humans, because the effective population size has been small and hence negative selection has been inefficient. If promoter evolution in the absence of positive selection is almost as fast as intronic evolution, a modicum of positive selection suffices to raise the overall rate of a promoter region above that of the associated intronic sequences. Thus, Taylor *et al.*'s interpretation of their "branch-only" results is questionable. (Indeed, these results closely parallel results we obtained ourselves, which we included in early versions of our manuscript. We removed them from the manuscript to shorten it, but we will gladly send them to anyone who requests them.)

Another reason to doubt Taylor *et al.*'s assessment is the incongruity between our results for humans and chimpanzees. As we mentioned in our Letter, the rank correlation between *P* values for the two lineages over all 6,280 analyzed genes is 0.27; over the 575 genes scoring high in humans, the correlation is 0.31, and over the 636 scoring high in chimpanzees, it is 0.29. These are not negligible, but neither are they large. This is expected if the scores predominantly reflect positive selection, which has presumably targeted different traits and genes in the two lineages (with anticipated exceptions such as some immunity-related traits and genes), but not if they reflect some primate-wide mutational bias.

Taylor *et al.*'s arguments (in their Supplementary Note) regarding 'housekeeping genes' are biologically naive and inconsistent with both our results and those of others. To begin with, genes transcribed in the germline include many kinds of genes that are decidedly not enriched with genes scoring high in our study. For example, the PANTHER molecular functions 'cytoskeletal protein' and 'ribosomal protein' have enrichment *P* values of 0.98 and 0.95, respectively. Moreover, although we did not emphasize them in our article, many immunity- and apoptosis-related genes do score high in our study. For example, if our Table 1a had included one more line, it would have been for the PANTHER biological process 'T-cell mediated immunity' with enrichment *P* value 0.053. Finally, we are not the only investigators to find enrichment of metabolic functions with signals of positive selection in humans. For example, one previous study[3] found enrichment of PANTHER biological processes including 'other carbohydrate metabolism' and 'steroid metabolism'; several such categories have also been found to show enrichment in other surveys[4,5]. Taylor *et al.* misconstrue us as supposing that metabolic adaptation in humans relates to dietary changes alone, but we recognize that other factors may well be relevant. In particular, the evolution of the energetically expensive human brain probably entailed metabolic adjustments throughout the body[6].

Thus, Taylor *et al.*'s analyses of our data do not affirm their contention that mutation is generally accelerated in primate promoters. Their assessment is also discordant with the contrast between humans and chimpanzees in our results. Taylor *et al.*'s belief that metabolic functions are implausible targets of positive selection in humans is biologically dubious and conflicts with studies besides ours. Accordingly, we remain confident that our results predominantly reflect positive selection.

(About our data filtering: contrary to what Taylor *et al.* state in their Supplementary Note, we masked both promoter and intronic sites in 50-site windows with extreme divergence between humans and chimpanzees or macaques. Over the 10,933 genes we were able to analyze at all, we masked only 0.067% and 0.052% of promoter and intronic sites, respectively. All intronic sites we used lie within 2.6 kb of coding sequences, whereas most promoter sites we used are more distant, which probably entails greater liability of the promoter sites to assembly errors in the chimpanzee and macaque genome sequences. We excluded from further analysis any promoter region with a masked-site frequency above 0.75%. We excluded genes for other reasons too, and of the 4,653 genes excluded, only 419, amounting to 9.0% of 4,653 and 3.8% of 10,933, failed the masked-site frequency cutoff. Any bias thereby introduced against rapidly evolving promoter regions is therefore minor.)

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Japonica rice carried to, not from, Southeast Asia

To the editor: The paper by Shomura *et al.*[1] infers that domesticated *japonica* rice originated in the tropical insular region of Southeast Asia and was then transferred to China. This hypothesis, however, is contradicted by a wealth of archeological data accumulated over the past couple of decades. Archeobotanical evidence provides a fossil record of the past evolution of crops under domestication, documenting the presence of species in regions at dates that can be confirmed by direct radiometric methods, and documenting aspects of the evolution of morphological domestication traits[2]. The earliest hard evidence for rice use, as well as evidence for the evolution of domestication traits in rice, is documented from the middle and lower Yangzte river valley in China. Evidence for the evolution of nonshattering, domesticated rice panicles, larger grain sizes and reduction of awns is documented as evolving between 7000 BC and 4000 BC[2,3]. In addition, artificial field systems indicating the creation of paddyfields have been discovered dating to just before 4000 BC[4].

By contrast, the earliest evidence for cultivation of rice in Southeast Asia, including both the mainland and insular regions, occurs between 3000 and 2000 BC. In Taiwan the earliest rice is found from 3000 to 2500 BC, whereas further south in the Phillippines or Thailand the earliest rice is closer to 2000 BC[5]. The earliest systematically documented rice remains from Thailand are younger than 2000 BC and possess fully domesticated, nonshattering spikelet bases of rice[6]. This can be contrasted with the evidence from 2,000 to 4,000 years earlier in the Lower Yangzte region where such domesticated forms increased gradually in proportion to wild types[3,7].

Several strands of anthropological data have long suggested that agricultural populations departed Taiwan and spread southwards to the Philippines and Indonesia only 4,000–5,000 years ago, and similar migrations probably brought rice agriculture to mainland Southeast Asia. This is supported by evidence based on historical linguistics, archeology and human genetics[5,8].

