

# Wild Rice Conservation Genetics in the Bay of Green Bay

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## Introduction



Figure 1. Native wild rice along the bay of Green Bay.

Northern wild rice (*Zizania palustris*; Fig 1) is a native aquatic plant found in wetlands of northcentral and eastern United States and Canada. Historically, wild rice was plentiful in Green Bay coastal wetlands; however, populations declined as European human settlement and industry developed along the shores of Green Bay (Woessner 1977).

The field of conservation genetics investigates how genetic information present in populations may affect a population's response to changing environmental conditions and whether a population will adapt to and survive, or if it will experience significant reduction in growth and reproduction, and likely face extinction (Frankham 1995). Knowledge of the current population genetics of native wild rice in Wisconsin will help to inform wild rice restoration management efforts.

Our objectives were to develop simple sequence repeat (SSR) loci for use in conservation genetic studies of *Z. palustris* by (1) evaluating previously published SSR loci developed for *Z. palustris* (Kern and Kahler, 2014), *Z. texana* (Richards et al 2007) and *Z. latifolia* (Quan et al 2009); (2) using the recently published genome sequence of *Z. palustris* to develop new SSR loci that can be used to evaluate population genetic variation.

## Materials & Methods

- Wild rice leaves were collected from 2017-2023 in previously re-seeded areas along Green Bay (Fig 2)
- SSR loci from previously published studies (listed above) were evaluated. Twenty loci were identified from the *Zizania palustris* genome sequence and are currently being tested.
- DNA was isolated from 61 leaves. Polymerase chain reaction (PCR) with 6-fam labeled forward primer was performed for 8 loci of the 20+ SSR loci currently in development.
- Allele representatives were Sanger sequenced to confirm SSR.
- Fragment analysis was conducted at the Cornell Institute of Biotechnology.
- Genotypes were determined using Geneious Prime 2019. (Biomatters; <http://www.geneious.com/>)
- Genotypes were analyzed using GenAlEx (Peakall and Smouse 2012).



Figure 2. Four of the *Zizania palustris* and *Z. aquatica* populations sampled.

