

The accumulation of deleterious mutations in rice genomes: a hypothesis on the cost of domestication

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The extent of molecular differentiation between domesticated animals or plants and their wild relatives is postulated to be small. The availability of the complete genome sequences of two subspecies of the Asian rice, *Oryza sativa* (indica and japonica) and their wild relatives have provided an unprecedented opportunity to study divergence following domestication. We observed significantly more amino acid substitutions during rice domestication than can be expected from a comparison among wild species. This excess is disproportionately larger for the more radical kinds of amino acid changes (e.g. Cys ↔ Tyr). We estimate that approximately a quarter of the amino acid differences between rice cultivars are deleterious, not accountable by the relaxation of selective constraints. This excess is negatively correlated with the rate of recombination, suggesting that ‘hitchhiking’ has occurred. We hypothesize that during domestication artificial selection increased the frequency of many deleterious mutations.

Introduction

How different are domesticated plants and animals from their wild progenitors at the molecular level? It is often postulated that only a few loci contribute to the process of domestication [1] and this has been corroborated in a few examples [2–9]. Therefore, the divergence at the molecular level might be relatively small, probably similar to the divergence between two individuals that are randomly sampled from the wild species.

This suggestion is based on the direct effect of artificial selection. However, domestication can also indirectly affect the genome by interfering with natural selection in two different ways. First, during domestication, there can be extensive relaxation of natural selection on characters that are important in the wild but not in cultivation [10]. Many mating characters are in this category, for example, the attraction of potential mates [11]. The reduction in the effective population size during

domestication can also contribute to the relaxation of selection [12,13].

Second, deleterious mutations can accumulate during domestication. It is common observation that nearly every domesticated breed of animal, or cultivar of plant, has a low reproductive fitness. The pleiotropic effects of genes selected for their desired traits by humans probably have a role in this fitness loss [14–16]. Nevertheless, we also wish to know how often deleterious mutations might hitchhike with genes that were selected by humans. Normally, the accumulation of deleterious mutations in sexually reproducing species is infrequent because recombination enables these mutations to be removed [17]. However, when the effect of recombination is reduced, the accumulation of deleterious mutations can be substantial, a good example being the degeneracy of the Y chromosome [18]. Although artificial selection by itself should not affect the crossover rate, the practice of inbreeding during domestication can reduce the effectiveness of crossover in breaking up linkage groups.

The complete genomic sequences of the two subspecies of the Asian rice, *Oryza sativa*, have made it possible to explore the dynamics of selection during domestication [19,20]. *O. sativa* comprises two major subspecies, *Oryza sativa* L. ssp. *indica* (referred to as indica) and *Oryza sativa* L. ssp. *japonica* (referred to as japonica). Unlike the single domestication event that occurred in maize [21], japonica and indica were thought to be domesticated separately from their ancestral species, *Oryza rufipogon* [22]. In addition to the many differences in physiological and morphological characters between the two subspecies, partial reproductive barriers in the form of hybrid sterility, hybrid inviability and segregation distortion also exist between them [22,23].

Two cultivar genomes have been sequenced: the 9311 strain of indica (referred to as Oi; [20,24]) and the Nipponbare strain of japonica (Oj; [19,25–28]). In addition, the wild rice *Oryza brachyantha* (Ob) has been sequenced extensively [29]. By analyzing the genomic sequences from rice cultivars and their wild relatives, we wished to know: (i) whether the rate of amino acid substitutions was increased during rice domestication; (ii) how much of the

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Table 1. The divergence between the domesticated- and wild-rice lineages^a

Comparison	Number of genes	A	S	$\bar{K}_a \times 100^b$	$\bar{K}_s \times 100^b$	\bar{K}_a/\bar{K}_s
All data						
Oj versus Oi	15 406	47 129	35 477	0.398 (0.002)	0.799 (0.003)	0.498
Oj versus Ob	4640	88 500	124 672	5.188 (0.017)	20.058 (0.058)	0.259
Lineage-specific divergence						
Oj	4182	1673	1477	0.129 (0.002)	0.299 (0.006)	0.431
Oi	4182	2349	1972	0.179 (0.003)	0.405 (0.007)	0.442
Ob	4182	65 413	94 331	4.977 (0.019)	19.705 (0.068)	0.253

^aAbbreviations: A, the number of nonsynonymous substitutions; Ob, *Oryza brachyantha*; Oi, *Oryza sativa* L. ssp. *Indica*; Oj, *Oryza sativa* L. ssp. *Japonica*; S, the number of synonymous substitutions.

