American Genetic Association

Journal of Heredity, 2019, 601–609
doi:10.1093/jhered/esz030
Original Article
Advance Access publication May 7, 2019

OXFORD

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

Original Article

Discordant Patterns of Introgression Suggest Historical Gene Flow into Thai Weedy Rice from Domesticated and Wild Relatives

Marshall J. Wedger, Tonapha Pusadee, Anupong Wongtamee, and Kenneth M. Olsen

From the Department of Biology, Washington University in St. Louis, 1 Brookings Dr, St. Louis, MO 63130 (Wedger and Olsen); Department of Plant and Soil Science, Chiang Mai University, Chiang Mai, Thailand (Pusadee); and Department of Agricultural Science, Naresuan University, Phitsanulok, Thailand (Wongtamee)

Address correspondence to Kenneth M. Olsen at the address above, or e-mail: kolsen@wustl.edu.

Received February 20, 2019; First decision April 7, 2019; Accepted May 4, 2019.

Corresponding Editor: Mark Chapman

Abstract

Weedy relatives of crop species infest agricultural fields worldwide, reducing harvests and threatening global food security. These weeds can potentially evolve and adapt through gene flow from both domesticated crop varieties and reproductively compatible wild relatives. We studied populations of weedy rice in Thailand to investigate the role of introgression from cultivated and wild rice in their evolution. We examined 2 complementary sources of genetic data: allelic variation at 3 rice domestication genes (*Bh4*, controlling hull color; *Rc*, controlling pericarp color and seed dormancy; and *sh4*, controlling seed shattering), and 12 previously published SSR markers. Sampling spanned 3 major rice growing regions in Thailand (Lower North, North East, and Central Plain) and included 124 cultivated rice accessions, 166 weedy rice accessions, and 98 wild rice accessions. Weedy rice strains were overall closely related to the cultivated varieties with which they co-occur. Domestication gene data revealed potential adaptive introgression of *sh4* shattering alleles from wild rice. Introgression of potentially maladaptive *rc* crop alleles (conferring reduced dormancy) was also detected, with the frequency of the crop allele highest in northern populations. Although SSR markers also indicated introgression into weed populations from wild and cultivated rice, there was little overlap with domestication genes in the accessions showing admixed ancestry. This suggests that much of the introgression we detected at domestication genes most likely reflects past introgression rather than recent gene flow. This finding has implications for understanding long-term gene flow dynamics between rice and its weedy and wild relatives, including potential risks of transgene escape.

Subject areas: Population structure and phylogeography; Molecular adaptation and selection
Keywords: agricultural weeds, dedomestication, domestication gene, *Oryza sativa*, southeast Asia, SSRs

© The American Genetic Association 2019. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Introduction

Agricultural weeds that are closely related to crop species are present in agroecosystems worldwide and pose a major threat to sustainable crop production (Ellstrand et al. 2010; Singh et al. 2013; Ziska et al. 2015). These weedy crop relatives are commonly restricted to agricultural habitats, where they aggressively outcompete crop varieties and can reduce harvests by 80% or more (Diarra et al. 1985; Singh et al. 2013). An important question in the study of weedy crop relatives is the extent to which their evolution and adaptive fitness is shaped by gene flow from co-occurring domesticated varieties and/or nearby populations of reproductively compatible wild relatives (Beebe et al. 1997; Warwick et al. 2008; Ellstrand et al. 2010; Engku et al. 2016). This question is often examined in the context of transgene escape, with recent studies largely focused on the contemporary movement of herbicide resistance alleles from transgenic crops into nearby wild and weedy populations (Warwick et al. 2008; Singh, Singh et al. 2017). Most such studies document transgene escape but do not assess the multigeneration impact of this crop-to-weed introgression (Morrell et al. 2005). Thus, less is known about the longer-term consequences of hybridization and gene flow between cultivated, weedy, and wild populations. From a practical perspective, introgression into weeds can elevate their competitive advantage, leading to strains that are much more difficult to control. It is therefore imperative to understand the evolutionary influence of these types of introgression and the timescale over which they occur.

A potentially useful approach for studying the long-term dynamics of gene flow into weedy crop relatives is to examine allelic variation at genes that control domestication-related traits. Because weedy relatives are specifically adapted to agroecosystems, some domestication traits would be expected to confer fitness benefits to weed strains; these include erect plant growth architecture and short stature (allowing weeds to grow competitively and inconspicuously in agricultural fields), as well as herbicide resistance. For such traits, the domestication (crop) alleles at the genes controlling these traits would be adaptive in weed populations. For other domestication traits, including reduced seed shattering, reduced seed dormancy and loss of structures promoting secondary seed dispersal (e.g., awns and barbs), crop alleles are likely maladaptive. For such traits, introgression from wild populations rather than crop varieties could be adaptive for allowing the weeds to persist and proliferate in agricultural fields. Comparisons of the distributions of crop versus wild alleles at multiple domestication genes can thus provide insights on patterns of adaptive introgression into weed populations from domesticated and wild relatives (Song et al. 2014; Cui et al. 2016; Huang et al. 2018).

As a complement to the gene-specific insights provided by domestication genes, genome-wide neutral markers can help to elucidate the broader genomic consequences of gene flow into weedy crop relatives. Depending on the frequency at which hybridization has occurred, and whether hybridization occurred recently or in the more distant past, the genetic composition of weedy relatives is expected to show greater or lesser overall levels of relatedness to the hybridizing source populations. Neutral markers can thus be informative for determining whether gene flow occurred extensively and in the recent past—in which case the weeds would show genome-wide evidence of admixture from the source population—or whether introgression occurred enough generations ago that evidence of the hybridization event is no longer apparent on a genome-wide scale.

In recent years, weedy rice (*Oryza sativa*) has emerged as a genomic model system for studying the evolution of weedy crop relatives (Guo et al. 2018; Wedger and Olsen 2018). Weedy rice is a conspecific form of cultivated Asian rice that is present in almost every world region where rice is cultivated, including both areas where the wild crop ancestor (*Oryza rufipogon*) is present (South and Southeast Asia), and areas without reproductively compatible wild relatives (e.g., Japan, North America, Europe) (Cao et al. 2006; Londo and Schaal 2007; Grimm et al. 2013). Weedy rice has evolved multiple times independently from different cultivated rice varieties, making the system highly amenable to studies on the parallel evolution of weediness (Qi et al. 2015; Li et al. 2017) In some rice growing regions, including Japan, Italy, and China (Akasaka et al. 2009; Grimm et al. 2013; Sun et al. 2013), weedy rice strains are closely related to local rice varieties, suggesting in-situ origins by dedomestication. In other regions, such as the United States, the weeds are genetically distinct from local crop varieties and likely evolved through dedomestication in Asia, with subsequent unintentional introductions into their present range (Reagon et al. 2010; Li et al. 2017). In areas of tropical Asia where wild rice is present, weedy rice strains have typically been found to show some evidence of introgression from wild populations, although they are still primarily descended from domesticated rice (Cao et al. 2006; Song et al. 2014; Huang et al. 2017).

