pi}$ is the average of π, the average number of pairwise differences, across SNP sites

Table 2: Correlation coefficients comparing rice diversity statistics to recombination rate (cM/100kb)

	$\pi_{(sSNPs)}$[1]		$\pi_{(dSNPs)}$		$\pi_{(dSNPs)}/\pi_{(sSNPs)}$	
Data Set	r^2	*p*-value[2]	*r*	*p*-value	*r*	*p*-value
BH *indica*	0.364	$2.35e^{-5}$	0.353	$4.26e^{-5}$	-0.098	0.271
BH *japonica*	0.199	$2.45e^{-2}$	0.214	$1.51e^{-2}$	-0.103	0.246
3K *indica*	0.397	$1.28e^{-8}$	0.353	$5.29e^{-7}$	-0.263	$2.33e^{-4}$
3K *japonica* (temperate)	0.310	$1.32e^{-5}$	0.272	$1.39e^{-4}$	-0.249	$5.04e^{-4}$
3K *japonica* (tropical)	0.376	$8.17e^{-8}$	0.308	$1.45e^{-5}$	-0.303	$1.98e^{-5}$

[1] $\pi_{(sSNPs)}$, $\pi_{(dSNPs)}$ and their ratio were calculated in non-overlapping 3Mb windows for the BH dataset and 2Mb windows for the 3K dataset.

[2] *r* is the Spearman correlation coefficient, with corresponding *p*-value.

Table 3: The number and percentage of SS regions identified by different methods, based on 3K data.

	indica		***japonica* (temperate)**		***japonica* (tropical)**	
	No.	**Extent[2]**	**No.**	**Extent[2]**	**No.**	**Extent[2]**
Huang et al (2012b)	84[1]	9.98%	103[3]	15.32%	103[3]	15.32%
SweeD	485	4.61%	461	4.76%	389	4.81%
XP-CLR	161	5.02%	160	8.41%	171	5.62%

[1] Based on 60 SS regions identified as specific to *indica,* which overlapped with 31 of 55 regions identified in the combined samples of *indica* and *japonica* rice, for a total of [60+(55-31)]=84.

[2] Extent = the percentage of the reference genome covered by SS regions.

[3] Based on 62 SS regions identified as specific to *japonica,* which overlapped with 14 of 55 regions identified in the combined samples of *indica* and *japonica* rice, for a total of [62+(55-14)]=103.

FIGURE LEGENDS:

Figure 1: The site frequency spectrum (SFS) for cultivated rice and *O. rufipogon*, based on BH data. The top row represents sSNPs, and the bottom row represents dSNPs. Additional SNP classes are graphed in Figure S1. The two columns represent *indica* rice on the left and *japonica* rice on the right. As per Huang et al (2012b), *indica* rice is contrasted to the accessions from wild population I (W_I) and *japonica* rice is contrasted to wild sample population III (W_{III}). The Density on the y-axis is the proportion of alleles in a given allele frequency. Each graph reports the *p*-value of the contrast in SFS between cultivated and wild samples.

Figure 2: The SFS for cultivated rice and *O. rufipogon*, based on 3K data for the indica and tropical *japonica* samples. The top row represents sSNPs, and the bottom row represents dSNPs. Additional SNP classes are graphed in Figure S2. The two columns represent *indica* rice on the left and tropical *japonica* rice on the right; temperate *japonica* is included in Figure S2.

Figure 3: Comparisons of the number of derived dSNP to sSNP between wild and cultivated samples based on their frequencies. The top row reports results based on the BH data. From left to right, the panels represent: *left*) the ratio of the number of dNSPs to sSNPs (y-axis) at each derived allele frequency (x-axis) between *indica* rice and the W_I sample; *middle*) the ratio of the number of dSNPs to sSNPs (y-axis) at each derived allele frequency (x-axis) between between *japonica* rice and the W_{III} sample and *right*) a measure $R_{(A/B)}$ of the relative accumulation of SNPs in *indica* or *japonica* rice compared to *O. rufipogon*, where a value > 1.0 indicates an increased population density of that SNP type relative to wild rice. Bars indicate standard errors. The bottom row reports the 3K data, and the three panels (left to right) are equivalent to those from the BH data.