^bStandard errors are given in parentheses.

excess, if any, might be due to the relaxation of selective constraints and how much might be advantageous or deleterious; and (iii) whether the accumulation of deleterious mutations in rice cultivars, if any, might be associated with artificial selection.

The phenomenon – an increase in nonsynonymous substitutions among cultivars

We obtained high-quality coding sequence (CDS) alignment for 15 406 genes between Oj and Oi and 4640 genes between Oj and Ob, after removing transposable element-related genes and those without matching EST sequences. The detailed sequence quality validation, sequence alignments, divergence calculations and statistical analyses are described in the supplementary material online. K_a and K_s are the number of nonsynonymous and synonymous substitutions per site, respectively. The genomic average of K_a/K_s between Oj and Oi is 0.498, much greater than the K_a/K_s of the domesticated and wild rice (0.259, Table 1). The difference is highly significant ($P < 0^{-10}$ by the χ^2 test; see supplementary material online). Our analyses of 4182 orthologous genes from Oj, Oi and Ob show the same pattern (Table 1, see supplementary material online).

A more direct comparison is to compare, for each orthologous gene, the K_a/K_s value between Oi and Oj and those between cultivars and Ob. Owing to the closeness

between Oi and Oj, K_a/K_s fluctuates wildly from gene to gene, often being 0 or infinity. Therefore, we grouped the 4182 orthologous genes in Oi, Oj and Ob by their physical proximity into 118 segments, each comprising 5000 codons. Figure 1 shows the K_a/K_s values of these 118 segments. 84.7% (100 out of 118) of these segments are above the 45° line, indicating that the increase in K_a/K_s in cultivars is widespread across the entire genome. This acceleration in protein evolution between rice cultivars is observable across all categories of molecular functions (Figure 1 in the supplementary material online).

It is important to know that the genome evolution of the particular strains, Oi (9311) and Oj (Nipponbare), chosen for whole genome sequencing do not deviate from the norm of their respective type (*indica* and *japonica*). Neither K_a nor K_s between Oi and Oj is statistically different from the mean statistics of multiple lines of *japonica* and *indica*, based on our results of sequencing 25 genes from 17 strains of cultivars and wild rice (paired-t test, for both cases, $P > 0.05$). Furthermore, the availability of large numbers of DNA sequences from two new rice cultivars (Guangluai 4 [25] and Kasalath [28]) has afforded us the opportunity to repeat the analysis of Table 1. In Table S1, we show that K_a/K_s is significantly greater in these two cultivars than in wild rice, similar to the patterns reported in Table 1. Oi and Oj are fairly representative of the two subspecies of domesticated rice.