Comparative analyses of domestication genes and neutral markers have proved particularly insightful in evolutionary studies of weedy rice (Song et al. 2014; Cui et al. 2016; Huang et al. 2018). These analyses have largely relied on 3 well-characterized rice domestication genes: *sh4* (controlling loss of shattering in the crop), *Rc* (controlling loss of pericarp pigmentation and seed dormancy in the crop), and *Bh4* (controlling loss of dark-pigmented hulls in the crop). In the case of *sh4*, strong selection during rice domestication led to the fixation in the crop of a nonsynonymous substitution in exon 1 that results in a reduction in grain shattering (Li et al. 2006; Zhang et al. 2009). Most weedy rice strains examined to date carry this domestication allele, confirming descent from domesticated ancestors (Thurber et al. 2010; Zhu et al. 2012). Despite carrying the reduced-shattering allele, however, weedy rice strains are typically highly shattering, and the re-emergence of the shattering phenotype appears to have occurred through multiple compensatory mutations throughout the genome (Qi et al. 2015; Li et al. 2017). In Southeast Asia, some weedy rice strains carry the wild *sh4* allele, a pattern consistent with adaptive introgression from local wild rice populations (Song et al. 2014; Huang et al. 2018).

Rc encodes a bHLH protein that pleiotropically controls both the proanthocyanidin pigment synthesis pathway and abscisic acid-mediated seed dormancy (Sweeney et al. 2006; Gu et al. 2011). Most modern cultivated rice varieties carry a 14-bp frameshift deletion in exon 7 that generates a nonfunctional gene product and nonpigmented or "white" pericarps (bran) (Sweeney et al. 2007). Unlike *sh4*, this *rc* domestication allele is not present in most weedy rice; instead, most weed strains carry functional *Rc* alleles (Gross et al. 2010; Cui et al. 2016). This suggests that most weedy rice strains are not descended from modern rice varieties, but rather that they evolved from dark-pericarp landraces that predate modern light-pericarp varieties. The high frequency of the functional *Rc* allele in weedy rice populations has been proposed to reflect strong selection for seed dormancy, as this is a critical trait for weed persistence in crop fields (Cui et al. 2016).

Bh4 encodes an amino acid transporter that is expressed in maturing rice hulls and generates the dark hull pigmentation that characterizes wild *Oryza* species. Most cultivated rice varieties carry a 22-bp frameshift deletion in exon 3 that results in the straw-hull

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

phenotype of domesticated rice (Zhu et al. 2011). Among weedy rice strains, both straw- and black-hull strains occur widely (Reagon et al. 2010; Grimm et al. 2013; Song et al. 2014; Merotto et al. 2016), with the former carrying the domestication allele and the latter carrying the functional *Bh4* allele of wild *Oryzas*. The widespread occurrence of both phenotypes in weedy rice has led to the hypothesis that this variation may represent 2 adaptive weed strategies: a crop-mimic (straw-hull) form, and a more wild-like (black-hull) form (Federici et al. 2001). Alternatively, this variation may simply reflect a lack of strong selection on hull color in weedy rice, with the 2 forms present as a legacy of independent weed origins from straw-hull and black-hull rice ancestors (Vigueira et al. 2013; Li et al. 2017).

In this study, we examined the distributions of wild and crop alleles at *Bh4*, *Rc*, and *sh4* to study patterns of adaptive introgression into weedy rice in Thailand, a region where both cultivated rice and local wild rice populations may contribute to weed evolution (Pusadee et al. 2013; Wongtamee et al. 2017). We then compared these patterns with genome-wide patterns inferred from previously reported neutral SSR loci (Wongtamee et al. 2017) to assess the time frame over which introgression has occurred. Thailand lies in the center of diversity for rice domestication. Additionally, Thailand is among the few rice growing countries where the wild progenitor is still abundant and present at the margins of fields. This wild–weed–crop complex allows for interactions among the 3 components and suggests that rice in Thailand forms an evolutionarily dynamic system.

We specifically asked the following questions: 1) Is there evidence of gene flow from wild or cultivated rice into co-occurring Thai weedy rice populations? 2) Do weeds that show evidence of crop allele introgression at domestication genes show increased genome-wide similarity to the crop based on SSR markers? 3) Do weeds that show evidence of adaptive introgression of wild alleles show genome-wide evidence of wild rice ancestry? Our results suggest that introgression into weedy rice has occurred from both wild and cultivated rice, but that this is likely a historical process with relatively little gene flow occurring on a contemporary time scale.

Methods

Sampling

Oryza leaf samples were obtained from 3 geographical regions of rice cultivation in Thailand: the North East (NE), Lower North (LN), and Central Plain (CP) (Figure 1). Samples included 166 weedy rice accessions (40 NE, 77 LN, 49 CP), 104 co-occurring cultivated rice accessions (10 NE, 54 LN, 40 CP), and 28 common wild rice accessions collected from natural habitats spanning the 3 geographical regions (Supplementary Table S1). Here, we use the term "accession" to refer to individual rice plants and their derived seed. We reserve "populations" for genetically distinct subgroups inferred from *STRUCTURE* analyses described below. Weedy rice accessions were collected by randomly selecting plants separated by 5–10 m intervals (to avoid collecting close relatives) from heavily infested agricultural fields (>50% infestation by visual inspection). Only *indica* rice varieties are cultivated in the sampled rice growing regions.

For weedy rice and wild rice collections, leaves and panicles of sampled plants were collected in the field and silica-dried following the method of Chase and Hills (1991). For cultivated rice samples, seeds were collected and germinated in petri dishes for 1 week and then transplanted to outdoor field plots at Chiang Mai University, with 10 plants per plot. Four weeks after transplanting, leaves of 10 individuals of each variety were harvested and dried in silica gel for DNA extraction.

