

Part 3:

Genetics and Reproduction

A GENETIC OVERVIEW OF WILD RICE FROM THREE DECADES OF BREEDING

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ABSTRACT

Wild rice breeding research has been conducted at the University of Minnesota since 1972. During that time, breeding research has yielded an improved understanding of wild rice genetics. Although the cultivation of wild rice has initiated the process of domestication, cultivation and domestication do not necessarily occur simultaneously. Some traits specific to crop domestication will be discussed in relation to wild rice. In addition, several key traits, such as seed shattering, yield, and disease resistance, have been especially emphasized in breeding efforts. A number of other traits, both quantitative and qualitative, have been investigated in breeding experiments, resulting in preliminary knowledge about their inheritance. Diseases of wild rice have been studied in cultivated and natural populations. Methods used in improving wild rice cultivated varieties (cultivars) have mainly focused on individual plant selection in diverse populations, as well as selection among related families of wild rice. Phenotypic variability in cultivars and breeding populations still appears to be quite high even after several decades of improvement. Quantifying genetic variability within and among natural populations has not yet been done in a systematic fashion. Recent developments in molecular gene mapping makes this kind of assessment more feasible. Since some natural stands have declined or disappeared, understanding their genetic uniqueness must be a high priority to guide those who make decisions related to preserving or restoring declining stands. Researchers of both cultivated and natural wild rice share a common goal of preserving natural stands of wild rice for present and future generations.

HISTORY AND CONCEPTS OF WILD RICE DOMESTICATION

Wild Rice Species' Natural Range

There are four species of wild rice, three of which are native to North America. *Zizania palustris* has been adapted to cultivation and is also the species that is gathered from the wild. Its natural range is primarily in the Great Lakes region. *Z. aquatica* is a much larger plant, sometimes reaching a height of 4.57 m, with smaller seed than *Z. palustris*. The natural range of *Z. aquatica* overlaps with *Z. palustris* but tends to lie more along the Eastern Seaboard of the United States. *Z. texana* is a perennial found only on a portion of the San Marcos River in Texas and is on the U. S. Endangered Species List. *Z. latifolia* is also a perennial and is native to Asia. It differs from the other three species by having 17 pairs of chromosomes instead of 15.

What Is Wild Rice?

Wild rice can be viewed from several perspectives, two of which are described below. Other perspectives on wild rice will likely be covered in other papers. It is not the purpose of this paper to challenge the validity of other viewpoints on the nature of wild rice. Rather, for the purposes of this paper, we will deal with the following perspectives.

From an agronomic/breeding viewpoint, wild rice is a cultivated crop selected from the species *Zizania palustris*. This crop is grown much like rice (*Oryza sativa*) in flooded soils. Being a monoecious, cross-pollinating plant, controlled pollination can be carried out in a manner similar to corn. However, the way it is managed under cultivation more resembles other cereal grains like rice, oats, and

wheat. The fact that it is only recently being domesticated means it still retains some wild characteristics like seed shattering and dormancy.

From a molecular/genetic viewpoint, wild rice is a diploid organism with 15 pairs of chromosomes. It is not a wild species of rice (*Oryza sativa*), as the common name suggests. Nevertheless, broad DNA similarity comparisons strongly suggest that wild rice is more closely related to rice than it is to corn, oat, barley, or wheat. More specific research on characterizing wild rice genetic material shows that large areas of its chromosomes parallel those of rice. So far, there have been 121 Restriction Fragment Length Polymorphisms (RFLPs) mapped on the wild rice genome.

Wild Rice Cultivation and Domestication

Since wild rice is considered a crop by those who produce it commercially, it is helpful to understand the processes by which it has become a crop. It is especially important to understand the concepts of cultivation and domestication, and the difference between them. "To cultivate means to conduct those activities involved in caring for a plant, such as tilling the soil, preparing a seedbed, weeding, pruning, protecting, watering, and manuring" (Harlan 1975). By this description, cultivation of wild rice began in 1950, when the first one-acre wild rice paddy was successfully harvested. Since then, agronomic practices have been developed for cultivation, at first only by growers, and subsequently with the aid of agronomic research.

However, domestication differs from cultivation. "Cultivation is concerned with human activities, while domestication deals with the genetic response of the plants being cultivated. It is quite possible to cultivate wild plants, and cultivated plants are not necessarily domesticated" (Harlan 1975). By this distinction, domestication began with the discovery of a nonshattering or "flag" plant within a wild population that was being cultivated in a paddy. A brief history of cultivated wild rice has to include this discovery as well as the initial successful attempt at cultivation. (See Table 1.)

Table 1. A brief history of cultivated wild rice.