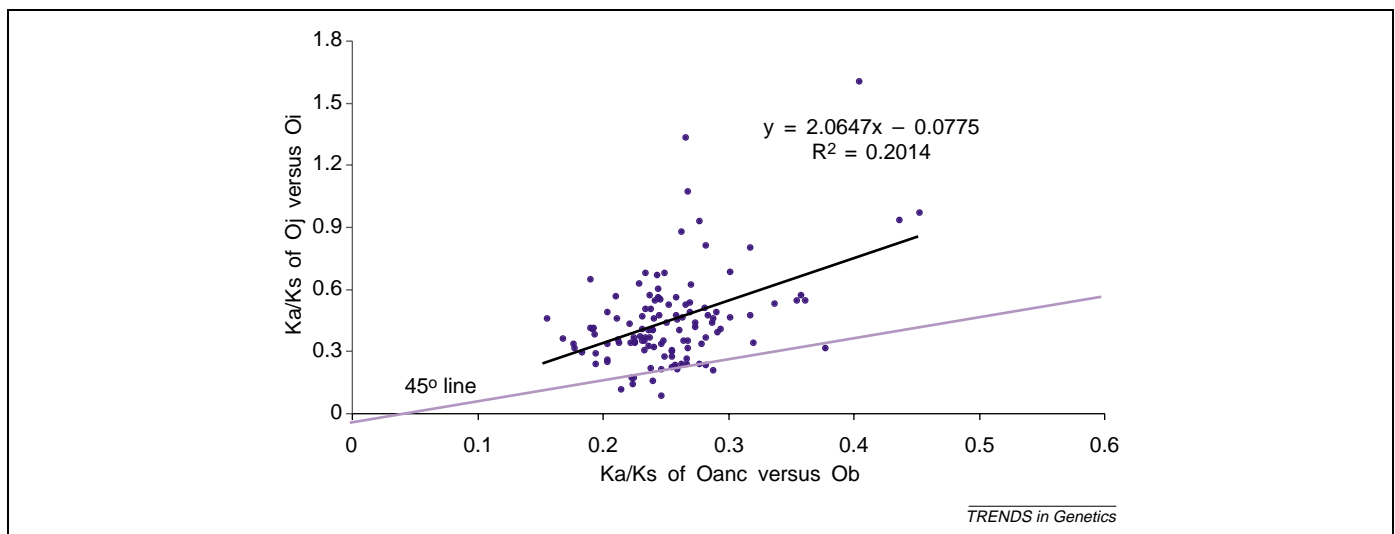


Figure 1. The K_a/K_s values from the comparison of the cultivars are plotted against the corresponding values from the comparison of the wild rice. Each point represents a contiguous DNA segment of ~5000 codons. Both the 45° line and the regression line are shown. Note that 100 (84.7%) of the 118 points are above the 45° line. Abbreviations: Oi: *Oryza sativa* L. ssp. *Indica*; Oj, *Oryza sativa* L. ssp. *Japonica*; Oanc, the inferred ancestral sequences of Oi and Oj.

The excess in nonsynonymous substitutions – are they neutral?

What forces might have driven the K_a/K_s ratio in the domesticated rice shown in Table 1 and Figure 1? Are these ‘extra’ amino acid substitutions mostly advantageous, neutral or deleterious? A most parsimonious explanation seems to be the relaxation of selective constraints. These extra nonsynonymous changes are effectively ‘neutral’ in the cultivars but deleterious in the wild rice. Changes in the environment and a reduction in the effective population size during domestication might contribute to the relaxation of selective constraints [12,13,30], which is a common occurrence among natural species as can be seen in the substantial variation in mean K_a/K_s among taxa [31].

We tested the hypothesis that the relaxation of selective constraints is the main cause in two different ways.

Neutrality test I (the effect of recombination)

If the excess in amino acid substitutions in cultivars is entirely due to the relaxation of selective constraints, the driving force should still be genetic drift and the excess would not depend on the recombination rate. We first determined the local recombination rates based on the integrated genetic and physical map of *O. sativa* (<http://www.tigr.org>). The genome is divided into 2-Mb segments. For each segment, we calculated the mean recombination rate and the mean K_a/K_s value between Oj and Oi (supplementary material online). As shown in Figure 2, the K_a/K_s value is negatively correlated with the mean recombination rate of that segment ($r = -0.192$, $P = 0.008$).

Because selection is generally less effective in regions of reduced recombination [32–37], the negative correlation in Figure 2 suggests that substitution of deleterious amino acids occurred during domestication. However, if the excess in amino acid substitutions is due largely to advantageous mutations, the correlation in Figure 2 should have been positive (i.e. more recombination resulting in more-effective selection and, hence, more advantageous amino acid substitutions [38]).