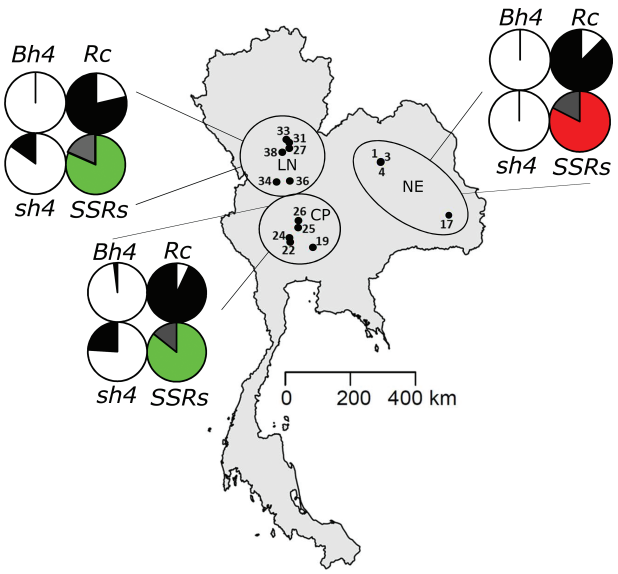

Figure 1. Sampling locations in Thailand. Black dots represent collection sites with numbers representing the field number. North East (NE) samples represent collections from Khon Kaen (1,3,4) and Ubon Ratchathani (17) provinces. Central Plain (CP) samples represent collections from Phra Nakhon Si Ayutthaya (19), Suphan Buri (22,24), Sing Buri (25), and Nakhon Sawan (26) provinces. Lower North (LN) samples represent collections from Phitsanulok (27), Uttaradit (31,33), Phichit (34,36), and Sukhothai (38) provinces. Pie charts labeled *Bh4, Rc,* and *sh4* represent allele frequencies in weedy rice, with white representing the domestication allele and black representing the wild allele. The pie chart labeled "SSRs" represents the proportion of weedy rice samples that *STRUCTURE* has unambiguously assigned to a population based on ≥0.80 membership assignment. The green and red colors represent the green and red *STRUCTURE* populations, while the grey represents plants that show evidence of admixture (<0.80 assignment to a single population).

Genotyping

DNA was extracted from leaf tissue at Chiang Mai University using a modified cetyltrimethyl ammonium bromide (CTAB) protocol from Doyle and Doyle (1987). Genotyping of domestication genes was performed at Washington University in St. Louis as described below.

Bh4

Polymerase chain reaction (PCR) genotyping was used to score all plants in the study for the presence/absence of the 22-bp deletion that distinguishes straw-hull rice (the common phenotype in most cultivated rice) from the black-hull phenotype (characteristic of wild rice). Four PCR primers, *Bh4-22F1*, *Bh4-22R1*, *Bh4_gt2F*, and *Bh4_gt2R*, were designed for this purpose (Supplementary Table S2). Thermocycler conditions were as follows: denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, and elongation at 72 °C for 30 s. PCR was finished with elongation at 72 °C for 7 min and held at 4 °C. Reactions were conducted at standard PCR concentrations with GoTaq (Promega) and 1M betaine added to reduce secondary structure formation. PCR amplifications were visualized and scored with ethidium bromide on a 2.5% agarose gel. A functional "black hull" allele would appear as a 114 bp band, whereas a nonfunctional "straw hull" allele would appear as a 92 bp band. Results were spot checked for accuracy by direct Sanger sequencing of PCR products

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

(using primers *Bh4_gt2F* and *Bh4_gt2R*). Sequencing was performed on an ABI 3130 capillary sequencer in the sequencing facility of the Washington University Biology Department.

Rc

A 14-bp frameshift deletion allele is the primary cause of the nonpigmented ("white") pericarp seen in most cultivated rice. Samples were genotyped for the presence/absence of the 14-bp deletion in 1 of 2 ways. For the first method, 3 primers, *Rc_wtF*, *Rc_delF*, and *Rc_gtR* (Supplementary Table S2), were designed and used together in PCR. Thermocycler conditions were as follows: denaturation at 94 °C for 2 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, and elongation at 72 °C for 30 s. PCR was finished with elongation at 72 °C for 7 min and held at 4 °C. Reactions were performed with PlatinumTaq (Invitrogen) and 1M Betaine for precision and stability. PCR amplifications were visualized and scored with Ethidium Bromide on a 2.5% agarose gel. A functional "red" *Rc* allele would appear as a 175 bp band, a nonfunctional "white" rc allele would appear as a 155 bp band, and any heterozygous genotypes would amplify both products.

The second method for scoring *Rc* was based off the protocol of Rysbekova et al. (2017) and used 2 sets of primer pairs: *Rc_wtF1* with *Rc_wtR1*, and *Rc_delF3* with *Rc_delR3* (Supplementary Table S2). Thermocycler conditions for both reactions were as follows: denaturation at 94 °C for 2 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and elongation at 72 °C for 30 s. PCR was finished with elongation at 72 °C for 7 min and held at 4 °C. Reactions for each primer set were conducted separately. Reactions with *Rc_wtF1* and *Rc_wtR1* were conducted with ExTaq and 2mM $MgCl_2$ to increase amplification. Reactions with *Rc_delF3* and *Rc_delR3* were conducted with ExTaq and 3 mM $MgCl_2$ to further increase amplification. PCR products were visualized and scored on a 0.8% agarose gel. A nonfunctional "white" allele would appear as a 400 bp band, while a functional "red" allele would appear as an 800 bp band.

sh4

Two primers, *Sh4_00F* and *Sh4_00R* (Supplementary Table S2) were used to PCR-amplify a portion of the gene for Sanger sequencing to genotype the domestication SNP (a G to T substitution in exon 1). The T substitution results in reduced shattering in cultivated rice and is present at 100% frequency in the crop. Thermocycler conditions for both initial PCRs were as follows: denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 1 min. PCR was finished with elongation at 72 °C for 7 min and held at 4 °C. Reactions were conducted at standard PCR concentrations with ExTaq and 1M Betaine for precision and stability. Resultant PCR products underwent a further sequencing reaction consisting of 5 µL template, 2 µL of forward or reverse primer, and betaine. Thermocycler conditions were as follows: 96 °C for 1 min followed by 30 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 1 min. Samples were then held at 4 °C until sequencing. PCR products were sequenced on an ABI 3130 capillary sequencer at Washington University and visualized using Geneious v. 8.0 (http://www.geneious.com, Kearse et al. 2012).

Data Analysis

SSR Loci

Genotypes from 12 microsatellite loci, distributed across 10 of the 12 rice chromosomes, were obtained for all cultivated and weedy rice samples in this study from a previously published dataset (Wongtamee et al. 2017) (Supplementary Table S2); these data were used to assess population structure and genetic relationships among accessions (Supplementary Table S3). Samples used in the study were chosen based on data availability from the previous study. Of the sampled accessions in Wongtamee et al. (2017), only those from fields with more than 10 accessions in that study were analyzed. SSR genotypes for an additional 20 cultivated and 70 wild rice SSR genotypes were obtained from the same study for inclusion in analyses. Population structure was first assessed using the Bayesian analysis in *STRUCTURE* (Pritchard et al. 2000) at K values ranging from 2 to 10 with a burn-in of 50,000 MCMC replicates and a run length of 50,000 replicates. Default parameters were used to identify the optimal number of populations (K), with the delta-K statistic (Evanno et al. 2005) used as the selection criterion for optimal K. A final *STRUCTURE* run was performed at the optimal K with a 500,000 burn-in length and 500,000 runs for final determination of population membership coefficients. Population membership coefficients were used as an indicator of ancestry to determine the extent to which a given accession unambiguously belonged to a population or showed evidence of genetic introgression from another group. Accessions with <80% membership assignment to a single population were considered to be admixed. As a complement to the *STRUCTURE* analysis, genetic relationships among accessions were further assessed by principal coordinates analysis (PCoA), using default parameters in GenAlEx (Peakall and Smouse 2006, 2012).

