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## Genetics and possible origins of California weedy rice

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### Introduction

Weedy rice (*O. sativa* f. *spontanea* Rosh.) is an emerging weed of rice in California. While weedy rice was present early in California rice culture (Bellue 1932), adoption of a continuously flooded growing system and use of certified seed allowed for presumed eradication of weedy rice in the 1950s (Miller 1979). After several decades of no weedy rice detected in California, weedy red rice was identified in a dry-seeded rice field in 2003 (Londo and Schaal 2007). Since then, weedy rice has since been identified in seven rice-growing counties in California infesting over 130 fields and 13,900 acres, as of 2018 (Luis Espino, personal communication, August 4, 2019).

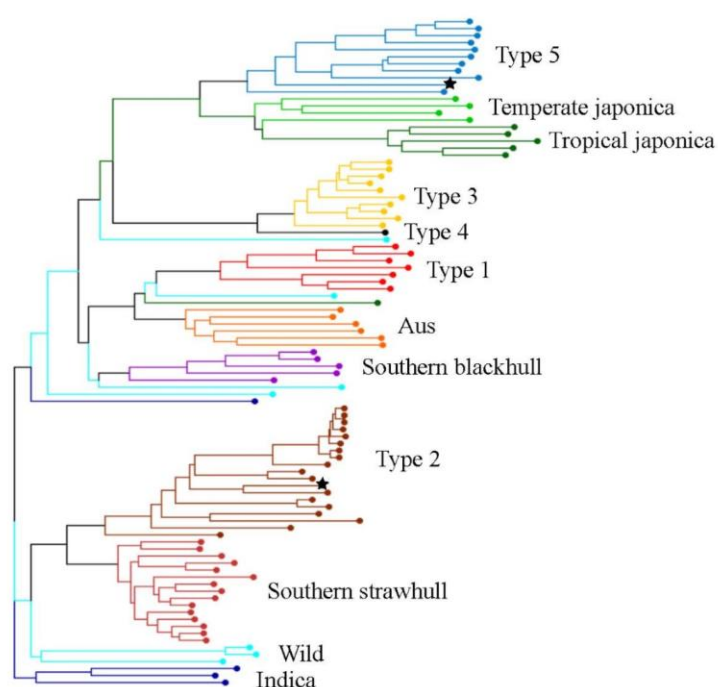
Weedy rice is a serious weed in other rice-growing regions of the world, leading to yield losses of up to 80% in the southern United States (Estorninos et al. 2005), and a greenhouse study of California weedy rice indicated possible yield losses of up to 69% (unpublished data). Currently, there are six known biotypes of weedy rice present in California, which are distinguishable by the presence of awns, grain size, plant height, and seed hull and pericarp color (Table 1). The recent re-discovery and rapid spread of weedy rice raises questions of where this weedy rice is coming from, and what factors may play a role in preventing its further spread. A genetics study was conducted to determine the relationships of weedy rice biotypes found in California to each other and to other weedy, wild, or cultivated rice types, and to investigate possible origins of the weed in California.

### Materials and Methods

Weedy, wild, and cultivated rice samples selected for genotyping analysis totaled 96 samples. Forty-six weedy rice samples from California were obtained from commercial rice fields in five counties (Glenn, Colusa, Butte, Yuba, and Sutter Counties), and



**Figure 1.** Weedy rice panicles in a field in Colusa County. (photo credit: Luis Espino)



**Figure 2.** Phylogenetic tree showing relationships among 96 rice samples, including 46 California weedy samples, 20 weedy rice samples from the southern United States, 8 wild rice species, and 22 rice cultivars. The starred individuals are two red-pericarped specialty rice varieties grown in California.

## Results and Discussion

In a phylogenetic analysis, individuals are connected to their closest relatives to form a genetic tree (Figure 2). Most cultivated and weedy rice individuals clustered by rice type, while the wild rice samples were scattered, which reflects the wide diversity in wild rice species. The California weedy rice samples were grouped into four major clusters, which correspond to five biotypes categorized by hull color, grain size, and presence of awn (Figure 2, Table 1). Type 1 weedy rice was placed near a wild rice sample and one temperate *japonica* rice variety, as well as *aus* rice varieties. Type 2 weedy rice was placed near southern strawhull weedy rice and some wild rice. Type 3 and Type 4 weedy rices were placed together, and are close relatives of each other. Type 5 weedy rice was placed near both temperate and tropical *japonica* rice varieties. The southern US weedy rice individuals were grouped into two clusters, the strawhull weedy rice and blackhull weedy rice.