Year	Event
1950	First one-acre wild-type (shattering) paddy established
1963	Nonshattering "discovered"
1968	Nonshattering Johnson cultivar first grown
1972	Kosbau Brothers release K2 cultivar
1972	Breeding project begins at University of Minnesota
1978	University of Minnesota releases first cultivated variety
1978	California is first state to produce 100,000 lbs of cultivated wild rice
1993	RFLP mapping project begins at University of Minnesota

Most crop species have a much longer history of domestication than this, usually several thousand years. This raises the question of whether cultivated wild rice can be considered fully domesticated. "Since domestication is an evolutionary process, there will be found . . . a range of morphological differentiations from forms identical to wild races to fully domesticated races. A fully domesticated plant or animal is completely dependent upon man for survival" (Harlan 1975). The kinds of adaptations that result from the activities associated with cultivation, and which characterize domestication, are summarized by Harlan (1975) as follows:

- I. Harvesting results in
 - A. increase in percent seed recovered through
 1. nonshattering (retention of seed on the plant as it ripens);
 2. more determinate growth (i.e., uniform maturation of seeds).
 - B. increase in seed production.
- II. Seedling competition results in:
 - A. increase in seedling vigor
 - B. more rapid germination (loss of dormancy)

Of these possible adaptations, there are only two that have so far been significantly impacted by the cultivation of wild rice—nonshattering and increased seed production (yield). The rest have not been significantly changed, either unconsciously by cultivation or through conscious breeding efforts. Therefore, cultivated wild rice probably should not be considered a fully domesticated crop. However, to the extent that shattering (a wild trait important for seed dispersal) has been greatly reduced, cultivated wild rice is developed to be less fit to survive in the wild.

KEY TRAITS EMPHASIZED IN BREEDING CULTIVATED WILD RICE

There are several key traits currently being emphasized in breeding wild rice cultivars: nonshattering (qualitative), shattering resistance (quantitative), yield, disease resistance, resistance to lodging (stem breakage), and shorter height. Although other traits are being investigated, these are currently receiving the most attention, and of these, reducing shattering and disease resistance have the highest priority.

Nonshattering

If the wild-type trait of shattering is the loss of seed at or near maturity, then nonshattering is its opposite: the retention of seed during the maturation process. Figure 1 follows the changes over time in seed tensile strength, or the force needed to pull a seed off the panicle. Nonshattering plants exhibit a qualitative difference in the retention of seed compared to wild plants, making it one of the primary traits distinguishing cultivated from natural wild rice. Although the term suggests an all-or-nothing response, nonshattering cultivars do still lose some seed due to shattering. Losses average around 15 to 25%, depending on the conditions, but under certain circumstances losses can be as high as 50%. This trait is associated with the retention of male florets as well.

As a result of earlier work done on the genetics of nonshattering (Elliot and Perlinger 1977), a two-

gene model for shattering has been the working hypothesis to explain the inheritance of this trait. According to this framework, shattering is dominant to nonshattering at each allele, but both genes must have a dominant shattering allele for the shattering phenotype to be expressed. If either locus lacks a shattering allele, the phenotype is nonshattering. Thus, if the two genes are designated A and B, then:

- $A_B_ = \text{shattering (Sh)}$;
- $A_bb = \text{nonshattering (ns)}$;
- $aaB_ = \text{nonshattering (ns)}$; and
- $aabb = \text{nonshattering (ns)}$.

All cultivars currently used in Minnesota are open-pollinated populations developed through individual plant selection. In an open-pollinated population, one nonshattering genotype could have a shattering gene that is complementary to another nonshattering genotype. Mating these could produce shattering genotypes. For example, if one individual in the population, $AAbb$ (ns), randomly mated with another individual, $aaBB$ (ns), the progeny would be $AaBb$ (Sh). Because of this, current open-pollinated cultivars with mixed genotypes have not been fixed for nonshattering (i.e., shattering genes eliminated). In order for cultivars to be fixed for nonshattering, the genotypes of individual plants need to be identified and only those that do not contain shattering alleles should be kept or crossed together to compose the cultivar.

This 2-gene model leads to several observations about shattering. First, shattering alleles are difficult to remove by phenotypic selection. Second, since shattering is dominant, it is more often expressed in a population with equal frequencies of shattering and nonshattering alleles. The ratio of phenotypes in the second generation of a cross between shattering ($AABB$) and nonshattering ($aabb$) plants would be 9:7 Sh:ns. Third, since shattering confers a selective advantage in the wild (being important for seed dispersal), it is favored over nonshattering under natural selection and, under some circumstances, even in paddies. Figure 2 illustrates how the cultivar K2 has reverted to the wild-type over time when cultivated continuously in paddies for 2, 4, 10, or 15