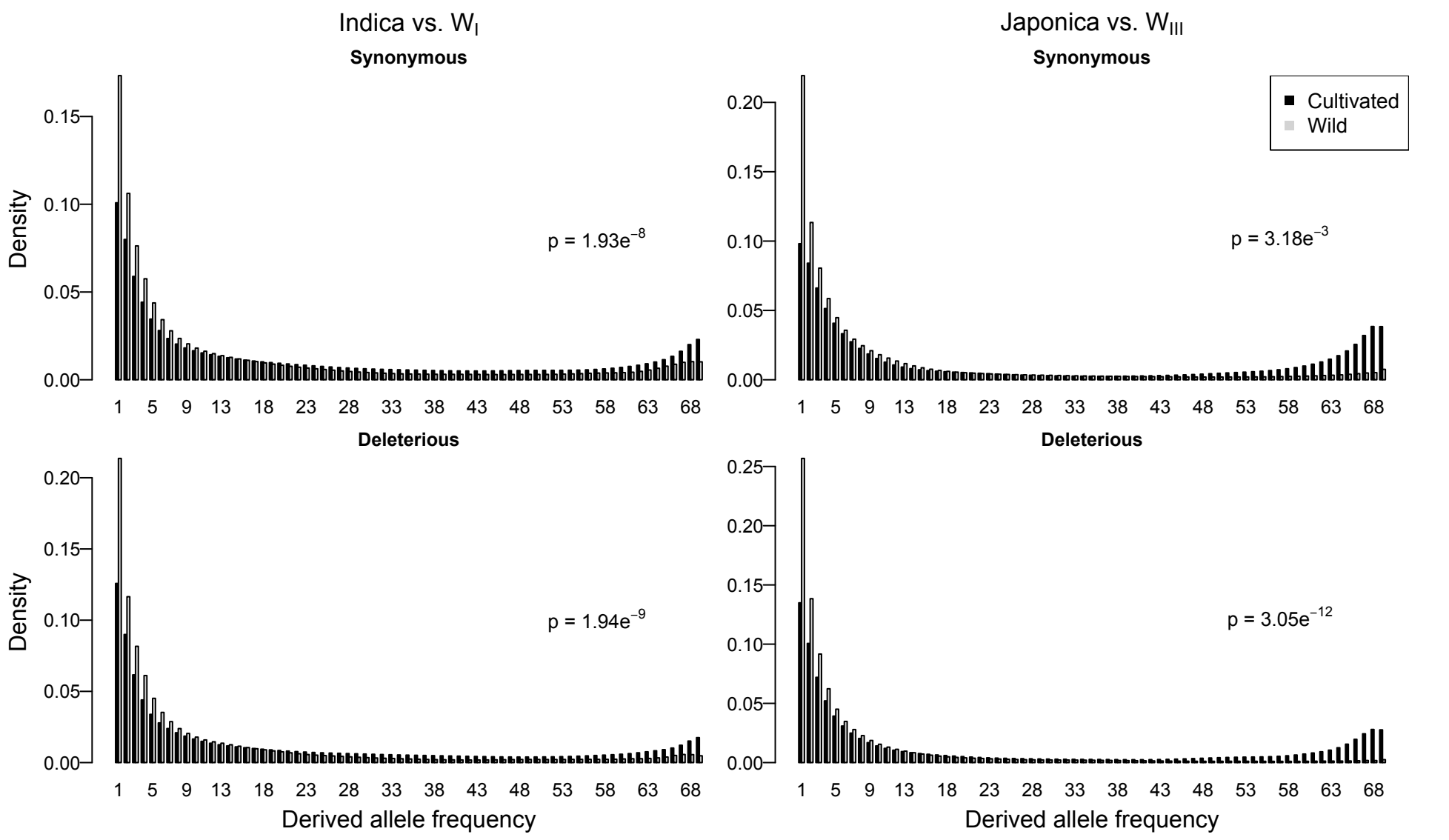
Figure 4: Patterns of genomic variation relative to recombination, based on the BH dataset. The x-axis for each graph is the recombination rate (x-axis) as measured by centiMorgans (cM) per 100 kb. The y-axis varies by row. The top row is the diversity of dSNPs, as measured by π in 3Mb windows; the middle row is the diversity of sSNPs in 3Mb windows; and the bottom row is the ratio of π for dSNPs to sSNPs in 3MB sliding windows. The *p*-values for correlations are provided in the plot, but also within Table 2. A similar figure for the 3K data is provided in Figure S6.