Neutrality test II (radical versus conservative amino acid changes)

When selective constraint is relaxed in nature, we can ask how the changes are distributed among amino acid types, ranging from the radical (e.g. Cys ↔ Tyr) to the

conservative (e.g. Val ↔ Ile). Box 1 presents the analysis using an evolutionary index (EI) for amino acid changes [39]. If all amino acid substitutions are neutral, the observed EIs are usually proportional to a universal measure, U (which can be considered the intrinsic relative exchangeability, determined by the amino acid properties). In other words, the regression line of EI over U should correlate and should pass near the point of origin.

The evolution of wild rice apparently follows this neutral pattern (Figure 3a), as do all other examples analyzed so far (Box 1). However, EIs from the Oi–Oj comparison yield a regression line against U that has a mild slope and intercepts the y-axis at 0.258 (Figure 3b). The amino acid exchangeability does not depend as much on the underlying physico-chemical properties of the amino acids between the two subspecies of domesticated rice in contrast to the situation in natural species. By extrapolation, even the extreme radical changes that should have an evolutionary exchangeability of <0.1 can have an EI value of ~0.3 between Oi and Oj.

Using the regression approach, it is possible to partition the excess in amino acid substitutions between Oj and Oi into two components – those that are neutral, attributable to the relaxation of selective constraint, and those that are non-neutral. We estimated that the relaxation of selective constraint in rice cultivars accounts for 16.3% of all amino acid differences between Oj and Oi (supplementary material online). Interestingly, 24.7% of the total is estimated to be non-neutral.

The excess in nonsynonymous substitutions – are they advantageous?

Approximately 25% of the amino acid changes between the two rice cultivars, which are biased towards the more radical kinds, cannot be accounted for by the relaxation of selective constraints. Although positive selection during domestication might have contributed to the increase in amino acid substitutions, it seems unlikely that artificial selection for desired mutations could directly affect so many nonsynonymous sites in the genome.

We used the likelihood ratio tests [40] to estimate positive selection in the domesticated rice (supplementary material online). We considered three possible scenarios: positive selection acting (i) only on Oj; (ii) only on Oi; and (iii) equally on both. Among the 118 orthologous fragments in Figure 1, we found that 55 (46.6%) bear the signature of positive selection in at least one domesticated rice lineage. In each segment, the proportion of amino acid substitutions that could be inferred to be under positive selection, however, averages to be <1%. Our estimation of the proportion of positively selected substitutions is compatible with a recent study that suggested 2–4% of the genes in the maize genome experienced artificial selection [41]. If positive selection is the predominant driving force for the excess in amino acid changes, we should have observed greater K_a/K_s on regions with greater recombination rates, contrary to the result of Figure 2.

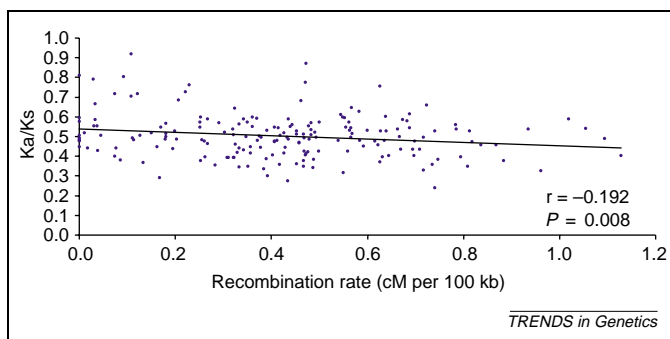


Figure 2. The K_a/K_s value for each 2-Mb fragment between Oj and Oi is negatively correlated with the average recombination rates of that fragment ($r = -0.192$, $P = 0.008$). Each dot represents the genes grouped in a fragment of 2 Mb.