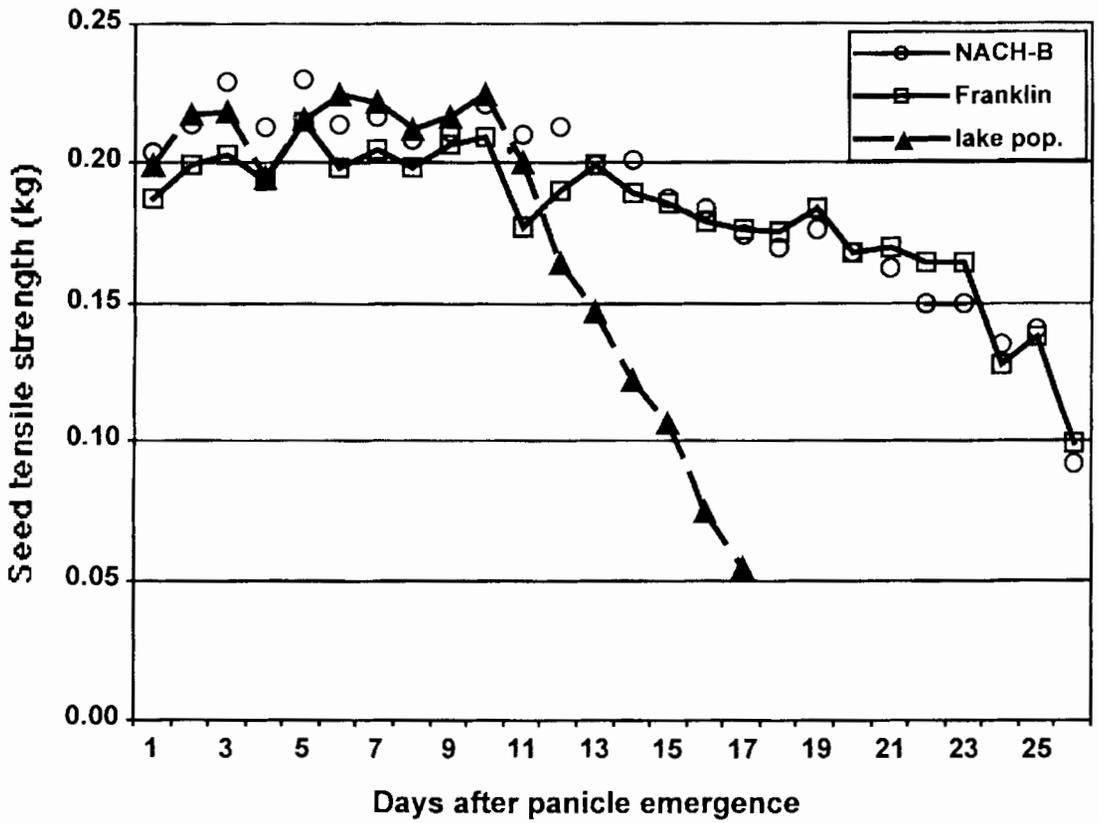


Figure 1. Tensile strength of one shattering and two nonshattering populations during seed maturation.

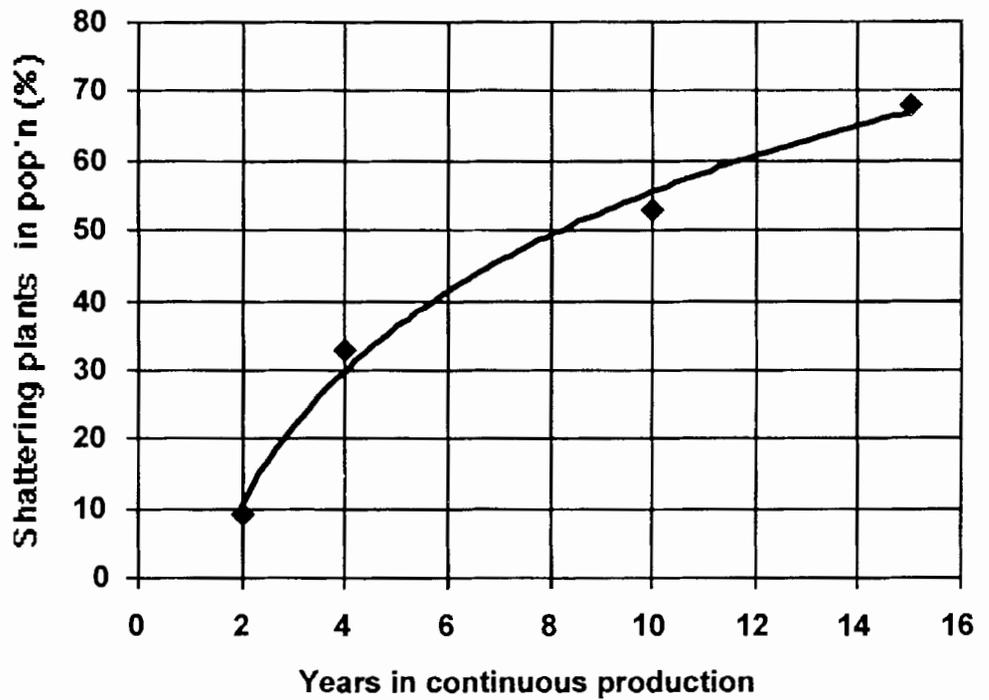


Figure 2. Seed shattering loss of the cultivar K2 under continuous production, 1990 data.

years. After 15 years, shattering (wild-type) plants have reached a frequency in the population of 70%.

