



Evolving insights on weedy rice

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A B S T R A C T

Agricultural weeds that have evolved from de-domesticated (feral) crop plants cause millions of dollars in annual yield losses worldwide and are one of the primary barriers to global crop productivity. Weedy rice (*Oryza sativa* f. *spontanea*) is a de-domesticated form of rice that has evolved multiple times independently from different cultivated rice varieties. This weedy crop relative has recently emerged as a genomic model system for studying the genetic basis of agricultural weed evolution and the mechanisms that govern the parallel evolution of independently-evolved weed strains. In this review we highlight findings from recent genetics and genomics studies that have led to our current understanding of weedy rice evolution.

1. Introduction

Crop fields account for more than one-tenth of the Earth's total surface area [1], making them one of the most widespread terrestrial habitats on the planet. Agricultural weeds have evolved to exploit these fertile habitats through a variety of different mechanisms. Some of the more striking of these include close mimicry of crop species by unrelated weeds [2] and weed evolution from feral crop varieties through de-domestication [3]. Regardless of the mechanism leading to their evolution, many cropland weed species share a suite of traits collectively called the “agricultural weed syndrome” [4]. These include some crop-like traits that allow them to thrive in agricultural habitats, such as the ability to grow upright in crowded, high-density crop fields and to reproduce within a narrow window of time. They also possess some wild-like traits, such as freely-dispersing seed and strong seed dormancy. This combination of crop-like and wild-like traits makes agricultural weeds particularly well suited for proliferation in croplands, while escaping human detection and eradication efforts. The repeated evolution of agricultural weed phenotypes is an active and important avenue of weed science research; it has also recently gained attention as a model system for studying the genetics of rapid convergent evolution [4–6].

On a genomic level, one of the best studied agricultural weeds is weedy rice (*Oryza sativa* f. *spontanea*), a de-domesticated form of cultivated Asian rice (*Oryza sativa* L.) (Fig. 1). Weedy rice infests rice fields in almost every world region where rice is cultivated, including the United States [7], Europe [8], Latin America [9], East and South Asia [10–12], and Africa [13]. Because of its close phenotypic similarity to cultivated rice, particularly in the seedling stage, weedy rice is difficult

to detect early in the growing season; if left unchecked, weedy rice infestations can reduce crop harvests by more than 80% [14]. Weedy rice has probably been present in the margins of rice fields since the inception of rice agriculture in southern Asia approximately 10 000 years ago. However, it has only become a major threat to global rice production in recent decades, due to shifts away from hand transplanting of rice seedlings (which, while labor-intensive, provides ample opportunities for hand-weeding of rice fields) toward direct-seeded mechanized farming. As described in section 2.1 below, weedy rice has evolved multiple times independently from different cultivated rice varieties. Although phenotypically diverse across its worldwide occurrences, it has convergently evolved traits associated with the agricultural weed syndrome, including highly shattering seed, strong seed dormancy, and competitive growth in agricultural fields. Identifying the genetic mechanisms underlying this convergent weediness evolution is an active area of research.

The cultivated Asian rice genome was the first reference genome published for a crop species, as well as the second angiosperm genome published after Arabidopsis [15]. Rice has become a genomic model system, particularly for cereal crops, due to its small genome size (~430 Mb) and ease of genetic modification. Since weedy rice is a direct descendant of cultivated rice, the wealth of genomic resources developed in cultivated rice can be easily transferred to the weedy rice system. We highlight some of the genetic and genomic studies that have led up to our current understanding of weedy rice and potential avenues for future research directions.