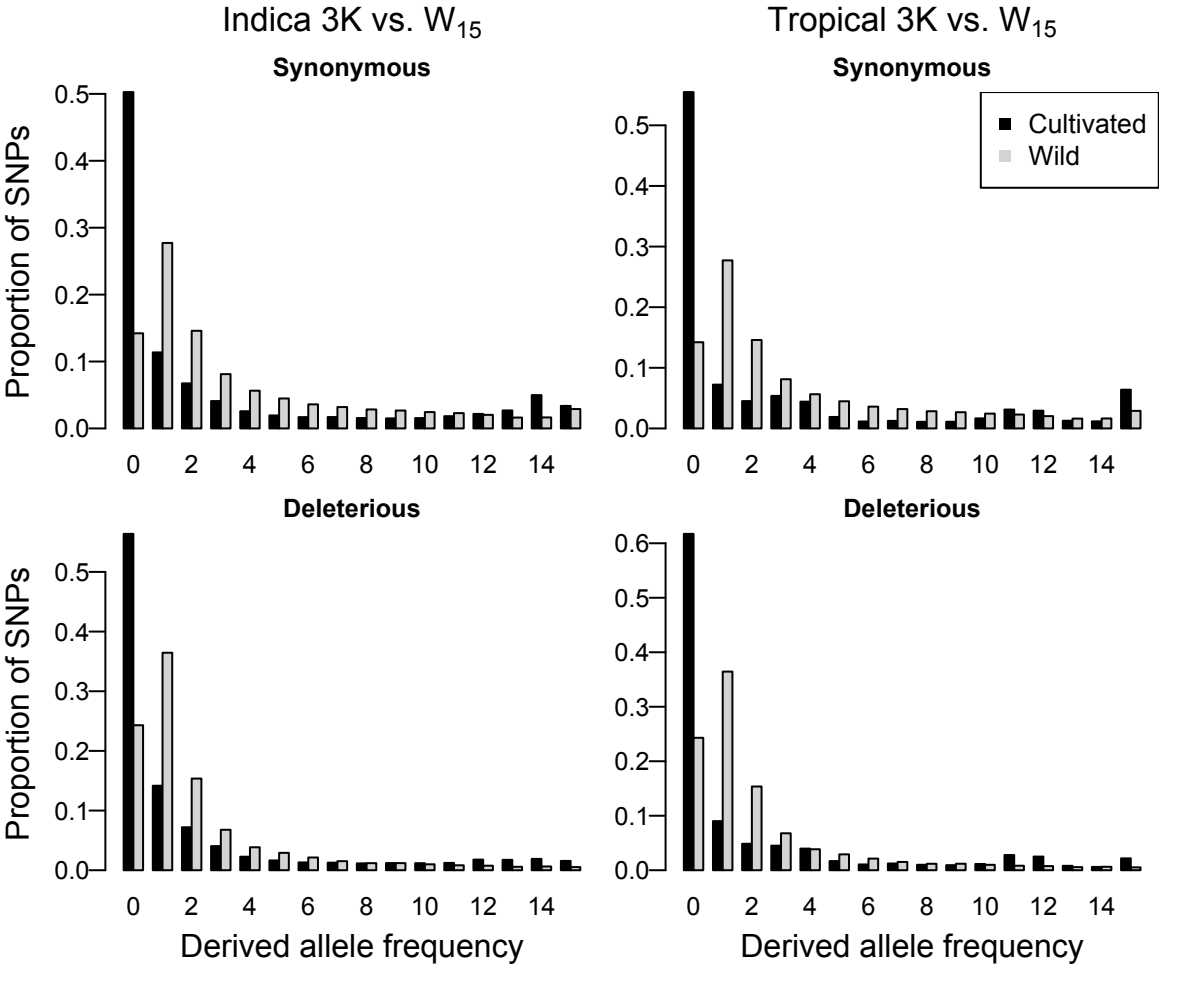
Figure 5: A graph of the location of inferred SS regions along Chromosome 1 for the 3K *indica* dataset. The *x*-axis is the location along the chromosome, measured in base pairs. The top graph (red) indicates the ratio of π for the *indica* accessions against a set of wild accessions. The (grey) background represents values for windows of 10kb with a step