Recent developments in molecular genetic research on wild rice have raised the possibility of revising the two-gene model for shattering (Kennard et al. 1998). Four loci (genes) influencing shattering have been identified from molecular genetic marker data. One locus, mapped to linkage group (chromosome) 2 has been found to have the most influence on shattering, accounting for more than one-third of the variability in shattering in the mapping population. Two other loci are found on chromosome 4, and a fourth on chromosome 9. This research reveals that the genetic control of shattering may be more complicated than previously thought. However, the molecular markers being developed through this research can be used to assist in selecting plants with the desired genotypes. This use of molecular genetics would not involve genetic engineering, or "gene-splicing." It would simply make use of molecular genetic information to allow more accurate identification of naturally occurring genetic variability within normal breeding lines.

Diseases and Breeding for Disease Resistance

Fungal Brown Spot (FBS) disease is the most important one for cultivated wild rice. It is caused by *Bipolaris oryzae*, a fungus that also causes a similar disease on rice. It also has been observed in wild populations (Porter, personal communication with J. Percich) but is not suspected of causing significant damage. In cultivated wild rice, it can cause a yield reduction of 12%. This level of loss due to this disease represents a decreased impact resulting from improvements in management practices, especially nutrient management (fertilization). However, to reduce the need for fungicide control, breeding has also emphasized selecting plants within populations that are more resistant to the disease. This involves inoculating the breeding lines in the research plots with a known concentration of spores of the fungus, evaluating the plants for lesion size, and selecting the plants or families with the fewest and smallest leaf lesions and the least stem breakage due to stem rot.

Other fungal diseases (and their causal agents) that have been observed and investigated to some extent include:

- spot blotch (*Bipolaris sorokiniana*);
- scab, found in cultivated and natural stands (*Fusarium* spp.);
- stem rot (*Nakatea sigmoidea*);
- anthracnose (*Colletotrichum* sp.);
- Phoma leaf spot (*Phoma* sp.); and
- diseases of water plantain for biocontrol of this weed in paddies.

INHERITANCE OF WILD RICE TRAITS

Over the years in which cultivated wild rice varieties have been developed, genetic studies of traits have been carried out, resulting in increased knowledge about how wild rice traits are inherited. Traits can be categorized in terms of whether they are quantitative or qualitative. Qualitative traits are generally thought of as having an "either-or" attribute. They are generally controlled by one or a few genes and environmental effects have less influence on their phenotype or expression. Some examples of known or suspected qualitative wild rice traits are:

- nonshattering (2 to 4 genes);
- pistillate panicle, all female florets (1 recessive gene);
- bottlebrush panicle, a form of male sterility (1 recessive gene);
- nondormancy (1 dominant gene?); and
- "Johnson brown"-type FBS lesion (1 gene?).

Quantitative traits are those that are thought to be controlled by many genes, each with minor effects. Shattering resistance is an example of a quantitative trait. Since plants that are nonshattering still incur seed shattering losses, the differences between the degree of those losses among nonshattering plants is due to heritable, minute differences in shattering resistance. The degree to which the variation in these measurable traits is due to genetic influence is called heritability, which is genetic variability as a

Table 2. Quantitative traits of wild rice and their heritabilities.

Trait	Author	Heritability	Methods
Shattering Resistance	Everett and Perlinger (1977)	0.55-0.58	Mass selection
	Boze (1985)	0.25-0.90	Broad-sense heritability, 10 pop.
Grain Yield	Palm (1984)	0.01-0.05	Half-sib family selection
	Hutomo (1986)	0.75-0.84	Covariance of half-sib families
Flowering Date	Foster and Rutger (1980)	1.06	Covariance of half-sib families
	Palm (1984)	0.52	Half-sib family selection
	Hutomo (1986)	0.47-0.64	Covariance of half-sib families
Plant Height	Foster and Rutger (1980)	0.88	Covariance of half-sib families
	Palm (1984)	0.27	Half-sib family selection
	Hutomo (1986)	0.58-0.67	Covariance of half-sib families
Tiller Number	Foster and Rutger (1980)	0.32	Covariance of half-sib families
Seed Length	Foster and Rutger (1980)	0.58	Covariance of half-sib families
Tiller Synchrony	Hayes and Stucker (1987)	0.32-0.56	Covariance of half-sib families
FBS Resistance	Porter, MacGregor, and Schumer (1999)	0.52	Covariance of half-sib families
Lodging Resistance	Porter, MacGregor, and Schumer (1999)	0.32-0.46	Covariance of half-sib families

