Using DNA fragments to measure genetic variability within a natural stand of wild rice in northwestern Wisconsin

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INTRODUCTION

Wild rice, *Zizania palustris*, is the only cereal grain native to North America. It is important to the people of Wisconsin who value wild rice as an integral part of their culture, for wildlife that depend on a healthy natural crop, and as an economic resource. Commercial cultivation of wild rice began in 1950 in Minnesota. Improvement of commercial cultivars depends on the genetic variability found in natural stands of wild rice. Genetic variability confers protection from migrating birds, stormy weather and other environmental stresses. Wild and cultivated stands of *Z. palustris* express a high degree of variability, such as maturing at different heights and at different times, seed shattering and dormancy. A prior study of 17 highly genetically diverse sites in Wisconsin showed that diversity can be lost from small populations due to genetic drift (Lu, *et al.*, 2005). Native populations of wild rice are in decline for many reasons, including shoreline development and heavier use of lakes for recreation. Preservation of isolated populations of wild rice is important to preserving genetic variation of the species.

This study sought to compare phenotypic characteristics of a natural stand of American wild rice with genetic variations found using a simple Restriction Fragment Length Polymorphism (RFLP) technique. In 1972, traditional controlled breeding of wild rice began at the University of Minnesota. Development of a genetic linkage map of the wild rice genome began at the University of Minnesota in 1993. *Z. palustris* is closely related to *Oryza sativa*, the white rice we usually see on our dinner tables. Similarity to *Oryza spp.*, as well as to other grass species such as maize, sorghum, and sugar cane, has been useful in comparing the genetic maps between species. These plants share large parallel areas of their chromosomes. Research techniques used to understand one species of grass can be used to understand another (Hass *et al.*, 2003). Even though there is considerable genetic colinearity between *O. sativa* and *Z. palustris* (nearly 85% of the RFLP markers that have been mapped) there is significant chromosomal differences between the two species: *O. sativa* has a chromosome number of n=12; *Z. palustris* has a chromosome number of n=15. *Z. palustris* also has twice the DNA content of *O. sativa* (Hass *et al.*, 2003). As of 1999, the wild rice genome had 121 RFLPs mapped.

Most previous genetic research focused on developing more productive commercial crops of wild rice. Concern for diminishing wild populations has prompted research that will help to understand genetic variation of wild populations (Lu, *et al.*, 2005). Understanding genetic uniqueness and environmental variability will help to preserve natural stands of wild rice.

MATERIALS AND METHODS

Site

The precise location is withheld out of respect for those who harvest rice at this site. The rice collected grows in an extended bay off a larger lake. The deepest part of the bay is about 5m. Rice is distributed along the shores of the bay until the open water is reduced to a channel only about 2m. deep and just wide enough for a canoe. Water in the marshy area of the back bay is much colder and clearer than in the deeper open water, suggesting the presence of springs. Rice dominates the marshy area at the edges of the open water. Where the water is more shallow wild rice is mixed with Swamp Loosestrife *(Decodon verticillatus)*, Cattail *(Typha spp.)*, Sedges *(Carex spp.)*, and other aquatic plants. The location of each plant sampled was recorded on a large scale map based on landmarks. We limited samples to those we could collect from the canoe and selected plants throughout the bay.

Plant material

Two leaves from each of 80 rice plants *(Z. palustris)* were collected on a single day in July 2006. Seven characteristics were recorded for each plant from which samples were taken: water depth, depth of the substrate, the height of the plant from the bottom, stem color, seed color, flower color, and panicle length (Lu, *et al.*, 2005). Fungus and insect damage was more evident in 2006 than in 2005. Many panicles contained empty hulls. Samples were kept on ice until they could be stored in a freezer (-20C°).

Genetic Analysis

Portions of the alcohol dehydrogenase *(Adh)* genes were tested for use as primers in this study (RCS1, RCH1, RCH2, and RCH3). These are middle repetitive sequences conserved throughout the *Poaceae* (Hass *et al.*, 2003). In three samples used for the selection of primers there were no bands from the RCH1 and RCH2 primers. This was consistent with the study done by Hass *et. al.* (2003) when using PCR to amplify DNA. Thus subsequent PCRs were done using only the RCS1 primers:
(5'-GTTCGCTTGTTGTTGTTG-3'and 5'-TCCTCATCATCCTCCCCATC-3') and RCH3 primers:
(5'-GATAATGAGCCACCGGAAGA-3' and 5'-TGCTAGCCAAGTTGTTGCAG-3').

100 mg of each leaf sample, minus the midrib, was chopped, and then ground in a mortar to a fine powder in liquid nitrogen. Genomic DNA was extracted using a Qiagen DNeasy Plant Mini Kit©. PCR reactions were run using GoTaq™ buffer and Taq polymerase (Promega), **3-6 ηg DNA** and 20 µM primers. Thermocycler settings were 7 min at 95°C followed by 25 cycles of 95°C for 1 min, 52°C for 2 min, 72°C for 2 min, and the final extension at 72°C for 10 min. 1% agarose gels were run to sort bands into distinct profile classes.