Domestication Genes

To assess the degree of concordance between domestication genes and SSRs for inferred introgression into weedy rice, weed accessions were separated into mutually exclusive groups based first on inferred population membership coefficients from *STRUCTURE*, and then on the distributions of wild and crop alleles at the 3 domestication genes. This allowed us to test the hypothesis that plants that showed introgression at domestication loci would also show differential similarity to the corresponding population at neutral loci.

Results

Domestication Genes

Bh4

All cultivated rice plants that were genotyped for *Bh4* variation (104 of 104 accessions) carried the 22-bp deletion allele that encodes the straw-hull phenotype found in most cultivated rice (Table 1). Similarly, nearly 100% of the genotyped weedy rice plants (165 of 166 accessions) also carried the crop allele, consistent with weed descent from domesticated ancestors. The sole weedy rice plant with a wild *Bh4* allele (conferring black hull color) was collected in the Central Plain; this accession does not appear to be a descendant of recent wild-to-weed introgression (see *STRUCTURE* results below). Among the genotyped wild samples, most accessions carried the wild allele (25 of 28 accessions), with the remaining 3 carrying the domestication allele. This pattern suggests a low level of unidirectional gene flow from cultivated into wild rice, a pattern that has been previously reported and is likely prevalent in wild rice populations (Wang et al. 2017).

Rc

All but one of the genotyped cultivated rice accessions (100 of 101 accessions) carried the 14-bp deletion domestication allele that

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

Table 1. Distributions of domestication alleles in the sampled rice groups. Numerators indicate the number of genotyped accessions that carry the domestication allele at each gene; denominators indicate the total number of genotyped accessions

	Bh4 22-bp deletion	*Rc* 14-bp deletion	*sh4* T substitution
Rice type			
Cultivated	104/104 (100%)	100/101 (99.0%)	59/59 (100%)
Weedy:			
Lower North (LN)	77/77 (100%)	16/75 (21.3%)	45/53 (84.9%)
North East (NE)	40/40 (100%)	5/40 (12.5%)	33/33 (100%)
Central Plain (CP)	48/49 (98.0%)	3/43 (7.0%)	19/25 (76.0%)
All regions	165/166 (99.0%)	24/158 (15.2%)	96[a]/111 (86.5%)
Wild	3/28 (10.7%)	2/7 (28.6%)	—

[a]Includes one heterozygote.

confers light-colored pericarps and is found in most rice varieties (Table 1). In the weedy rice samples, 134 of 158 genotyped accessions (84.8%) carried the functional wild *Rc* allele that confers dark pericarp pigmentation and seed dormancy, with the remaining 24 accessions carrying the light-pericarp *rc* allele. The frequency of the crop allele in weedy rice showed a general north-to-south decrease across the sampled regions (21.3% in the Lower North, 12.5% in the North East, and 7% in the Central Plain) (Figure 1). This occurrence of the *rc* allele in weedy rice suggests that there has been introgression of the crop allele that is likely maladaptive for the weeds, since the functional *Rc* allele confers seed dormancy, an important trait for weed fitness. In the wild samples, genotyping could only be successfully performed in 7 accessions; among these, 2 accessions (28.6%) carried the wild allele. As with *Bh4*, the identification of crop alleles in the wild samples supports an inference of crop-to-wild gene flow.

sh4

The reduced-shattering *sh4* domestication allele, which is fixed in all cultivated rice (59 of 59 accessions), was present and homozygous in the majority of weedy rice accessions (95 of 111 accessions, or 85.6%) (Table 1); this pattern is consistent with descent from domesticated ancestors. The remaining 16 accessions (14.4%), one of which was a heterozygote, carried the wild allele. The presence of the wild *sh4* allele in weedy rice has been observed in other regions of tropical Asia where wild rice is present (Song et al. 2014; Cui et al. 2016), and is potentially consistent with adaptive introgression of the free-shattering allele into the weeds. The presence of wild *sh4* alleles varies widely by region, ranging from zero instances in the North East to 24% in the Central Plain. Because of difficulties in amplifying *sh4* gene in wild rice, no wild samples were genotyped at this locus.

SSR Loci

The SSR genotype data from Wongtamee et al. (2017) were highly polymorphic in the study populations, with expected heterozygosity values ranging from H_e = 0.347 to 0.544 among the 12 loci (Supplementary Table S4). *STRUCTURE* analysis and delta-K assessments revealed an optimum at K = 3 populations, with a smaller secondary peak at K = 6 (Supplementary Figure S1, Supplementary Figure S2). At K = 3, wild rice formed its own unique group while cultivated and weedy rice were grouped by geography rather than plant type (Figure 2). These patterns of differentiation were also broadly supported by the PCoA; the first coordinate (accounting for 17.4% of the total variation) primarily distinguishes wild rice from weedy and cultivated rice, while the second coordinate (accounting for 12.1% of the variation) broadly separates out the 2 geographical population groups that are present within cultivated and weedy rice (Figure 3).

For individual accessions, *STRUCTURE* membership coefficient values revealed no evidence of admixed ancestry in cultivated rice (all membership coefficient values >98%). Weedy rice accessions showed the greatest evidence of admixture, with 29 accessions (17.5%) showing <80% assignment to a single population (Supplementary Table S1). Among these, more than half (15 accessions) showed >20% assignment to the "blue" population characteristic of wild rice. This pattern is consistent with previous reports of introgression into Thai weedy rice from local wild rice populations (Pusadee et al. 2013). In addition, 10 weed accessions that were assigned primarily to the "green" population showed >20% assignment to the "red" population, and 1 weed accession that was assigned primarily to the

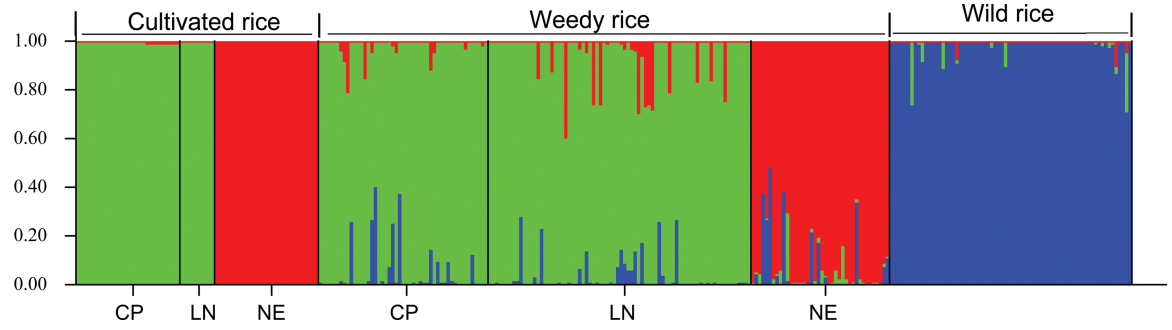

Figure 2. *STRUCTURE* output at K = 3 populations. Accessions analyzed include 67 cultivated, 165 weedy, and 70 wild rice plants. Cultivated and weedy rice plants are separated into 3 geographical regions; North East (NE), Central Plain (CP), and North East (NE). Accessions with a population membership assignment <0.80 to any one population are considered admixed.