proportion or percentage of total variability (genetic plus environmental variability). Some quantitative traits with their estimated heritabilities are listed in Table 2, along with the method that was used in estimating each heritability. These heritabilities often differ from study to study, but, in general, flowering date and plant height have higher heritabilities than the other traits, and, in practice, they are easily changed by mass selection. Grain yield, shattering resistance, seed length, FBS resistance have more moderate heritabilities and require more sustained efforts to change. Tiller number, tiller synchrony, and lodging resistance have lower heritabilities and should be more difficult to improve unless family selection is used to increase accuracy of selection.

SUMMARY OF OBSERVATIONS

Several observations can be summarized from three decades' experience of breeding *Zizania palustris*.

A number of genetically controlled traits have been identified, and some have been characterized. These traits were derived from natural genetic variability, not by mutation breeding or genetic engineering.

The methods that *were* used involve selection of individual plants or half-sib families of plants from within populations that already had a large amount of natural genetic variability.

More recent methods are employing controlled crosses using the gene pools already available within wild rice. Genetic variability within these gene pools is organized by self-pollination to develop distinct lines, which can then be crossed to each other for new combinations of traits.

As breeding of cultivated wild rice has progressed, genetic variability still appears to remain quite high in cultivars and breeding populations. There appears to be no significant narrowing of the genetic base within cultivated wild rice varieties. Rather, for the traits of interest, there is adequate genetic variability to continue to improve breeding methods.

Genetic variability among and within lake populations has not yet been adequately estimated. There have been reports of natural stands that have declined or disappeared. This is a concern of both cultivated and natural wild rice interests.

Further research needs to be done to quantify the genetic variability in natural populations, perhaps making use of molecular genetic tools that have recently become available. There is a need to collect and preserve seed of declining populations before they are lost, but genetically unique populations first need to be identified by reliable genetic methods.

Finally, there is research currently being done that would allow longer-term ex situ seed storage of wild rice germplasm. Such seed storage would allow not only the preservation of current germplasm and breeding lines for cultivated wild rice researchers, but also the collection and preservation of seed by natural wild rice researchers for future efforts to restore declining natural stands. Seed collected for the latter purpose can easily be kept separate from seed collected for the former, allowing each group to benefit from research-based improvements in seed storage in order to address its unique goals.

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MOLECULAR GENETICS OF WILD RICE

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ABSTRACT

Molecular genetics research on wild rice has been underway for the past seven years at the University of Minnesota. This work has been devoted to the development of a genetic linkage map of the genome. Development of a genetic map of wild rice will benefit breeding programs at the University of Minnesota and elsewhere by assisting in the breeding of traits pivotal to cultivation. The genetic map will also be an important tool in assessing and protecting the genetic diversity present within natural stands throughout the upper Midwest and Canada.

In comparison to many of its other relatives in the grass family (*Gramineae*), domestication of wild rice is still in its infancy. Crops such as maize, wheat, barley, and rice have been domesticated for several thousand years. In that time, many of the traits common to wild species, such as seed size, shattering, and dormancy, have been altered. Genetic studies have shown that the areas of the genome that control these traits are conserved among many of the *Gramineae* species. This genetic conservation is pivotal in the development of the wild rice genetic map. Our hypothesis is that DNA markers already mapped in rice will show a similar relationship in wild rice in regard to their chromosome locations.

Probes from the Rice Genome Project in Japan, the Maize Genetics Cooperative in Columbia, Missouri, and from Cornell University were specifically chosen for their ability to identify regions of genetic commonality. Utilization of both the genetic and intellectual resources of these large genome projects will assist breeding and help protect this important food source.

INTRODUCTION

The development of a molecular genetics program in wild rice at the University of Minnesota was started with the goal of developing a molecular genetic map of the genome. This map would be useful in the breeding of traits important to cultivation as well as in assisting with assessing the genetic diversity that remains within the species. Wild rice is the only cereal grain that is native to North America. The cultivated species, *Zizania palustris* L., remains widely scattered throughout its native habitat in the upper Mississippi watershed and central Canada, indicating that much of the genetic diversity of the species is still available. However, since its native range is not immense, the assessment and analysis of existing genetic diversity is still feasible.