RESULTS

Characteristics for each sample:

No correlation between the plant or site characteristic and genetic profile was obvious. Additional analyses may show a link. It is much more difficult to link genetic characteristics to morphological characteristics from plants grown in the wild than in those grown in controlled conditions. Human activities were not controlled for: recreational boating and fishing occur throughout the study area.

Aeration of sediments by springs, or siltation due to boat traffic impact the quantity of oxygen available to the plant and water clarity will affect light availability. Boat traffic varies with location within the study area. Grazing by animals affects plant height. It was clear that some stems had been sheared off or damaged by grazing animals. Geese and some ducks are particularly fond of the newly emerging rice plants early in the summer. Plants may survive, but often height is reduced and they may have little or no seed production. Temperature will have many impacts on the ability of a plant to grow to its full potential. If temperatures are too warm wild rice becomes susceptible to fungal diseases. If water is too cold growth will be slower and stems may show more intense coloration with anthocyanin. Variations in nutrients throughout the area also impacts plants.

Water depth. Depth of the water is a physical characteristic of the growth circumstances of each plant and can limit height. Fluctuations in water depth appear to benefit rice when timing is right. If water is too shallow when plants mature they may topple under their own weight. Average depth at this site on the date samples were collected was 0.8m. Depth ranged from 0.3 to 1.2m deep.

Depth of the substrate. Average depth of substrate was 6.7cm. with some plants in substrate as deep as 20cm. and a few growing out of apparently solid ground – although most of those were noted as growing out of or near submerged wood.

Height of the plant measured from the bottom. The average height of plants measured 1.8m. Plants ranged from 1.0m. to 2.6m. tall. Taller plants were consistently found in deeper water. Under controlled growing conditions W. C Kennard (2002) has shown that there are linkages between height and genotype.

Stem color, seed color, flower color. Kennard (2002) found that stem color was linked to genetic variation in the two cultivated *Z. palustris* lineages he studied. I was not able to relate color to genetic variation in this study.

Panicle length. Kennard (2002) also found that panicle length could be attributed to genetic variation. Average panicle length of samples measured on this date was 19.5cm. Maximum length was 30.5cm. and minimum was 12.7cm.

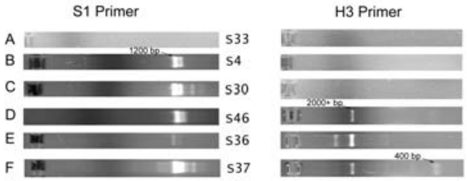

Figure 1. Representative PCR results of wild rice samples. Samples were divided into six genetic classes. *(A)* Leaf sample 33 was not amplified using either the RCS1 or RCH3 primers; *(B)* Leaf sample 4 was amplified with RCS1 (2 bands) but not RCH3; *(C)* Leaf sample 30 was amplified with RCS1 (3 bands) but not RCH3; *(D)* Leaf sample 46 was amplified with RCS1 (2 bands) and RCH3; *(E)* Leaf sample 36 was amplified with RCS1 (2 bands) and RCH3 (2 bands); *(F)* Both RCS1 and RCH3 primers amplified with multiple and various bands.

Genetic variability:

When the results of the genetic analysis were examined they could be arranged into six classes (Fig. 1.). Approximately 9% of the samples *(A)* did not amplify at all using PCR and the two primer pairs (RCS1 and RCH3), see Table 1. Nearly 63% of the samples did amplify with the RCS1 primers (both *B* - two bands and *C* - three bands), but not the RCH3 primers. The remaining 29% of samples amplified with the RCH3 primer as well as the RCS1 primer. Class (*F*) was a conglomeration of many variants of the RCH3 primer.

Class	Percent
A	8.8%
B	50.0%
C	12.5%
D	15.0%
E	5.0%
F	8.8%

Table 1. Percentage of wild rice samples that grouped into each genetic class.

DISCUSSION

Working under controlled growing conditions with cultivars of commercially grown *Z. palustris*, Kennard (2002) was able to show genetic linkages to traits desirable for commercial production. We were not able to make such linkages with our analysis. Lu (2005) found that genetic variability within wild stands of *Z. palustris* was related to the size of the stand. Larger areas showed greater genetic variability. He also found that populations of wild rice that were geographically isolated showed significant variation from distant populations. Populations that grew in closer proximity were more similar to each other. Even though this site is not a large population of *Z. palustris*, it does show considerable morphological and genetic variability. Although half the plants sampled are genetically similar using the RCS1 and RCH3 probes, there is considerable variation in the remaining half. This matches observed variation in the appearance of the plants at this site. Lu (2005) warns that small populations are at risk of losing genetic diversity through random genetic drift. This small site retains genetic diversity either because of its unique characteristics or some other unknown means. It argues for heightened concern for its preservation.

Field studies are not the most efficient way to look for genetic variation within a population of plants because of travel constraints, observation time required, and the lack of environmental controls. However, the use of genetic markers is a useful way to record a snapshot of a population of wild growing plants at a moment in time. This place now has a genetic signature which will certainly change over time as genetic drift, changing environmental conditions and the possible addition of seeds and pollen from outside sources are introduced.

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