Box 1. Evolutionary index (EI) of amino acid exchangeability, the universal EI (U) and the pattern of neutral substitution

The 'evolutionary index' (EI) for amino acid substitutions [39] is the equivalent of the K_a/K_s ratio for each pair of amino acids that have underlying codons that differ by only one base. There are 75 such 'elementary changes', for example, Leu↔Ile or Ser↔Pro. Between closely related species, EI can be accurately computed when many DNA sequences are available. Tang *et al.* [39] computed the EI values for two species of yeast, two species of *Drosophila*, mouse and rat, and human and the macaque monkey. The correlation coefficient between any two taxa (e.g. yeasts versus rodents) ranges between 0.8 and 0.9, although the mean EI for each data set is different from one another. Therefore, the set of 75 EI values between any pair of species is linearly correlated with a constant universal scale, U (see Table 2 of Ref. [39]), and the slope of the regression line is defined by the average EI (equivalent to \bar{K}_a/\bar{K}_s). The line almost always intercepts near the point of origin.

Tang *et al.*, [39], produced regression lines of EI over U for four different comparisons. We added a further example from human versus chimpanzee (Figure 2 in the supplementary material online). The comparison of orthologous sequences among different species of wild rice, for which we used *O. brachyantha* (Ob) and the ancestral sequences of Oj and Oi are most relevant to our current study (supplementary material online). Again, the regression of the observed EI over U has a strong correlation ($r^2=0.92$) and an intercept at 0.05 (Figure 3a). Thus, all six comparisons (four from Ref. [39], one in Figure S2a and one shown Figure 3a) behave similarly, despite different selective constraints.

Thus, if amino acid substitutions between species are neutral, the regression of EI on U should be linear and pass near the point of origin. Different taxa can have different degrees of selective constraint that will be reflected in the slope of the regression line.

The excess in nonsynonymous substitutions – are they deleterious, and at low frequency?

Although positive selection is an interesting scenario, it seems prudent to conclude that the excess in non-neutral changes is composed mainly of deleterious amino acid

changes. How could there be so many deleterious changes? A trivial possibility is that there is an accumulation of deleterious mutations before purifying selection. In other words, the K_a/K_s between Oj and Oi might largely be contributed by the polymorphism of deleterious mutations, which could not reach significant frequency or become fixed. If this is true we would expect to observe a large excess of amino acid mutations at low frequency in *O. sativa*.

To survey the frequency spectrum of the amino acid mutations in *O. sativa*, we sequenced 25 genes in seven strains of japonica and ten strains of indica (supplementary material online). Figure 4 shows the result of the survey. There is a large excess of amino acid polymorphisms at the frequency of 30–50% and the observed low-frequency polymorphisms are fewer than expected under the neutral equilibrium ($P<0.001$, χ^2 test). Low-frequency polymorphisms would have proportionately less effect on our analysis because they are usually not represented in the sample (only one strain from each subspecies was sampled). In addition, the comparisons between the genomes of two closely related species (human versus chimpanzee) or two strains of the same species