BACKGROUND

In order to develop a molecular genetic map we first had to compile any information that existed about the genome of wild rice. Next, we assessed what genetic tools best fit our available resources and eventual goal of developing a genetic map. Cultivated wild rice has a chromosome number of 30, with 15 pairs of different chromosomes. Breeding efforts have shown that some traits segregate as if controlled by a single gene. This supports the hypothesis of wild rice being a diploid organism.

Wild rice is a highly heterozygous and heterogenous plant. Its open-pollinated nature and short history of domestication (less than 30 years) have allowed for even cultivated varieties to remain heterozygous as well as heterogenous. This is especially evident when looking at traits associated with domestication such as height, maturity, and seed shattering (Paterson et al. 1995).

Taxonomically, wild rice is classified in the tribe *Oryzaceae*. It has three more pairs of chromosomes and twice the total DNA content of its closest cultivated relative (Kennard et al. 1999), *Oryza sativa*, white rice. This relationship is integral to our mapping effort as will be explained shortly.

TECHNIQUES

When this project was started in 1991, a variety of new biotechnological advances were being made. Restriction Fragment Length Polymorphisms (RFLP) technology had evolved as the most widely utilized at that time and was used for both molecular mapping and genetic diversity studies. This technology utilizes enzymes that naturally cut DNA at sites determined by the base pair content. This provides segments of DNA of a variety of lengths that are unique to the particular genotype of that plant. By using the correct enzyme in conjunction with genetic probes, which are DNA segments that are from a known location in the genome, specific genes can be located (UMC RFLP Manual 1989). From that point, the presence or absence of alleles of these genes can be detected.

In the late 1980s, RFLP technology was being used to study another new finding. Genetic content and arrangement of genes in chromosomes appeared similar across many grass species. As species diverged from a common ancestor, blocks of genes tended to be maintained (Ahn and Tanksley 1993). Genetic analysis was beginning to show conserved regions that were identifiable across many of the grass species, suggesting that the *Gramineae* are more closely related than is suggested by morphological appearance (Nadeau and Sankoff 1998).

COMPARATIVE MAPPING

Similar traits, such as yield components, and physiological changes between grass species were being shown to frequently be controlled by orthologous genes (Paterson et al. 1995). Also, many of these genes map to homoeologous positions across many grass genomes. Height, maturity, seed

size, and seed shattering map to homoeologous positions in sorghum, rice, and maize. Storage protein, photoperiod response, and leaf rust resistance genes all map to similar positions in wheat, rice, maize, and oat.

The extent to which the genomes of the different grasses are similarly organized can be shown by aligning their common segments (Moore et al. 1995 and Devos and Gale 1997). This organization of *Triticeae*, maize, sorghum, sugar cane, foxtail millet, and rice by linkage groups, as opposed to chromosomes, shows the extent of homology within the grasses. This type of organization is also not effected by DNA amount, where *Triticeae* species contain about 40-fold more than rice.

Increased understanding of the organization of the grass genomes has allowed the development of a new technique for molecular mapping of cereal genomes: comparative mapping. Comparative mapping involves the use of genetic information from one species and applying that to another, often distantly related, species (Bennetzen and Freeling 1993). This has proven very effective among the grass species where conservation of gene content and gene order has been used to develop comparative maps among several of the cereals (Kennard et al. 1999).

Rice has become the pivotal genome for comparative mapping. This is due to its relatively low amount of total DNA, high basic chromosome number, and diploid nature. Also, due to its extensive use in Asia, rice is studied by many research groups both agronomically and genetically and has resulted in the publication of several high density genetic maps (Causse et al. 1994 and Harushima et al. 1996).

Comparative mapping has provided a tremendous resource to the wild rice molecular mapping project. Taking advantage of the extensive genetic information that has been collected on the other grasses and applying it to a crop that has not been as widely studied and is just beginning to be domesticated makes our molecular genetic work

more efficient. The information also continues to validate the theories that surround comparative mapping as it applies to the grasses.

Comparative mapping is important to the wild rice molecular marker work because of the minimal genetic tools available in wild rice. In wild rice, there are few morphological or molecular markers, no known chromosomal variations, and no genetic association of traits. Consequently, there was no clear starting point for our genetic work.

MAPPING OF WILD RICE

Initial work on genomic DNA hybridization showed a much stronger hybridization of wild rice to rice than to oat, barley, wheat, or maize (Kennard et al. 1999). This solidified the taxonomic classification of wild rice being more closely related to rice than many of the other grasses. With rice being the pivotal genome for comparative mapping work, utilization of rice probes was a good starting point. Probe sets from Cornell University, Ithaca, New York, and the Rice Genome Project, National Institute of Agrobiological Resources, Tsukuba, Japan, includes probes that have been mapped in rice and have been shown to hybridize well to other species in the grass family. With the lack of molecular markers available in wild rice, these probes were critical to establishing an initial framework for a molecular map of the genome of wild rice.