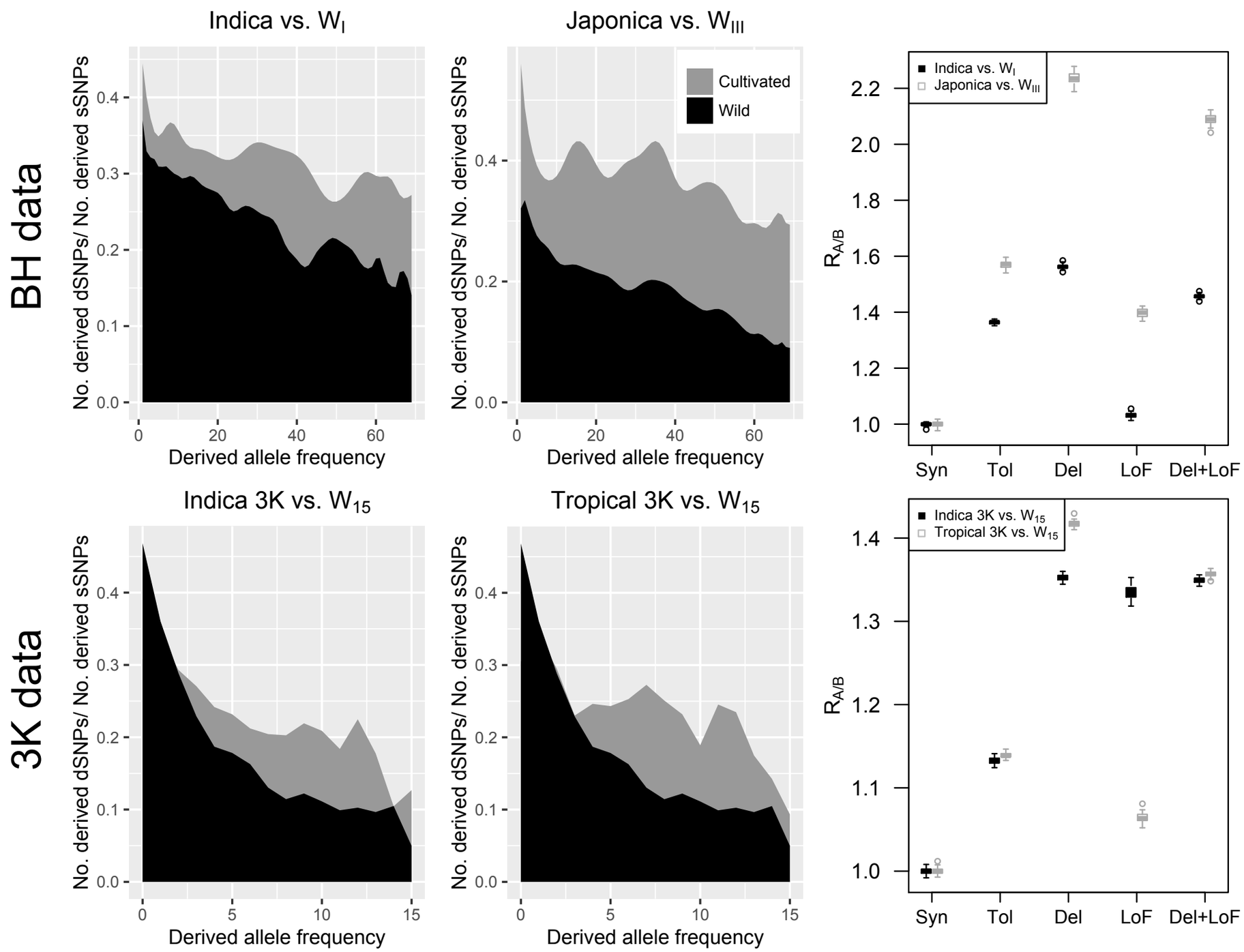
size of 1kb. Values > 2.0 were omitted for ease of presentation, and the line was smoothed. The middle graph shows values of π for the *indica* accessions. The bars at the bottom represent inferred SS regions using SweeD and XP-CLR, along with predefined SS regions (BH) defined by Huang et al. (2012b). The red and blue colors are included to help differentiate SS regions; the orange bars represent additional SS regions defined by Huang et al. (2012b) on the basis of their combined *indica*+*japonica* dataset. The width of each bar is proportional to the length of the corresponding SS region along chromosome. Similar graphs for chromosomes 2 through 12 are available as supplemental figures (Figures S7 to S17).