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

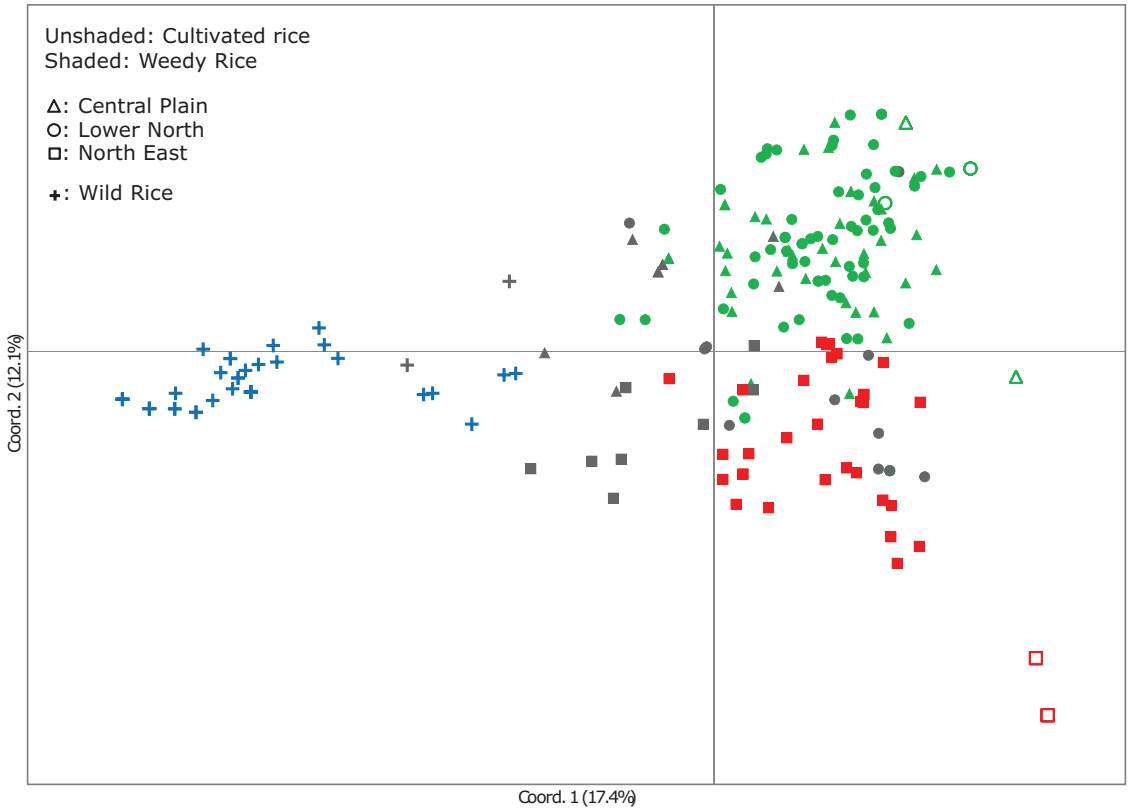

Figure 3. PCoA based on SSR markers. Symbol shape represents collection location. Colors correspond to *STRUCTURE* populations in Figure 2, with gray denoting admixed individuals. Filled shapes are weedy rice, while open shapes represent cultivated rice accessions.

"red" population showed 27.8% assignment to the "green" population. As both cultivated and weedy rice are assigned to the red and green populations, this evidence of red–green admixture in the weeds could either represent crop-to-weed introgression or admixture between the 2 weed groups. For wild rice, 2 accessions showed potential evidence of introgression by the <80% membership assignment criterion; these accessions both showed evidence of shared ancestry with the "green" population present in cultivated and weedy rice (Figure 2; see also Supplementary Table S1). However, as wild rice is genetically more diverse than either cultivated or weedy rice (both of which are ultimately derived from this wild species), the apparent admixture in the wild accessions could also be reflecting its more heterogeneous gene pool rather than introgression per se.

Comparison of Domestication Genes and SSRs

If the introgression of alleles at domestication genes were the result of hybridization in the recent past, weed accessions with introgressed alleles would be expected to show evidence of admixed ancestry in the genome-wide SSR markers. Instead, we found very little overlap between the patterns of introgression from neutral and domestication loci. For *Bh4*, only a single plant carried the wild allele (see above), despite 17.5% of the weedy rice accessions showing some potential evidence of wild introgression at the neutral loci. The sole plant with the *Bh4* wild allele has a membership coefficient of 98.5% to the same population as majority of weed and crop accessions in the region where it was collected (Table 2), suggesting no recent interpopulation hybridization in its ancestry. Similar results were found at *Rc*. The 17% of weedy rice plants that carried crop-like *rc* alleles were genetically indistinguishable from other local weed accessions by the SSR markers; membership assignments to the local majority population were 92.1% and 92.5% for putatively introgressed and nonintrogressed weeds, respectively (Table 2). Weedy rice plants that carried the putatively introgressed (wild) *sh4* allele also showed little evidence of recent admixture from wild rice at the neutral loci; their average membership assignment to their local weed populations was 95.0% (Table 2). Among the 15 weed accessions with the wild *sh4* allele, only one accession (2205A, from the Central Plain) appears to be derived from recent weed–wild admixture; this accession has a 40% membership assignment to the wild rice population, consistent with descent from a recent wild–weed hybridization event (Supplementary Table S1).

Discussion

The long-term evolutionary consequences of gene flow into agricultural weeds from cultivated and wild relatives has important implications for weed adaptation and competitive success. Here, we used a combination of data from domestication genes and neutral SSR loci to assess the history of introgression into weedy rice in the major rice growing regions of Thailand. This combination of data sources has allowed us to analyze complementary aspects of gene flow in this system. Analysis of allele frequencies at domestication genes revealed a low level of potentially adaptive introgression from wild rice at the *sh4* locus, where the wild allele confers seed shattering, and potentially maladaptive introgression from cultivated rice at the *Rc* locus, where the light-pericarp *rc* allele is associated with reduced seed dormancy (Table 1). Interestingly, comparison with genome-wide neutral SSR loci reveals that very few if any of these putative

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

Table 2. Comparison of *STRUCTURE* membership coefficients for weedy rice accessions with and without putatively introgressed alleles at domestication genes. Membership assignment values in the left column would be expected to be significantly lower than values in the right column if the domestication gene introgression occurred through recent hybridization. A 2-sample, equal variance *t*-test indicates no significant differences ($P > 0.75$ in all cases)

	Membership coefficients[a]	
	Accessions with putatively introgressed allele ± SE (N)	Accessions with majority allele ± SE (N)
Bh4	0.980 ± n/a (1)	0.923 ± 0.009 (163)
Rc	0.921 ± 0.025 (24)	0.925 ± 0.009 (132)
sh4	0.950 ± 0.026 (15)	0.899 ± 0.012 (95)

[a]Values are shown with respect to the population that the majority of weed accessions in a given region are assigned to.

introgression events at domestication loci involve recent hybridization; plants showing admixture at neutral loci are by and large not the same plants that show introgression at domestication genes (Table 2; Supplementary Table S1). Thus, introgression at the domestication genes appears to reflect past hybridization events more than contemporary gene flow dynamics. Below we discuss these findings and their implications for understanding processes of evolution and adaptation in weedy rice.