## Results

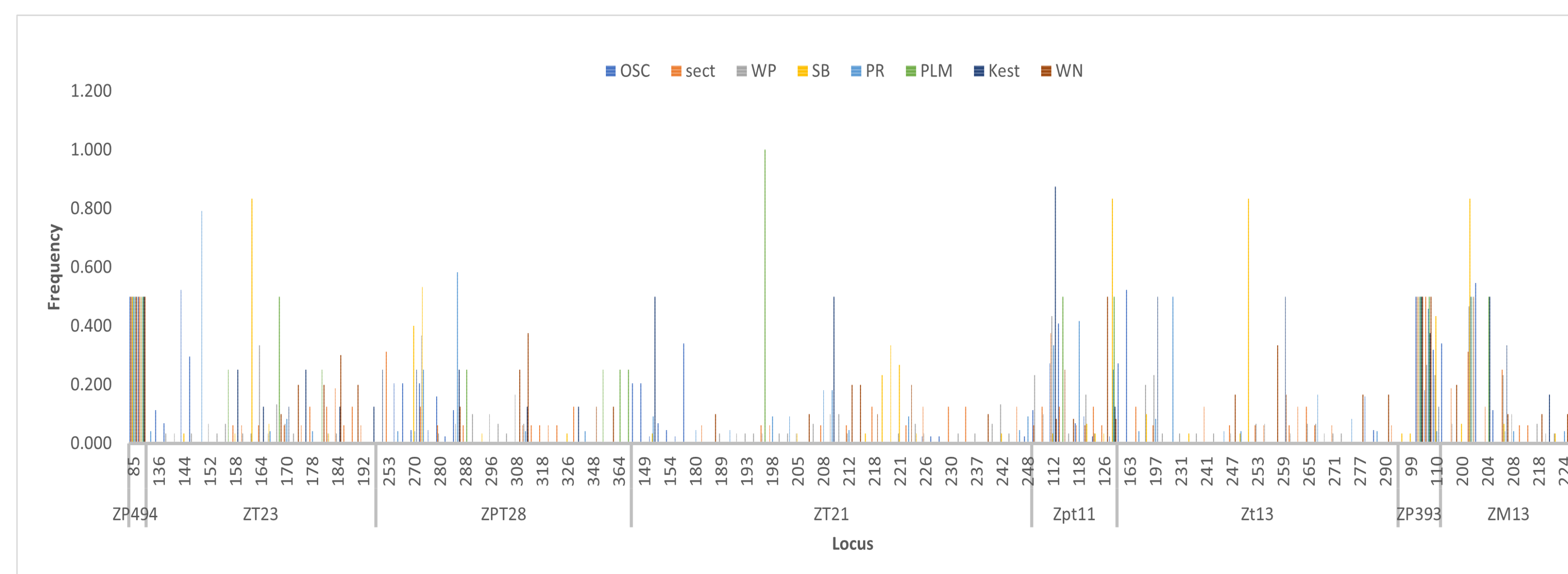


Figure 5. Allele frequency across all populations for the eight SSR loci tested. Five of the eight loci studied here, show high levels of allelic diversity within and across the eight populations. Allele size (i.e., PCR product size) is shown as a whole number. OSC=Oconto Sportsman Club, sect=Duck Creek Delta, WP=Winegar Pond, SB=Seagull Bar, PR=Peštigo River, PLM=Prairie Land Management, Kest= Kesters, WN=wildlife nurseries.

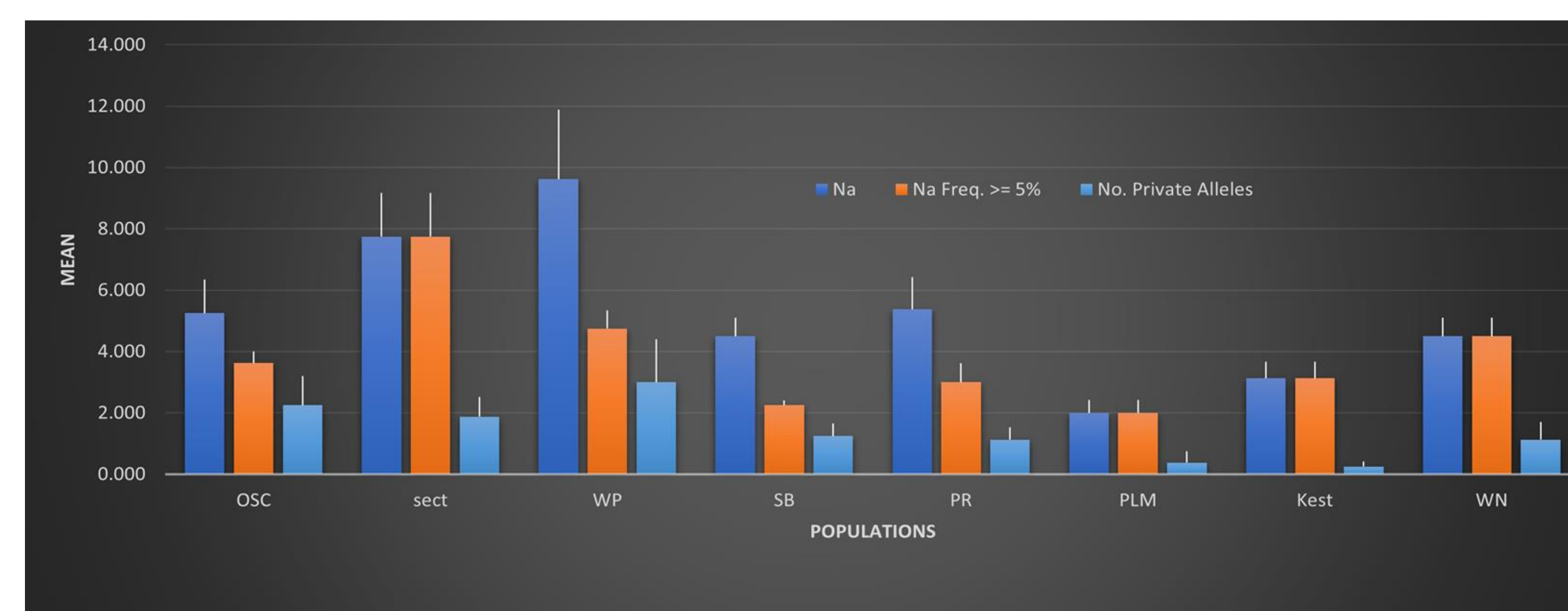


Figure 4. Allelic patterns across populations for eight SSR loci show moderate to high allelic diversity. Na=number of alleles; NaFreq. >=5%=number of alleles with a frequency greater than or equal to 5% in the population; No. Private Alleles=alleles that only occur in one population.