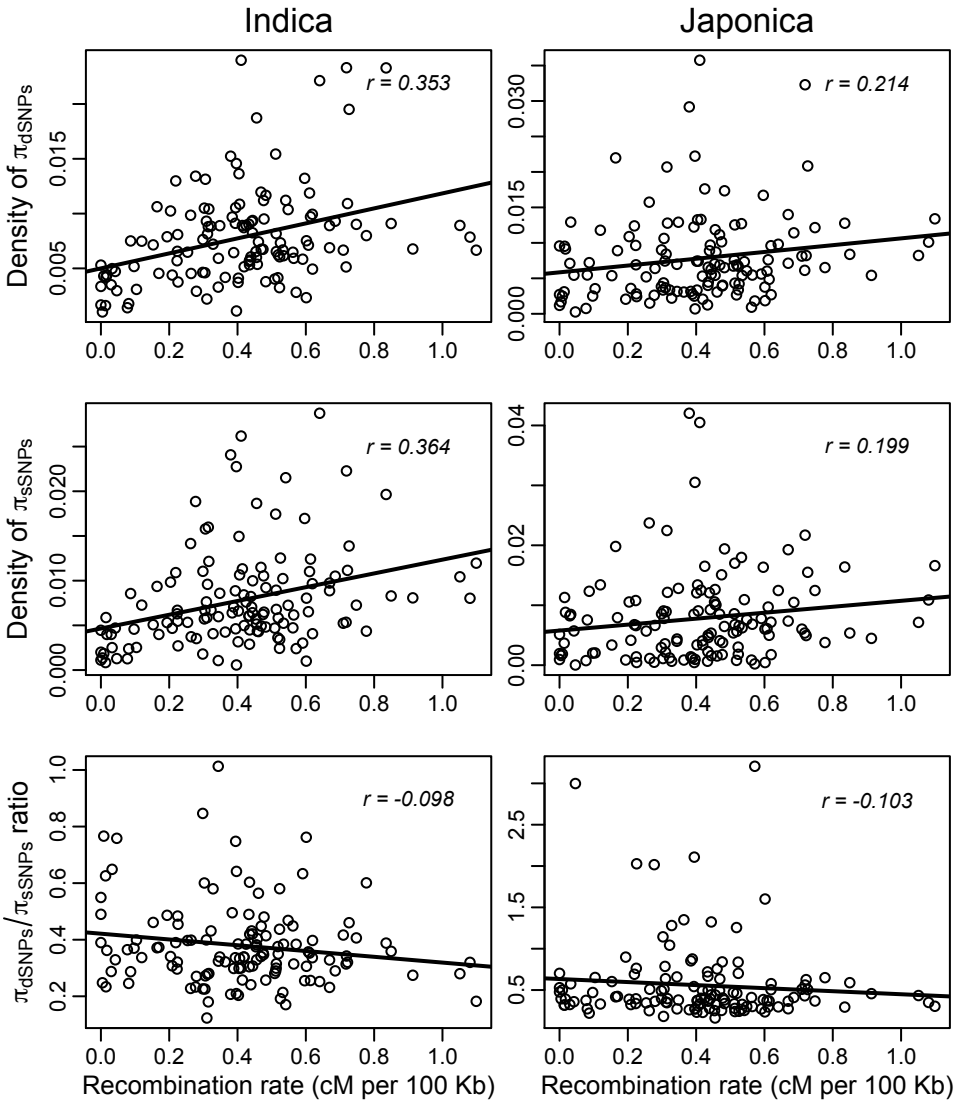
Figure 6: A comparison between selective sweep (SS) and non-SS regions based on the *indica* 3K dataset. The rows correspond to different methods employed to detect sweeps, including SS regions from Huang et al (2012b) (top row), SweeD (middle row), and XP-CLR (bottom row). The set of histograms on the right compare the derived allele count (DAC) of segregating or fixed synonymous site or putatively deleterious sites between SS regions and the remainder of the genome (non-SS regions).