In light of the archeological evidence, these new genetic data must be interpreted as part of the complex history of rice spreads into Southeast Asia from the north. This paper

makes the assumption that the genes for primitive traits are preserved in the putative homeland, as in the case of the dominant allele *qSW5*, whereas humans have selected for the recessive *qsw5* encoding a broader seed size[1]. Whether this was the only major gene involved in grain-size increase during domestication is unknown, and other grain-size QTLs have been identified[9]. However, the high percentage of dominant carriers (*qSW5*) in the Philippines and Indonesia may be explained by local selection conditions and/or introgression from wild populations. The archeological record from the Lower Yangzte region in China charts an evolutionary trend toward increasing grain size between 6000 and 4000 BC[2]. Although nonshattering was a key domestication trait in rice, *qsh1* documented by Shomura *et al.*[1] is only one of the alleles that may have caused this; *sh4* is another important target of early selection and is more widespread in rices, including *indica,* and might therefore have been selected earlier[9]. The evolution of nonshattering genes in rice is therefore complex.

The third mutation considered by Shomura *et al.*[1] is the *wx* mutation, which results in sticky, low-amylose rice grains. This is not a trait related to initial domestication but rather a later diversification allele that has been the target of selection under cultural food preference. The dominant allele *Wx* is seen not only in wild rice but also in widespread cultivated rices in South, East and Southeast Asia. High frequencies of *wx* are correlated with cultural preferences for sticky cereals, which also extends to waxy genotypes of *Setaria italica, Panicum miliaceum* and several other species within Eastern Asia[10]. Thus, all the genes discussed here, *qsw5*, *qsh1* and *wx*, have been targets of selection within some cultivated rices, but none of them are clearly linked to the beginnings of cultivation or domestication. Because all of the genes considered by Shomura *et al.*[1] were targets of cultural selection, they have been subjected to differing selection histories within different regional cultural histories. As such, they are perhaps less useful for phylogeographic reconstruction than neutral loci.

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Izawa, Shomura, Konishi, Ebana and Yano reply: In our study[1], we cloned a quantitative trait locus (QTL) termed *qSW5*, which controls seed width in rice. Deletion of the *qSW5* gene region resulted in greater yield, allowing us to infer its artificial selection in rice domestication. We therefore used genome-wide and neutral RFLP data to classify various rice cultivars and mapped the functional nucleotide polymorphism (FNP) of *qSW5* and two other FNPs of domestication-related genes *Wx* (a taste-related gene) and *qSH1* (a seed-shattering gene)[2].

Recently, we examined three more FNPs of two domestication-related genes, *Rc* (an anthocyanin-regulator gene for pericarp color) and *Rd*[3]. The heritage landraces, which contain the original (or nonselected) FNPs of all the domestication-related genes that we tested, often originated in island Southeast Asia. *rc* or *qsw5* single-mutant (or selected) and *rc qsw5* double-mutant landraces were distributed more widely than the heritage landraces; moreover, selection for the *wx* (or wx^b) mutation helped establish some landraces of Indochina origin[3]. These triple FNPs—of *rc*, *qsw5* and *wx*—were distributed in most *japonica* landraces with other origins (for example, China or Japan). We often found the FNP of *qsh1* and two FNPs of *rd* in the landraces and cultivars of China and Japan.

The presence of heritage landraces originating in island Southeast Asia, plus the local distribution of single-, double- and triple-mutant landraces and the further spread of landraces with triple *rc qsw5 wx* FNPs, led us to propose a model of domesticated *japonica* rice originating in island Southeast Asia. Rice-genome analyses suggest that there was a single domestication process for *japonica* rice and that tropical *japonica*, to which the heritage landraces belong, is more closely related to *japonica*-like wild rice than to temperate *japonica*[4–6]. However, as Fuller and Sato have said[7], many archeological data suggest that evolution of rice domestication traits occurred in the Yangzte river valley in China.

Therefore, if these traits were carried elsewhere from China, how many times did this happen? After the distribution of these heritage landraces, single, double and triple mutants should have followed the same path from China to give the current landrace distribution. Such multiple migrations are not yet supported by archeological data[7]. Although crossing of some triple-mutant landraces with a wild rice could also have given this distribution, the gradual changes in genome-wide RFLP data associated with the FNP distributions in *japonica* landraces exclude this possibility[1,3]. Therefore, we prefer the simplest model—that of an island Southeast Asian origin[1,3].

Strong evidence comes from the evolution of nonshattering in China[8]. Both a wild type and a *japonica* type of abscission-layer formation have been observed in excavated short rachillae of paddy rice grain (7,000 years old) at Yangtze river sites, as Fuller and Sato also cited[7]. Many *japonica* rice varieties have lost the abscission layer[2]. So far, two domestication-related genes for seed-shattering traits, *qSH1* (ref. 2) and *sh4* (refs. 9,10), have been identified. The *sh4* mutation preceded and spread more widely in landraces than the *qsh1* mutation[1,2,9,10]. Because the *qsh1* mutation, but not the *sh4* mutation, caused abscission-layer loss[2,9,10], it is very likely that the archeological data on the short rachillae indicate that the *qsh1* mutation had already been selected by 7000 BC[2,8]. Therefore, the FNPs of *sh4*, *rc*, *qsw5* and *wx* probably occurred sometime before 7000 BC (the time to which the Chinese archeological evidence has been dated), and maybe not in China. If ancient humans began cultivating rice in transient riverside or swampy sites on a home-garden scale, then it would be very difficult to find relevant archeological data. The duration of this small-scale rice-cultivation period might have been longer than we thought. Whatever FNPs were the targets of cultural selection, and whatever other QTLs were involved, the wide distribution of the *rc*, *qsw5* and *wx* FNPs in various landraces strongly suggests that these selections contributed critically to rice domestication. Traits related to initial domestication should therefore be identified by an approach like ours, based on neutral genome-wide DNA data of an unbiased collection of landraces, as we have already delimited the timing of

selection of the initial trait of rice domestication to before *rc* and *qsw5* selection. Most importantly, we should not forget to validate the collection continuously for the best model[3].

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