STRUCTURE analysis was used to examine population structure and identify hybridization or gene flow, by assigning each individual's genome to genetic populations. While most individuals assign to one population, some individuals assign to more than one, indicating gene flow (Figure 3). For example, some Type 2 weedy rice individuals show gene flow from strawhull weedy rice from the southern US, *indica* rice, or wild rice species. Despite some gene flow, the rice types are distinct from each other. A principal component analysis based on overall genetic differences shows that weedy rice biotypes are more similar to other rice types than to each other (Figure 4).

represent five weedy rice biotypes (Table 1). The sixth weedy rice biotype was not included in this study, because it was not identified until after this study began. To allow comparison with other rice groups, we included 20 weedy rice samples from the southern US (Arkansas, Mississippi, Missouri, Louisiana, and Texas), 8 wild rice accessions, and a total of 22 cultivated rice accessions: 6 temperate *japonica*, 4 tropical *japonica*, 5 *indica*, 5 *aus*, 1 aromatic group V, and 2 red-pericarp specialty rice accessions. These samples were obtained from USDA collections and from the Rice Experiment Station (Biggs, California). Genomic DNA was extracted from leaf tissue from each sample, and genotyped with 98 microsatellite (SSR) markers and 1 *Rc* gene-specific marker. The *Rc* gene is responsible for the red-pigmented pericarp of wild, weedy, and cultivated specialty red rices. Following amplification and detection of marker alleles, neighbor joining phylogenetic, STRUCTURE, and principal component analyses were performed to examine relationships and genetic structure among rice samples.

**Table 1.** Descriptions of the five weedy rice biotypes from California used in this study, and the counties where each biotype has been identified, as of 2018. Type 6 was not included in this study, as it was not discovered until after the study began.

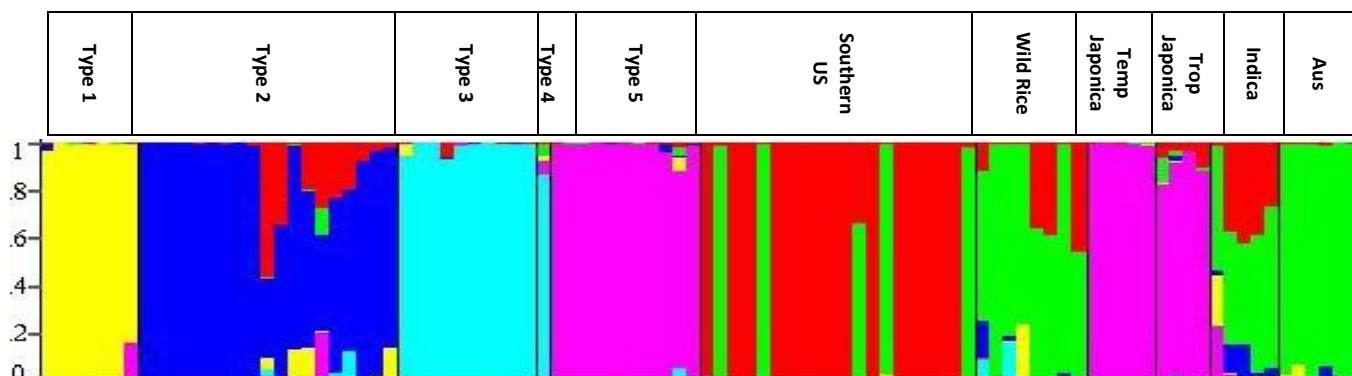
Biotype	Hull Color	Awns	Grain size	Plant height	Counties present
Type 1	Strawhull	Absent	Short grain	Tall	Butte, Glenn, Placer, San Joaquin, Sutter, Yuba
Type 2	Bronzehull	Absent	Medium grain	Tall	Butte, Glenn, Sutter, Yuba
Type 3	Strawhull	Long	Medium grain	Tall	Colusa, Glenn
Type 4	Blackhull	Long	Short grain	Short	Glenn
Type 5	Strawhull	Partial or absent	Medium or long grain	Tall	Butte, Sutter, Yuba
Type 6	Blackhull	Long	Medium	Tall	Butte

The recent increase in detected weedy rice cases raises the question of where this weedy rice has come from. Most biotypes, with the exception of Type 5, are genetically related to rice groups not grown in California, meaning that they were most likely imported into California from elsewhere, likely through contaminated seed. Type 5 weedy rice was shown in phylogenetic and STRUCTURE analyses to be closely related to *japonica* cultivars, but is still distinct from both, and it is unclear whether it could be derived directly from the temperate *japonica* cultivars grown in California or from *japonica* cultivars outside California and imported. It is possible that this biotype is derived from California cultivars of rice and went unnoticed for some time prior to detection, but it is also quite likely that this biotype originated.