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Fig. 1. Seeds of weedy rice sampled from rice fields in the southern United States. Photo credit: Kenneth M. Olsen

2. Insights on weedy rice evolution

2.1. Insights on weedy rice origins from microsatellites and other neutral markers

One of the most basic questions about weedy rice evolution has been understanding the extent to which different weed strains around the world have evolved independently or have shared origins. Related to this question is whether weedy rice is descended directly from cultivated ancestors, or whether wild *Oryza* populations have contributed to its evolution. The earliest molecular studies of weedy rice evolution relied on neutral markers such as microsatellites to compare weed strains to cultivated and wild rice samples. A common theme to emerge from these studies is that populations of two or more genetically distinct weed strains often co-exist in a single geographical region, and that these have evolved independently from different cultivated rice varieties. This basic pattern has been detected, for example, in the United States [16], Italy [17], China [18], Korea [6], and South America [13].

Specifically in the United States, an analysis of 16 microsatellites and neutral sequence haplotype networks revealed that the two ecotypes found there SH (strawhull awnless) and BHA (blackhull awned), cluster with the genetically distinct cultivated varieties, *O. sativa indica* and *O. sativa aus*, respectively [16]. The authors noted that neither of these rice varieties was ever grown commercially in the US, suggesting that weedy rice was inadvertently imported from elsewhere. In contrast, a study of Italian weedy rice using 19 microsatellites showed that some weedy rice strains there cluster with the locally grown *O. sativa japonica* cultivars [17]. As with US weeds, however, two genetically distinct groups of weedy rice were identified. The authors of this study were able to use weed appearance records and the fact that no wild *Oryza* grows in Italy to rule out hybridization with the wild ancestor (*O. rufipogon*) as a potential cause of origin. They were not, however, able to rule out crop-crop hybridization. From these and other studies worldwide (e.g. [19,20]), weedy rice was shown to have evolved repeatedly and independently. Although many of these studies suggested de-domestication as the primary cause for the origin of weedy rice, none had strong evidence for that mechanism over another.

2.2. Insights from candidate genes

With the repeated independent evolution of weedy rice worldwide, many of the same phenotypic traits have convergently evolved, including highly shattering seed, dark-pigmented pericarps (and associated seed dormancy), and highly competitive growth against crop varieties. This phenotypic convergence of weedy traits raises questions on the extent to which similar underlying genetic mechanisms have been involved in this convergent phenotypic evolution. A wealth of previous work exists in cultivated rice that has characterized so-called domestication genes and causal mutations that underlie domestication traits (e.g. *sh4*, *Wx1*, *Rc*, etc.). For the wild-like traits that have emerged during weedy rice evolution, these domestication genes provide prime candidates to assess whether mutational reversions at the domestication loci underlie the phenotypic reversions occurring during de-domestication, or whether other genes or regulatory regions are responsible. In this section we compare inferences from two well studied candidate genes, *sh4* (controlling seed shattering) and *Rc* (controlling pericarp pigmentation).

The re-acquisition of seed dispersal mechanisms is one of the most important steps in escaping dependence on humans, and as such, seed-shattering is among the most ubiquitously evolved traits in weedy rice worldwide. Previous work in cultivated rice has identified several shattering-related genes, of which *sh4* is the major causative gene (reviewed in [21]). Sequencing this gene in weedy rice worldwide showed that most weedy rice strains carry the non-shattering domestication allele, suggesting the importance of other parts of the genome in the reversion to shattering [19,22–24]. Further quantitative trait locus (QTL) mapping of the shattering trait in two crop-weed hybrid mapping populations representing the two major US weedy rice ecotypes (SH, BHA) identified 7 QTL [25]. Interestingly, none of the QTLs identified in this weed × crop cross overlap with *sh4* or other well-characterized cultivated rice shattering loci. These findings also suggest that many different underlying genetic mechanisms can lead to convergent phenotypic evolution for quantitative traits such as shattering.