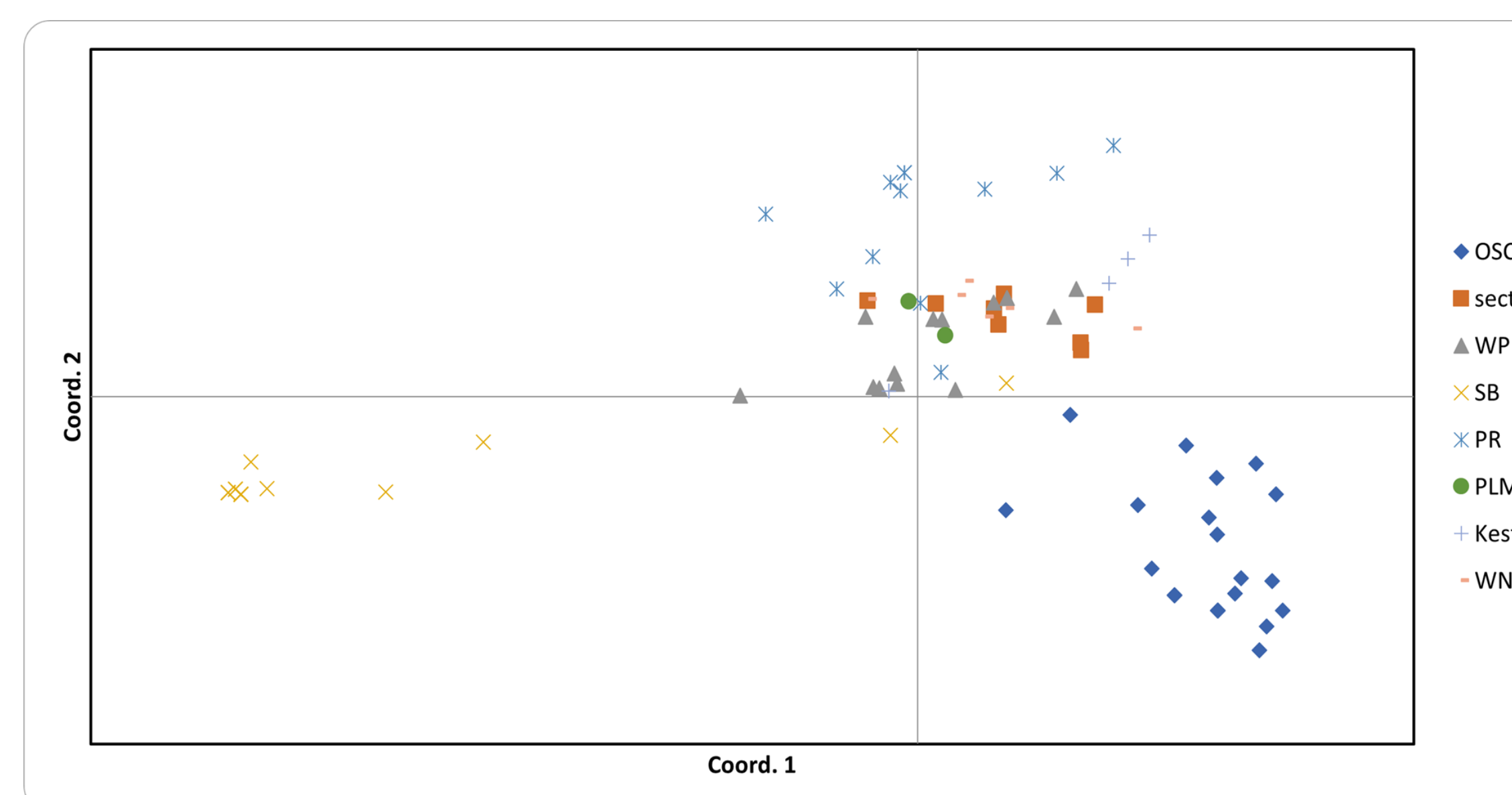


Figure 6. Principal coordinates (PCoA). Samples with similar genotypes are clustered together. Overall, there appears to be some level of gene flow among the *Z. palustris* populations. The Seagull Bar population appears to be distinct, for the most part, from the other *Z. palustris* populations requiring further investigation. These loci identify the one *Z. aquatica* (OSC) site as genetically isolated from the *Z. palustris* populations, as would be expected for two closely related species.

## Conclusions

The data presented here suggests these SSR loci will be useful for conservation genetic study of Wisconsin wild rice populations. Moderate to high levels of allelic diversity was verified by random Sanger sequencing.

Lu et al. (2005) reported limited gene flow between Wisconsin northern wild rice populations from different lakes or rivers based on isozyme markers. While we have not yet performed more extensive data analyses, once we have a few more populations and loci, we shall consider drainage system.

Recently Single Nucleotide Polymorphism (SNPs) were used to assess genotypic variation of genomes (Shao et al. (2019). This is promising marker system that can provide numerous variable sites for data analysis.

The next steps in this study include (1) include populations sampled in 2023 in analyses; (2) continue to test additional SSR loci; (3) conduct more comprehensive data analyses; and (4) continue to use this study to mentor undergraduate research students.

The information that will be acquired from these studies will be valuable to wetland managers for restoration of native wild rice populations.

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