Gene Flow Into Thai Weedy Rice

One clear finding from these analyses is that introgression into weedy rice is detectable at both the domestication genes and the neutral SSR markers. Pooling across the 3 domestication genes, 23% of the weedy rice plants examined had putatively introgressed alleles at one or more loci (38 out of 165 accessions). Similarly, by the <80% membership coefficient criterion in the *STRUCTURE* analysis, 29 weedy rice plants (17.6%) were inferred to have introgression from wild or cultivated rice (Supplementary Table S1). These results are consistent with previous studies in Thailand which report evidence for gene flow as a major force driving the evolution of the *Oryza* complex (Pusadee et al. 2013; Wongtamee et al. 2017).

Nonetheless, only 6 weedy rice plants show evidence of introgression at both domestication and neutral loci (Supplementary Table S1). Taken together, these results suggest the following: first, there is a low, yet detectable, level of contemporary gene flow in this system; and second, the majority of introgression detected at the domestication genes is historical, with enough generations having passed since the hybridization event for recombination to break up any genome-wide signatures of introgression. Thus, while we detect relatively low levels of hybridization in the very recent past, our insights from the domestication genes suggest that past introgression—even if at low levels—can have a lasting effect on the composition of the weedy rice genome.

The first reported observation of invasive weedy rice in Thailand was in the Central Plain in 2001. After just 5 to 6 cropping seasons, weedy rice had overtaken entire production areas in this region. Weedy rice has since spread to every region of Thailand where high-yielding varieties are grown. Additionally, Thai weedy rice has become insensitive to photoperiod, a trait presumably inherited from modern rice varieties (Maneechote 2004). The rapid expansion and apparent selection for introgressed individuals could help explain the results described above.

Adaptive and Maladaptive Introgression

One potential benefit in focusing on well-characterized domestication genes, including the 3 loci examined here, is that the allelic variation at these genes can in principle provide insights into patterns of adaptive or maladaptive introgression into weedy relatives. In the present study, our ability to draw definitive inferences in this regard are fairly limited. The strongest evidence for adaptive introgression comes from *sh4*, where the wild rice (G) allele (conferring freely shattering seeds) is present in nearly one-quarter of the weedy rice plants sampled in the Central Plain (Table 1). This frequency is far higher than has been reported in most weedy rice populations worldwide, the majority of which carry the reduced-shattering (T) allele as a legacy of descent from domesticated ancestors (Thurber et al. 2010; Zhu et al. 2012). Given the importance of seed dispersal for the persistence of weedy rice seeds in crop fields, the presence of the wild allele seems a plausible case of adaptive introgression. However, most weedy rice strains worldwide have highly shattering seeds despite carrying the domestication allele (Thurber et al. 2010; Zhu et al. 2012); the presence of the shattering phenotype in weedy rice appears to reflect the combined effects of multiple other shattering loci throughout the genome (Qi et al. 2015). Thus, allelic variation at *sh4* may not by itself have major phenotypic or fitness impacts in weedy rice. Empirical studies that explicitly measure seed shattering in the Thai weed samples as related to *sh4* variation would be useful for assessing the potential adaptive significance of wild rice *sh4* introgression.

In the case of *Rc*, we find potential evidence of maladaptive introgression of crop alleles into the Thai weed populations. Whereas most modern rice varieties carry the 14-bp loss-of-function mutation at *Rc*, most weedy rice worldwide carries the functional *Rc* allele that is associated with dark-pigmented pericarps and seed dormancy (Sweeney et al. 2006; Gu et al. 2011). In the United States, for example, weedy rice is nearly fixed for the functional *Rc* allele, and the dark-pericarp phenotype is so closely associated with weedy rice that it is commonly referred to by farmers as "red rice" (Gross et al. 2010). Seed dormancy is generally considered a critical fitness trait for agricultural weeds, as it promotes weed persistence in crop fields over multiple growing seasons. Thus, one would expect there to be strong selection against the *rc* allele in weedy rice populations. Nonetheless, we found this allele to be present in Thai weedy rice at an overall frequency of 15.2% (Table 1). Interestingly, this *rc* allele frequency is similar to that observed in weedy rice in a neighboring Southeast Asian country, Malaysia, where it was found to be present in a homozygous state in 17% of genotyped weeds (Cui et al. 2016). One possible explanation for the higher *rc* allele frequency in Southeast Asia is that selection for dormancy may be weaker in this climate compared to temperate climates. In a tropical climate, the cycle of wet and dry seasons rather than summer–winter determines the period of rice cultivation. In this type of climate, water availability directly coincides with favorable periods of weed growth, as the arrival of the wet season triggers rice cultivation at the same time

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

weedy rice would be germinating anyway. In contrast, weedy rice seeds in temperate climates must remain dormant through periods of wet but cold weather in order to survive. Thus, it is plausible that dormancy could be more strongly favored in temperate than tropical climates. Another possible explanation for the apparent lack of strong selection against *rc* in Southeast Asia is that, similar to *sh4*, there are other genes and pathways that contribute to seed dormancy (Marzougui et al. 2012; Zhang et al. 2017). Follow-up studies that explicitly measure seed dormancy levels in Southeast Asian weedy rice would be useful for testing these hypotheses.

Another interesting feature of *Rc* allelic variation in our samples is the apparent north-to-south decrease in frequency of the domestication allele (Figure 1). This cline could be due to a number of factors. Cultural and agricultural practices in Central Thailand are much different than in the Lower North and North East (Pusadee et al. 2013). In Central Thailand, some high-yielding modern rice varieties are direct seeded with up to 3–4 crop plantings per year. Conversely, rice in the North East is planted only once per year, which coincides with the wet season. It is possible that the more intensive agricultural practices of Central Thailand could impose stronger selection for dormancy in local weeds. Additionally, geography is much different from one region to the other. Soil quality and elevation differences might also contribute to the observed pattern for unknown reasons.

Potential Limitations and Avenues for Future Research

A potential limitation of our study is the relatively limited genetic sampling (3 domestication loci and 12 SSRs). Although having more markers is always better, several aspects of these data suggest that they are sufficient to detect and analyze gene flow in this study system. For the SSR loci, polymorphism is quite high for a self-fertilizing species, with H_e ranging from 0.347 to 0.544 (Supplementary Table S4; see also Pusadee et al. 2013). Additionally, we were able to successfully detect introgression and admixture in both weedy rice and wild rice using both SSRs and domestication genes (Figure 2). Thus, these data allow us to successfully infer that both historical and contemporary gene-flow have contributed to the evolution of weedy rice in Thailand. Follow-up studies using whole genome resequencing or reduced-representation SNP genotyping will be useful at answering these questions at finer-scale resolution.