These probes, along with several barley, oat, and corn probes have been used to produce the first molecular linkage map of wild rice (Kennard et al. 1999). This map contains 121 linked RFLP-based markers. Currently, three rice chromosomes are represented twice and only 11 of the 12 rice chromosomes are represented. Also, only two unlinked markers have been found, suggesting that although the map is not yet saturated, it is highly representative of the overall genome.

TRAITS OF INTEREST

As work started on the molecular map there were

several traits of interest that we wanted to locate on the map and describe genetically. Quantitative Trait Loci (QTLs), which describe areas of the genome that contain the genes that control the trait of interest, were located for maturity, height, panicle length, stem color, and shattering. Multiple QTLs have been identified for these traits. This is not uncommon since complex quantitative traits such as maturity are often controlled by multiple genes.

One of the most significant traits we are studying is shattering. Due to the long history of domestication of most of the other grains, traits such as shattering have been reduced. Since domestication of wild rice is still in its infancy, shattering still represents a significant problem in cultivated stands. A model has been proposed for the mode of action of the identified shattering genes. We currently believe that this is a three-gene system, with non-shattering being a recessive trait at all three loci. This model most closely fits the observed data. Preliminary research in rice suggests as many as five QTLs are involved in the shattering trait with non-shattering being recessive at four of the five loci (Fukuta et al. 1996).

The location of the three QTLs that control shattering as well as the extent to which each QTL describes the phenotype or the extent to which the trait is expressed in the plant have been identified. (See Table 1.) These values suggest a large degree of genotype by environment interaction with regard to the shattering trait. This may also suggest that not all QTLs for shattering have been identified. Also important to note are the collinear traits that have been identified in rice in these same areas. In this particular case, in the same region on Linkage Group 4 as the QTL that describes 7% of the variation in the shattering trait is a gene in rice, *Sh-3*, which is believed to have an effect on shattering in rice (Causse et al. 1993). These collinear traits have been one of the real highlights of doing comparative mapping work and greatly adds to the amount of information available to the researchers as was mentioned earlier.

Another trait that has been identified in a separate

Table 1. Areas showing significance to control of shattering along with linkage group location (Kennard et al. 1999), R² value, and colinear trait loci found in rice.

Interval	Linkage Group	R ²	Colinear Trait Loci in Rice
RG139b-UMC305	2	39%	QTL. Paterson et al. 1995
RZ590-CDO244	4	7%	Sh-3, Causse et al. 1993
RZ87-RZ467	4	15%	

population is a pistillate trait. The pistillate plant has no male flowers and tends to have a long panicle. Raymond Porter, the wild rice breeder at the University of Minnesota, has provided useful lines for the mapping of the pistillate trait. The pistillate trait is controlled by a single recessive gene, and finding markers linked to this trait would aid in its use in the breeding program and would represent the first application of molecular marker work to the wild rice breeding program.

CONCLUSION

Comparative mapping has provided many advantages to the molecular marker project. A large number of markers from a variety of different species have been provided that would not have been available otherwise. The taxonomical classification of wild rice has been reaffirmed as being closely related to rice. Addition of a molecular map of another member of the *Graminea* family reaffirms the conservation of genomic information that exists among those species. The presence of colinear loci existing between species may provide a means of predicting the location of genes of interest in the future. This would increase the efficiency of molecular mapping work on wild rice. In addition, as has been described earlier, the compilation of knowledge collected from different species will add to the knowledge of other species within the family and further understanding of the family as a whole on genetic, physiological, and evolutionary levels.

The techniques mentioned were chosen to maximize progress of the wild rice molecular marker program at the University of Minnesota. The current goal of this research is to assist the breeding program in utilizing traits pivotal to cultivation. Also, this

works sets a foundation for starting the analysis and cataloging of available genetic diversity that remains within the species.

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GENETIC VARIATION AMONG POPULATIONS OF WILD RICE (*ZIZANIA PALUSTRIS* VAR. *PALUSTRIS*) IN NORTHERN WISCONSIN

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ABSTRACT

Wild rice (*Zizania palustris* var. *palustris*) is a wind-pollinated aquatic annual grass that densely occupies local patches of habitat across a broad range in North America. To assess patterns of genetic variation, we compared isoenzyme variation among 17 populations of wild rice across northern Wisconsin. Seven of the 13 allozyme loci analyzed are highly polymorphic, and 2 to 8 loci vary within each population. Levels of genetic diversity and polymorphism are similar to other annuals and to those found in other studies of regional genetic variation in plants. Gene diversity varies widely among wild rice beds from only 0.023 at Blaisdell Lake to 0.278 at Totogatic Lake (mean: 0.15). Gene diversity is higher in larger rice beds and those containing denser populations of wild rice. Inbreeding within populations is limited (mean $F_{is} = 0.117$, s.d. = 0.10) as expected in wind-pollinated plants.