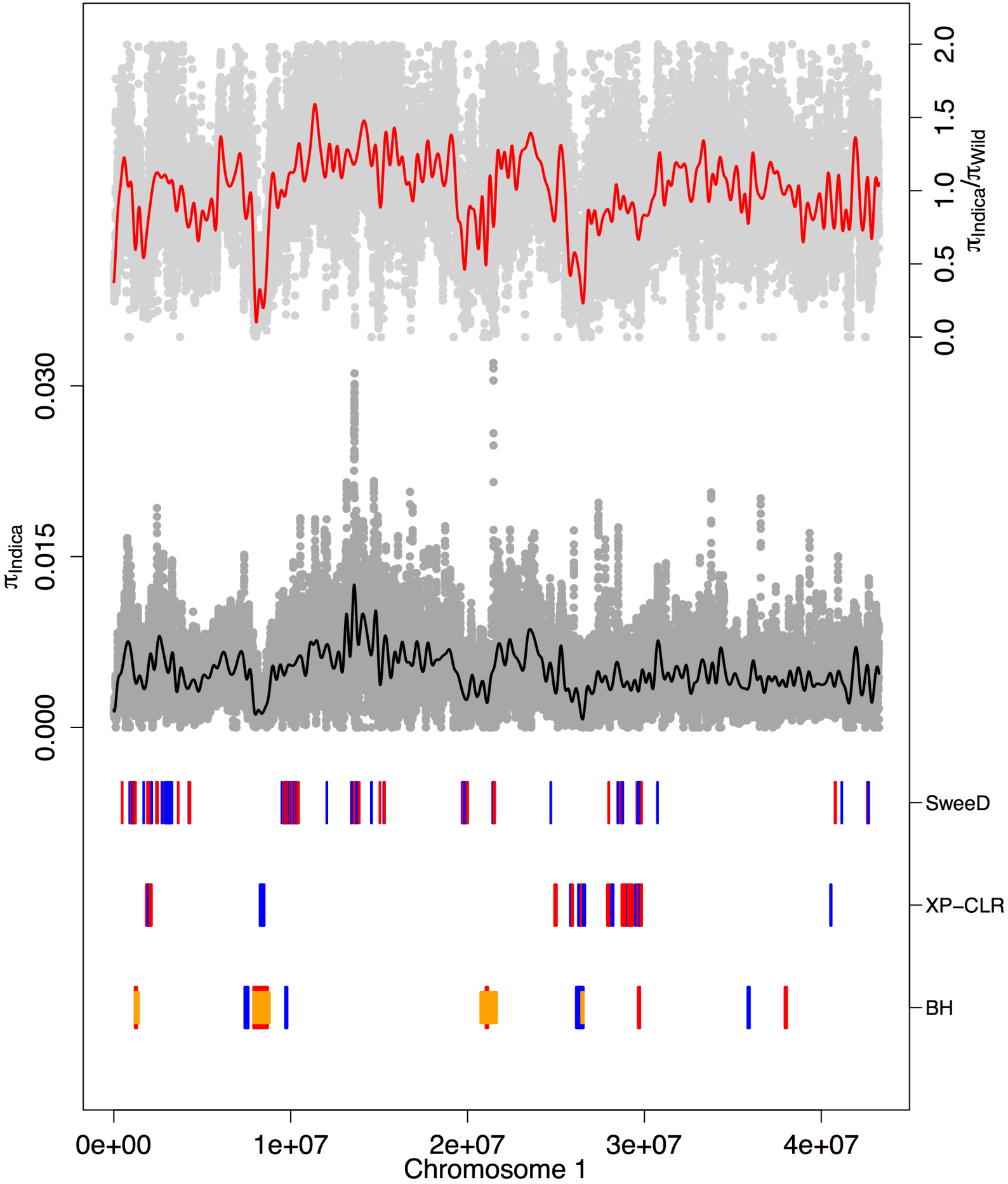
Figure 7: Mean derived allele frequency (MDAF) of empirical (A) and simulated data (B). The x-axis in Fig. 7B defines the six models; 'out' represents an outbred (random mating) population with a constant population size; 'inb' represents an inbred (selfing) population with a comparable population size; 'bot+out' and 'bot+inb' represents outbred and inbred populations with a domestication bottleneck; 'bot+out+pos' and 'bot+inb+pos' include positively selected alleles.