In many world regions where weedy rice is present, there are 2 or more independently evolved strains of weedy rice that coexist (Wedger and Olsen 2018). Interestingly, we have detected a similar pattern in the sampled Thai weedy rice populations, with 2 genetically distinct weed groups that are closely related to the crop varieties with which they co-occur (Figures 2 and 3). As only *indica* rice varieties are cultivated in the region of our sampling, the 2 weed strains appear to represent 2 independent domestications from *indica* backgrounds. Independent weedy rice origins from *indica* rice have also been detected in a number of other regions, including China (Qiu et al. 2017), Korea (Vigueira et al. 2019), Malaysia (Song et al. 2014), and the United States (where the weeds are of Asian origin) (Reagon et al. 2010; Li et al. 2017). Pooled analyses that compare these different *indica*-derived weeds could be especially insightful for understanding the genetic mechanisms that underlie dedomestication, and the role of introgression from modern crop varieties and wild relatives in this process.

Lastly, transgene escape is a serious issue for crop breeding and sustainable crop production. Although transgenic rice has not been commercialized, the requisite technology is well advanced and could be rapidly put into practice on a large scale, for example with the production of herbicide-resistant rice cultivars. Both cultivated and weedy rice are primarily selfing, but outcrossing and hybridization does occur (Singh, Burgos et al. 2017; Singh, Singh et al. 2017), and our analyses in the present study indicate that this hybridization can have multigeneration impacts on the composition of the weedy rice genome (Figure 2; Supplementary Table S1). Recent studies have suggested that weedy rice can act as a bridge for gene flow between cultivated and wild rice due to its extended period of flowering and weak postzygotic barriers to reproduction (Qiu et al. 2017; Singh, Burgos et al. 2017). Based on our results, one can conclude that if transgenic rice were to be introduced in Thailand, eventual escape into wild rice would be likely, and weedy rice could well serve as the conduit.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

Funding

M.J.W. is supported through the NSF Graduate Research Fellowship Program (Fellow ID: 2017221153). Weedy rice research in the Olsen lab has been supported through the NSF Plant Genome Research Program (IOS-1032023). This study was facilitated through funding from Chiang Mai University, which has generously provided support for an adjunct faculty position to KMO for collaborative research with TP on weedy rice.

Acknowledgments

The authors would like to thank members of the Olsen lab group for helpful comments on earlier drafts of this manuscript.

Data Availability

Sample and population IDs for all accessions used in the study are presented in Supplementary Table S1. Genotype data for *Bh4*, *Rc*, and *sh4* are presented for each genotyped accession in Supplementary Table S1. Membership coefficients for all accessions included in the *STRUCTURE* analysis are presented in Supplementary Table S1. SSR genotype data obtained from from Wongtamee et al. (2017) are presented in Supplementary Table S3.

References

Akasaka M, Ushiki J, Iwata H, Ishikawa R, Ishii T. 2009. Genetic relationships and diversity of weedy rice (*Oryza sativa* L.) and cultivated rice varieties in Okayama Prefecture, Japan. *Breed Sci*. 59:401–409.

Beebe S, Toro Ch. O, González AV, Chacón MI, Debouck DG. 1997. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. *Genet Resour Crop Evol*. 44:73–91.

Cao Q, Lu BR, Xia H, Rong J, Sala F, Spada A, Grassi F. 2006. Genetic diversity and origin of weedy rice (*Oryza sativa* f. spontanea) populations found in North-eastern China revealed by simple sequence repeat (SSR) markers. *Ann Bot*. 98:1241–1252.

Chase MW, Hills HH. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon*. 40:215–220.

Cui Y, Song BK, Li LF, Li YL, Huang Z, Caicedo AL, Jia Y, Olsen KM. 2016. Little white lies: pericarp color provides insights into the origins and evolution of Southeast Asian weedy rice. *G3 (Bethesda)*. 6:4105–4114.

Diarra A, Smith RJ, Talbert RE. 1985. Interference of red rice (*Oryza sativa*) with rice (*O. sativa*). *Weed Sci*. 33:644–649.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19:11–15.

Ellstrand NC, Heredia SM, Leak-Garcia JA, Heraty JM, Burger JC, Yao L, Nohzadeh-Malakshah S, Ridley CE. 2010. Crops gone wild: evolution of weeds and invasives from domesticated ancestors. *Evol Appl*. 3:494–504.

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019

Engku AK, Norida M, Juraimi AS, Rafii MY, Abdullah SNA, Alam MA. 2016. Gene flow from Clearfield® rice to weedy rice under field conditions. *Plant Soil Environ*. 62:16–22.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol*. 14:2611–2620.

Federici MT, Vaughan D, Tomooka N, Kaga A, Wang XW, Doi K, Francis M, Zorrilla G, Saldain N. 2001. Analysis of Uruguayan weedy rice genetic diversity using AFLP molecular markers. *Electron J Biotechnol*. 4:42–57.

Grimm A, Fogliatto S, Nick P, Ferrero A, Vidotto F. 2013. Microsatellite markers reveal multiple origins for Italian weedy rice. *Ecol Evol*. 3:4786–4798.

Gross BL, Reagon M, Hsu SC, Caicedo AL, Jia Y, Olsen KM. 2010. Seeing red: the origin of grain pigmentation in US weedy rice. *Mol Ecol*. 19:3380–3393.

Gu XY, Foley ME, Horvath DP, Anderson JV, Feng J, Zhang L, Mowry CR, Ye H, Suttle JC, Kadowaki K, *et al.* 2011. Association between seed dormancy and pericarp color is controlled by a pleiotropic gene that regulates abscisic acid and flavonoid synthesis in weedy red rice. *Genetics*. 189:1515–1524.

Guo L, Qiu J, Li LF, Lu B, Olsen K, Fan L. 2018. Genomic clues for crop-weed interactions and evolution. *Trends Plant Sci*. 23:1102–1115.

Huang Z, Kelly S, Matsuo R, Li L-F, Li Y, Olsen KM, Jia Y, Caicedo AL. 2018. The role of standing variation in the evolution of weediness traits in south Asian weedy rice (*Oryza* spp.). *Genes|Genomes|Genetics*. 8:g3.200605.2018.

Huang Z, Young ND, Reagon M, Hyma KE, Olsen KM, Jia Y, Caicedo AL. 2017. All roads lead to weediness: patterns of genomic divergence reveal extensive recurrent weedy rice origins from South Asian Oryza. *Mol Ecol*. 26:3151–3167.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, *et al.* 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 28:1647–1649.

Li LF, Li YL, Jia Y, Caicedo AL, Olsen KM. 2017. Signatures of adaptation in the weedy rice genome. *Nat Genet*. 49:811–814.

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

Londo JP, Schaal BA. 2007. Origins and population genetics of weedy red rice in the USA. *Mol Ecol*. 16:4523–4535.