Another possibility for the origin and spread of California weedy rice is from the cultivation of red-pericarp specialty rice varieties within California. While the majority of rice-growing acreage in California is devoted to non-colored pericarp rice production, some specialty colored-pericarp rice varieties are grown at a commercial scale. Two non-certified introduced cultivated red-pericarp specialty rice varieties grown in California were clustered with Type 2 and Type 5 weedy rice (Figure 2), indicating possible genetic contributions from red-pericarp varieties into weedy populations. These red rice varieties have not gone through California's third-party variety certification and inspection process and have been previously implicated in rice contamination (Timothy Blank, personal communication, March 7<sup>th</sup>, 2017). This potentially problematic relationship calls for careful management of rice seed and certification to avoid the contamination of white-pericarp cultivated rice acreage with red-pericarp rice.

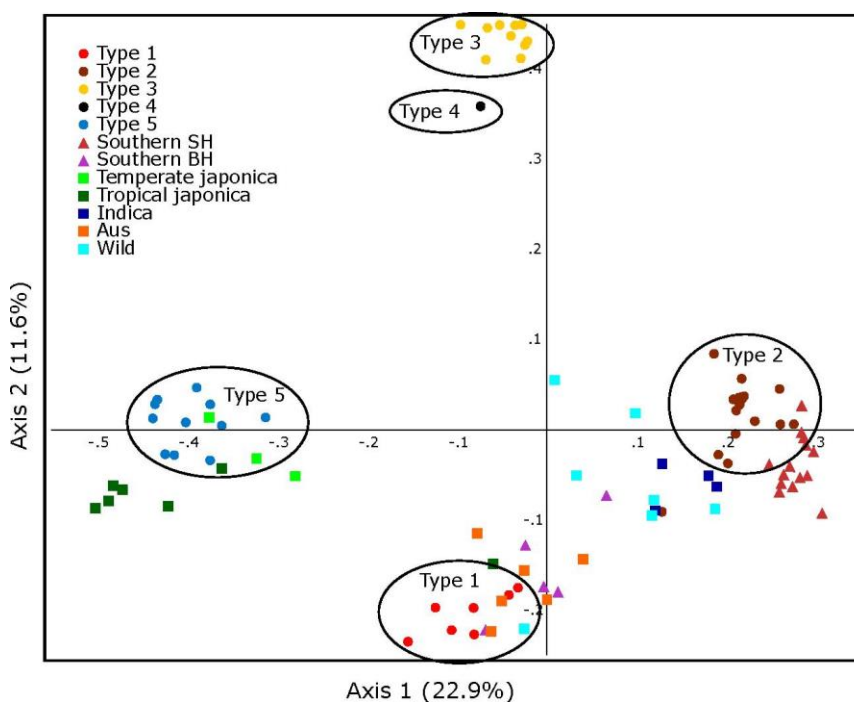
Overall, the genetic analyses provide some insights into the ancestry of California weedy rice. Type 1 weedy rice is likely derived from *aus* rice or possibly a wild rice species, as is the blackhull weedy rice from the southern US. These two American weedy rice biotypes may have a single origin from Asian rice or separate origins followed by hybridization with each other. Type 2 weedy rice is most closely related to strawhull weedy rice from the southern US, and these two groups are likely derived from *indica* rice, with possible contributions from wild rice. Type 4 weedy rice was only found in one field in California, and is likely derived from Type 3. Type 3 and Type 4 weedy rice are highly differentiated from other groups and have unclear origins, but may be related to wild rice. Type 5 weedy rice is derived from *japonica* rice.

**Figure 3.** Genetic assignments of rice individuals into six genetic clusters in STRUCTURE analysis. Colors represent genetic clusters. The vertical bars represent the portion of an individual's genotype assigning to each cluster, meaning that vertical bars with multiple colors represent admixed or hybrid individuals.



The four major biotype groups all contained samples from multiple different counties, and most counties that have weedy rice have more than one type. (Table 1). The spread of biotypes across a large geographic area is likely the result of seed movement by humans, either through contaminated seed stocks or equipment within California or through accidental importation of weedy rice from other rice-growing regions. This highlights the need for growing weed-free certified seed in California and encouraging growers to prevent the spread of weedy rice on contaminated equipment. Recent regulations regarding the importation of used equipment and the requirement for only planting certified seed or seed from a third-party quality assurance program should aid in these efforts (Prevention and Eradication of Weedy Rice, CA 3 CCR §2852.5, 2018).

More information on weedy rice in California can be found at <https://caweedyrice.com/>



**Figure 4.** Genetic clustering of rice samples in principal components analysis. Separation on the two axes account for 22.9% and 11.6% of the total variation present in the sample set.

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