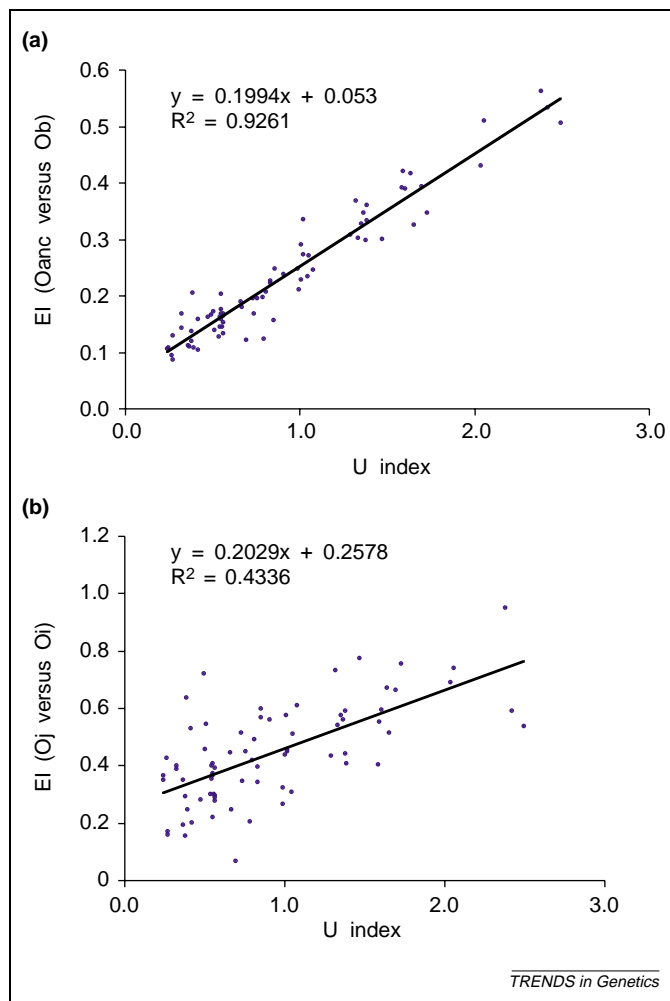


Figure 3. EI for amino acid changes in Ob, Oj and Oi. (a) EI versus U for all 75 elementary amino acid changes that we observed between Oanc and Ob. (b) EI versus U for all 75 elementary amino acid changes between Oj and Oi. For more details on EI, U and elementary changes, see Box 1. The regression line for each comparison is shown. Note that the regression line in Figure 2b does not intercept near the point of origin, unlike all other comparisons between natural species (see main text). Both comparisons are based on the same orthologous set of 4182 genes.

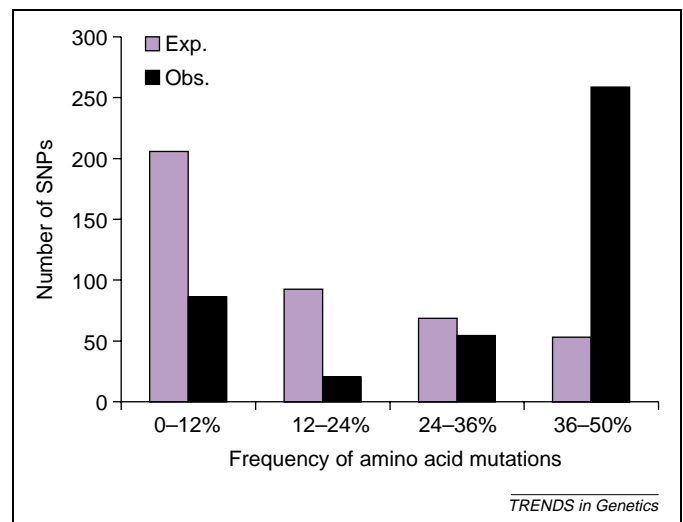


Figure 4. The observed (obs.) and expected (exp.) frequency spectrum of nonsynonymous single nucleotide polymorphisms in 17 lines of rice cultivars. There is a large excess of mutations at intermediate frequencies compared with the expected value under the neutral equilibrium ($P<0.001$, χ^2 test).

(*Drosophila simulans*) do not have a large excess in radical amino acid differences, as observed between Oj and Oi (Figure 3; Figure S2a in the supplementary material online).

After excluding various possible explanations, we suggest the excess in radical amino acid changes between Oi and Oj can best be accounted for by the accumulation of deleterious mutations at intermediate to increased frequency among rice cultivars.

A hypothesis on the accumulation of deleterious mutations – the domestication-associated Hill–Robertson (dHR) effect

We hypothesize that the accumulation of deleterious mutations we observed is due to domestication. When a desired genome is selected for propagation, all the mutations in that genome, beneficial or deleterious, can also propagate. Artificial selection therefore interferes with natural selection. The mutual interference between natural and artificial selection is a manifestation of the Hill–Robertson effect [33], which states that, in general, selection is most effective when variants freely recombine. We refer to this special form of interference as the dHR effect (for domestication-associated Hill–Robertson effect). The two main factors of the dHR effect are:

(i) Recombination – effective recombination can be strongly curtailed during the process of domestication, not because the crossover rate would be reduced but because inbreeding reduces the efficacy of crossover in recombining the genomes. It seems plausible that breeders would avoid diluting the good characters by intense inbreeding or selfing whenever possible. Intercrosses to combine desired characters from different strains are certainly practiced regularly; but even in those cases, selection with little intercrosses must be the norm once the right combination of characters has been assembled. In crops that are capable of selfing, reduction in recombination during domestication can be particularly strong. (Rice cultivars are selfers, whereas their wild relatives generally have an elevated outcrossing rate [22]).