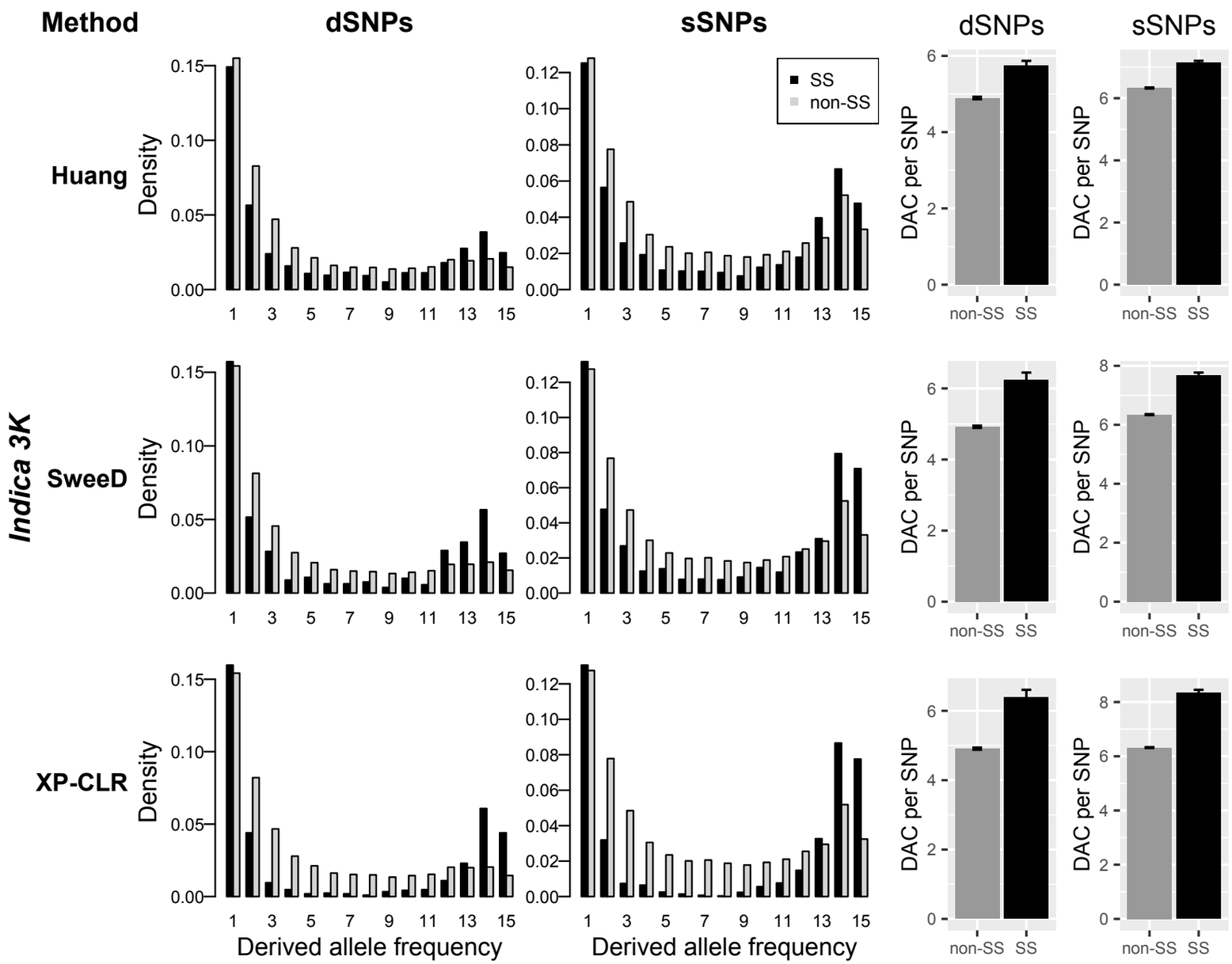

Indica vs. W_I
Japonica vs. W_{III}
Synonymous
Deleterious
Cultivated
Wild
Density
Derived allele frequency
$p = 1.93e^{-8}$
$p = 3.18e^{-3}$
$p = 1.94e^{-9}$
$p = 3.05e^{-12}$
0.00
0.05
0.10
0.15
0.20
0.25
1 5 9 13 18 23 28 33 38 43 48 53 58 63 68


Indica 3K vs. W15
Tropical 3K vs. W15
Synonymous
Synonymous
Deleterious
Deleterious
Cultivated
Wild
Proportion of SNPs
Proportion of SNPs
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
2
4
6
8
10
12
14
Derived allele frequency
Derived allele frequency