Like shattering, re-acquiring seed dormancy is an important step in the evolution of weedy rice. The gene *Rc* encodes a transcription factor that has been shown to pleiotropically control both pericarp color and seed dormancy [26]. The non-functional domestication *rc* allele results

in white pericarps and a reduction in dormancy, while the functional *Rc* allele results in red pericarps and variable dormancy. Sequencing of this gene in US weedy rice revealed that these weed strains contain a functional *Rc* allele. Unlike shattering and the *sh4* domestication allele, the white pericarp *rc* allele was not universally under selection during rice domestication, and some rice landraces still have pigmented pericarps and functional *Rc* alleles [27]. Gross et al. [27] proposed that the presence of functional *Rc* alleles in US weeds is a legacy of these weeds having evolved from landraces that never underwent selection for white pericarps. Functional *Rc* alleles can also be found in some Asian weed strains. For those growing in Southeast Asia, the functional alleles have likely originated in part through introgression from local wild rice populations; the high frequency wild-derived *Rc* alleles in these weeds may reflect strong selection for seed dormancy [6,24].

Candidate gene studies have furthered our understanding of weedy rice evolution by suggesting (as in the case of shattering) that many convergent phenotypic traits show no evidence of convergent molecular evolution. Conversely, genes like *Rc* have shown that similar underlying genetic mechanism can play a large role in convergent phenotypic evolution, but that the origins of the haplotypes should be investigated further.

2.3. Insights from whole genome sequencing

Recent advances in DNA sequencing technologies have made population-level genome-wide sequencing projects relatively cheap and easy to undertake. One recent weedy rice study that capitalized on this technology was Li et al. [5] in which 38 US weedy rice genomes (18 SH, 20 BHA) were compared to 145 previously published *Oryza* genomes including 89 cultivated rice accessions, 53 *O. rufipogon* accessions and three Chinese weedy rice accessions. Results from this study re-confirmed the origins of US and Chinese weedy rice as de-domesticated forms, provided relative divergence times, and identified regions of the weedy rice genomes that show signatures of selection (decreased π) and selective sweeps. The relative divergence times suggest that BHA weeds are older than SH and Chinese weeds, which suggests that BHA diverged from the very earliest ancient crops while SH and Chinese weeds diverged much later. This study identified 121 and 118 100-kb windows of low diversity in SH-*indica* and BHA-*aus* comparisons, respectively. Of these, only 12 windows were shared between the two comparisons. These 12 windows would be of particular interest for further study, as they are evidence of limited convergent molecular evolution. The broader implication of these data, however, is that the two US weed strains have convergently evolved phenotypes using largely different underlying genetic mechanisms. Although weedy rice accessions from more places around the world should be sequenced and analyzed in a similar manner, the results from this study provide a useful foundation for future comparative studies.

2.4. Avenues of future work

The next steps in the study of weedy rice evolution follow easily from the work described in section 2. Each weedy ecotype world-wide should be probed for independent origins and placed in a framework describing where and how many independent origins have occurred. Molecular and phenotypic evidence should be used to pin down relative divergence times similar to Li et al. [5]. With origins and relative divergence times we can begin to answer important questions related to the circumstances leading to the evolution of weedy rice.

Advanced techniques can also be used to continue identifying the genetic basis of the agricultural weed syndrome traits. Connecting phenotype to genotype is not easy, but combining transcriptome, methylome and conventional QTL techniques should be used in a broad range of weedy rice ecotypes to begin to understand the genetic basis of important weedy traits.

Another obvious avenue of future work is more whole genome

studies. Li et al. [5] focused largely on US weedy rice, but more accessions from world-wide occurrences can be collected and analyzed in a similar manner. Additionally, weedy rice is evolving in a rapidly changing agricultural environment. The introduction of both hybrid cultivated rice and herbicide resistant cultivars is changing how weedy rice interacts with its environment and thus, how its genome is evolving [28,29]. Whole genomes of post-introduction weedy rice should be sequenced and used to evaluate how weedy rice is adapting to this new environment. Studies characterizing these rapidly evolving genomes could provide important insights not only for understanding the genetic underpinnings of weed adaptation, but also for devising more effective weed control strategies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.egg.2018.03.005>.

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