(ii) Selection and hitchhiking – the dHR effect has a strong tendency to increase the frequency of deleterious mutations through hitchhiking because the intensity of artificial selection is often extreme. For example, breeders can pick a single specimen with desired characters that might not be fit in nature, and have it multiplied to its reproductive capacity over generations. Strong artificial selection could facilitate hitchhiking of deleterious mutations. Furthermore, when breeders desired a particular trait in a line and propagated it, all deleterious mutations in that line should have had a comparable chance of hitchhiking to increased frequency. Hence, strongly constrained mutations (usually those causing radical amino acid changes) would receive a greater boost in their fixation probability than those weakly constrained during domestication (Figure S5 in supplementary material online).

By contrast, natural examples for the accumulation of deleterious mutations, including Y-chromosome degeneracy [18] and fitness loss in asexual populations [42], can involve different mechanisms. Hitchhiking of the

deleterious with the advantageous mutations might not be prevalent in nature for two reasons [43]. First, the advantage has to be stronger than the combined effect of the deleterious mutations. Second, in the absence of recombination, most chromosomes in a population will probably be the ‘least-loaded’ kind. Thus, when an advantageous mutation emerges, it is likely to be on a genome with few deleterious mutations. Neither limitation applies to hitchhiking during domestication. In domestication, the intensity of artificial selection can often be sufficiently strong to overwhelm the deleterious effects. Furthermore, there should be a ‘normal’ amount of deleterious mutations common to sexual species at the onset of artificial selection. For natural systems, it might be the reduction in population size driving Muller’s ratchet forward [44] that explains the fitness loss.

Caveats to the dHR hypothesis

There are several caveats that apply to the dHR hypothesis. (i) Where did these deleterious mutations come from? The history of domestication is far too short for the genome-wide accumulation of *de novo* mutations. Hence, deleterious mutations have to arise from the polymorphisms in the wild rice. But how are so many deleterious polymorphisms swept to increased frequency by artificial selection? It seems necessary to have multiple cycles of artificial selection during domestication. In each cycle of selection and hitchhiking, new desirable traits, and many deleterious mutations, must have been introduced by the mixing of lines. The process, repeated many times, might have resulted in the accumulation of deleterious mutations in many domesticated breeds. (ii) Can the increase in nonsynonymous substitutions be attributed solely to inbreeding? Under strict neutrality, inbreeding, similar to haploidy, does not lead to a reduction in effective population size. The main effect of inbreeding is through the reduction of effective recombination that subsequently interferes with selection. By itself, inbreeding does not elevate the rate of amino acid substitution. There is no evidence for elevated K_a/K_s values in *Arabidopsis thaliana*, a selfer, in comparison with the outcrossing *Arabidopsis lyrata* [45]. (iii) Severe bottleneck during domestication: if the bottleneck is sufficiently severe, it is possible that selective constraints would be grossly relaxed, enabling even radical changes to become numerous. Nevertheless, this explanation does not apply to rice cultivars because their genetic diversity is not much lower than that of their presumed wild progenitor, *O. rufipogon* [46]. At certain loci, cultivars are much more variable than *O. rufipogon*, suggesting repeated introduction of novel genetic materials into rice cultivars (T. Tang *et al.*, unpublished).

Concluding remarks

The reduction in fitness, or the genetic cost of domestication, is a general phenomenon. Our analysis of two rice genomes suggested that several amino acid substitutions occurred during domestication, many of them deleterious. We hypothesize that during domestication artificial selection increased the frequency of many deleterious mutations in the absence of effective recombination

(the dHR effect). This hypothetical 'cost of domestication' might soon be testable in other domesticated species.

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Supplementary data

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