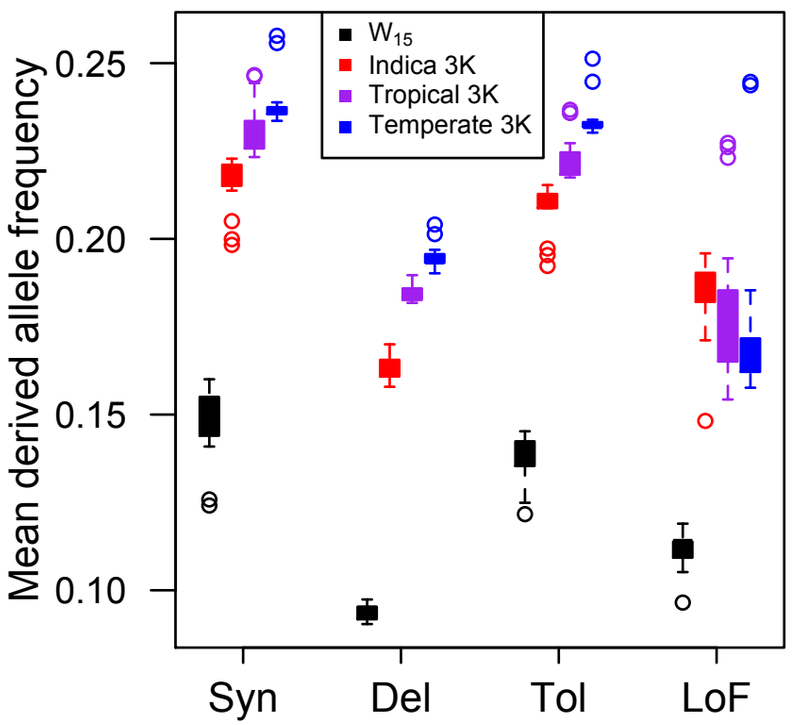
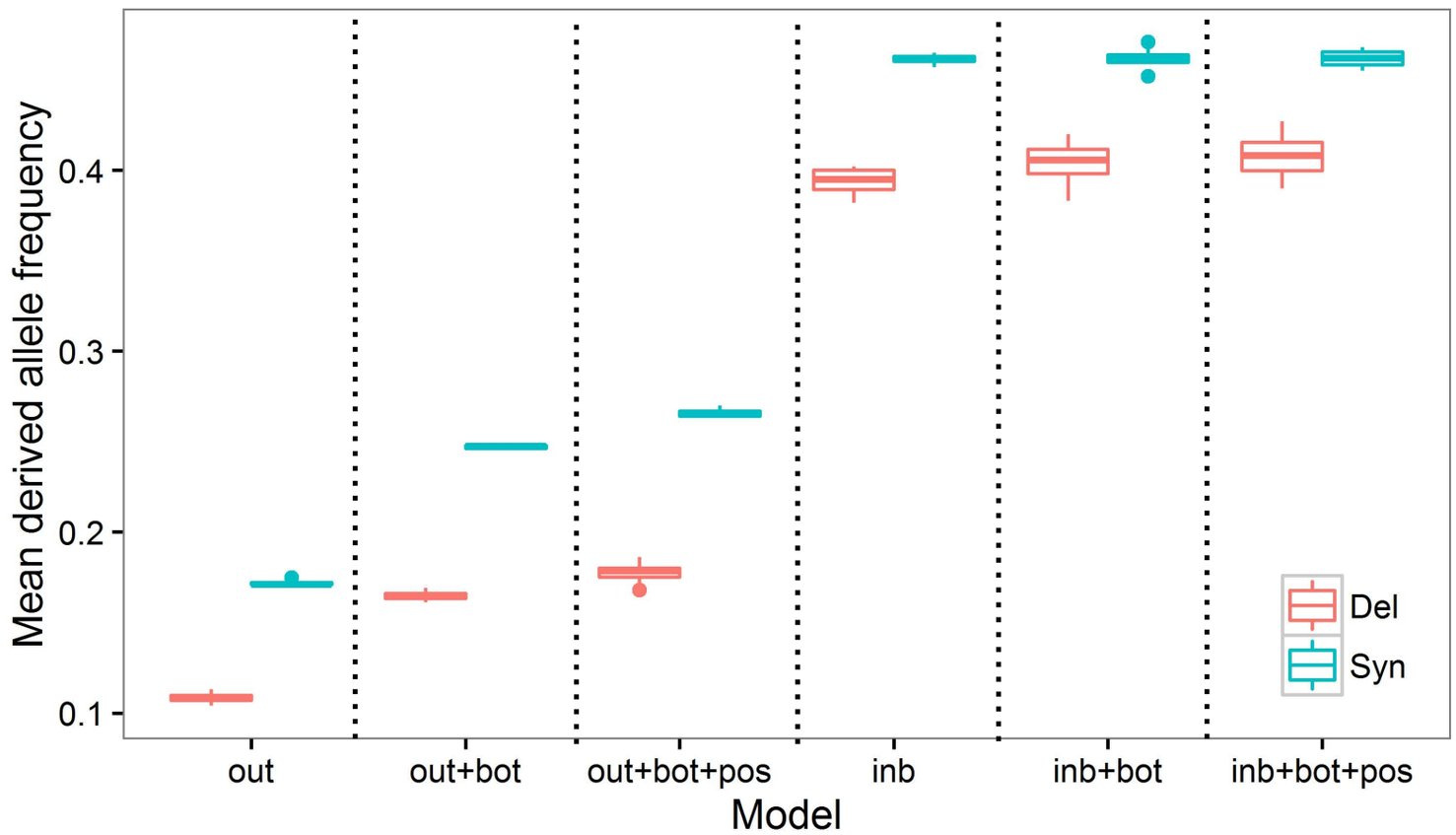

BH data
3K data
Indica vs. W_I
Japonica vs. W_{III}
Indica 3K vs. W_{15}
Tropical 3K vs. W_{15}
No. derived dSNPs/ No. derived sSNPs
Derived allele frequency
Cultivated
Wild
$R_{A/B}$
Syn
Tol
Del
LoF
Del+LoF


Indica
Japonica
Density of π_{dSNPs}
Density of π_{sSNPs}
π_{dSNPs}/π_{sSNPs} ratio
r = 0.353
r = 0.214
r = 0.364
r = 0.199
r = -0.098
r = -0.103
Recombination rate (cM per 100 Kb)
Recombination rate (cM per 100 Kb)


2.0
1.5
1.0
0.5
0.0
π_{Indica}/π_{Wild}
0.030
0.015
0.000
π_{Indica}
SweeD
XP−CLR
BH
0e+00
1e+07
2e+07
3e+07
4e+07
Chromosome 1


Method
dSNPs
sSNPs
dSNPs
sSNPs
Huang
SweeD
XP-CLR
Indica 3K
Density
SS
non-SS
DAC per SNP
non-SS
SS
Derived allele frequency

A. 3K data



B. Simulations