Maneechote C. 2004. Invasion of weedy rice in rice fields in Thailand. Rice Genet. Newsl. 29: 20–22.

Marzougui S, Sugimoto K, Yamanouchi U, Shimono M, Hoshino T, Hori K, Kobayashi M, Ishiyama K, Yano M. 2012. Mapping and characterization of seed dormancy QTLs using chromosome segment substitution lines in rice. *Theor Appl Genet*. 124:893–902.

Merotto A Jr, Goulart IC, Nunes AL, Kalsing A, Markus C, Menezes VG, Wander AE. 2016. Evolutionary and social consequences of introgression of nontransgenic herbicide resistance from rice to weedy rice in Brazil. *Evol Appl*. 9:837–846.

Morrell PL, Williams-Coplin TD, Lattu AL, Bowers JE, Chandler JM, Paterson AH. 2005. Crop-to-weed introgression has impacted allelic composition of johnsongrass populations with and without recent exposure to cultivated sorghum. *Mol Ecol*. 14:2143–2154.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes*. 6:288–295.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. *Bioinformatics*. 28:2537–2539.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155:945–959.

Pusadee T, Schaal BA, Rerkasem B, Jamjod S. 2013. Population structure of the primary gene pool of *Oryza sativa* in Thailand. *Genet Resour Crop Evol*. 60:335–353.

Qi X, Liu Y, Vigueira CC, Young ND, Caicedo AL, Jia Y, Gealy DR, Olsen KM. 2015. More than one way to evolve a weed: parallel evolution of US weedy rice through independent genetic mechanisms. *Mol Ecol*. 24:3329–3344.

Qiu J, Zhou Y, Wang Y, Mao L, Ye C, Wang W, Zhang J, Yu Y, Fu F, Wang Y, *et al.* 2017. Genomic variation associated with local adaptation of weedy rice during de-domestication. *Nat Commun*. 8:1–12.

Reagon M, Thurber CS, Gross BL, Olsen KM, Jia Y, Caicedo AL. 2010. Genomic patterns of nucleotide diversity in divergent populations of U.S. weedy rice. *BMC Evol Biol*. 10:180.

Rysbekova AB, Kazkeyev DT, Usenbekov BN, Mukhina ZM, Zhanbyrbaev EA, Sartbaeva IA, Zhambakin KZ, Berkimbay KA, Batayeva DS. 2017. Prebreeding selection of rice with colored pericarp based on genotyping Rc and Pb genes. Russ J Genet. 53: 49–58.

Singh V, Burgos NR, Singh S, Gealy DR, Gbur EE, Caicedo AL. 2017. Impact of volunteer rice infestation on yield and grain quality of rice. *Pest Manag Sci*. 73:604–615.

Singh K, Kumar V, Saharawat YS, Gathala M, Ladha JK, Chauhan BS. 2013. Weedy rice: an emerging threat for direct-seeded rice production systems in India. *J Rice Res*. 1:1–6.

Singh V, Singh S, Black H, Boyett V, Basu S, Gealy D, Gbur E, Pereira A, Scott RC, Caicedo A, *et al.* 2017. Introgression of Clearfield™ rice crop traits into weedy red rice outcrosses. *Field Crops Res*. 207:13–23.

Song BK, Chuah TS, Tam SM, Olsen KM. 2014. Malaysian weedy rice shows its true stripes: wild *Oryza* and elite rice cultivars shape agricultural weed evolution in Southeast Asia. *Mol Ecol*. 23:5003–5017.

Sun J, Qian Q, Ma DR, Xu ZJ, Liu D, Du HB, Chen WF. 2013. Introgression and selection shaping the genome and adaptive loci of weedy rice in northern China. *New Phytol*. 197:290–299.

Sweeney MT, Thomson MJ, Pfeil BE, McCouch S. 2006. Caught red-handed: Rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. *Plant Cell*. 18:283–294.

Sweeney MT, Thomson MJ, Yong GC, Yong JP, Williamson SH, Bustamante CD, McCouch SR. 2007. Global dissemination of a single mutation conferring white pericarp in rice. *PLoS Genet*. 3:1418–1424.

Thurber CS, Reagon M, Gross BL, Olsen KM, Jia Y, Caicedo AL. 2010. Molecular evolution of shattering loci in U.S. weedy rice. *Mol Ecol*. 19:3271–3284.

Vigueira CC, Li W, Olsen KM. 2013. The role of Bh4 in parallel evolution of hull colour in domesticated and weedy rice. *J Evol Biol*. 26:1738–1749.

Vigueira CC, Qi X, Song BK, Li LF, Caicedo AL, Jia Y, Olsen KM. 2019. Call of the wild rice: *Oryza rufipogon* shapes weedy rice evolution in Southeast Asia. *Evol Appl*. 12:93-104.

Wang H, Vieira FG, Crawford JE, Chu C, Nielsen R. 2017. Asian wild rice is a hybrid swarm with extensive gene flow and feralization from domesticated rice. *Genome Res*. 27:1029–1038.

Warwick SI, Légère A, Simard MJ, James T. 2008. Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. *Mol Ecol*. 17:1387–1395.

Wedger MJ, Olsen KM. 2018. Evolving insights on weedy rice. *Ecol Genet Genom*. 7–8:23–26.

Wongtamee A, Maneechote C, Pusadee T, Rerkasem B, Jamjod S. 2017. The dynamics of spatial and temporal population genetic structure of weedy rice (*Oryza sativa* f. spontanea Baker). *Genet Resour Crop Evol*. 64:23–39.

Zhang L, Lou J, Foley ME, Gu XY. 2017. Comparative mapping of seed dormancy loci between tropical and temperate ecotypes of weedy rice (*Oryza sativa* L.). *G3 (Bethesda)*. 7:2605–2614.

Zhang LB, Zhu Q, Wu ZQ, Ross-Ibarra J, Gaut BS, Ge S, Sang T. 2009. Selection on grain shattering genes and rates of rice domestication. *New Phytol*. 184:708–720.

Zhu Y, Ellstrand NC, Lu BR. 2012. Sequence polymorphisms in wild, weedy, and cultivated rice suggest seed-shattering locus sh4 played a minor role in Asian rice domestication. *Ecol Evol*. 2:2106–2113.

Zhu BF, Si L, Wang Z, Zhou Y, Zhu J, Shangguan Y, Lu D, Fan D, Li C, Lin H, *et al.* 2011. Genetic control of a transition from black to straw-white seed hull in rice domestication. *Plant Physiol*. 155:1301–1311.

Ziska LH, Gealy DR, Burgos N, Caicedo AL, Gressel J, Lawton-Rauh AL, Avila LA, Theisen G, Norsworthy J, Ferrero A, *et al.* 2015. An emerging constraint to global rice production: weedy (red) rice. *Adv Agron*. 129:181–228.

Downloaded from https://academic.oup.com/jhered/article-abstract/110/5/601/5486338 by Washington University School of Medicine Library user on 04 September 2019