The 17 populations were genetically differentiated from each other ($F_{st} = 0.298$, s.d. = 0.047), corresponding to low estimated levels of gene flow ($Nm = 0.6$). Genetic and geographic distances between populations are only weakly correlated but there appear to be four regional population groups that share alleles. Because gene exchange between drainages appears limited, it may be important to maintain nearby or continuous patches of suitable habitat to sustain regional populations and genetic diversity in wild rice.

INTRODUCTION

The aquatic grass known as wild rice is native to less disturbed wetlands across much of eastern North America. Wild rice has been exploited for

centuries as a traditional staple by indigenous American Indians and now also represents a popular specialty crop (Hayes et al. 1989). Although many Indian tribes traditionally relied on hundreds of plant species for sustenance and medical treatment, wild rice remains particularly important within Ojibwa culture (Vennum 1988; Meeker 1988, 1993).

Despite wild rice's importance as a traditional Indian food and domestic grain, little was known about its ecological responses to habitat conditions until recently. These are all the more relevant as natural populations of wild rice have declined dramatically across much of its original range due to habitat loss and disruptions of historical disturbance and hydrological regimes (Rogosin 1951; Fannucchi et al. 1986; Meeker 1993). As wild rice becomes scarcer across the landscape and its habitats become more fragmented, we should be concerned that wild rice populations may be losing both demographic resilience and, potentially, the genetic variation they need to persist and thrive. Both theory (Lynch and Gabriel 1990; Lande 1995; Lynch et al. 1995) and empirical evidence from plant populations (Menges 1991; Heschel and Paige 1995; Newman and Pilson 1997) suggest that demographic performance diminishes when genetic variation declines within local populations. Genetic variation decreases as small populations suffer genetic drift and when populations harboring particular alleles are lost.

Because wetland habitats are patchily distributed, aquatic plants may be prone to patchy distributions that could limit gene flow among populations (Sculthorpe 1967; Cook 1990; Barrett et al. 1993). With limited gene flow, populations could become genetically differentiated, supporting unique alleles or genotypes in different locations. The loss of such

populations could then eliminate locally-adapted ecotypes or alleles, diminishing both evolutionary flexibility and benefits from future applied breeding programs (Simmons et al. 1976; Franklin 1980; Lande 1988; Barrett and Kohn 1991).

Earlier work concerned with *Zizania* has emphasized species boundaries (Warwick and Aiken 1986; Duvall and Biesboer 1988) or the relationship between phenotypic plasticity and genetic variability (Counts 1993). This study pursued three goals as part of an overall effort to assess how vulnerable wild rice in northern Wisconsin might be to the loss of genetic variation. Our first goal was to characterize the mating system of wild rice so as to determine whether it more closely resembled annuals that frequently self-fertilize or other wind-pollinated grasses like corn that reproduce primarily via outcrossing. Our second goal was to quantify overall patterns of genetic variation in the region and characterize how that variation is structured within and among populations in response to gene flow. Our final goal was to relate these patterns of genetic variability to features of the environment and plant distribution and abundance. In this regard, we sought to learn whether particular populations harbor unique genotypes or greater variation than other populations and particularly whether small or more isolated populations maintain less genetic diversity than large central populations. To accomplish these goals, we surveyed isoenzyme (isozyme) variation within and among 17 wild rice populations across northern Wisconsin that varied in location and population size.

Suitably interpreted isozyme banding patterns in diploid plants provide convenient markers for estimating patterns of genetic variability. These markers almost always follow Mendelian patterns of segregation and their co-dominance allows one to distinguish heterozygotes and so estimate patterns of inbreeding. Isozymes are also usually interpreted to represent neutral or near-neutral markers useful for characterizing overall patterns of genetic variation.

This study represents a collaboration between the

resource biologists at the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) and scientists at the University of Wisconsin-Madison.

MATERIALS AND METHODS

Wild Rice

Zizania palustris var. *palustris* (Fassett 1924) Dore is broadly distributed across the upper Great Lakes region and locally abundant in certain lakes and sloughs (Fassett 1924). It is differentiated from *Z. palustris* var. *interior* in being smaller in stature and having a wider distribution, narrower leaves, fewer spikelets, and a shorter awn length than var. *interior* (Dore and McNeill 1980; Warwick and Aiken 1986; Counts 1993).

Sampling and Isozyme Analysis

To evaluate the nature and extent of genetic variation within and among populations of wild rice in the upper Midwest, we sampled plant leaves and analyzed patterns of isoenzyme variation in 17 populations distributed across northern Wisconsin. (See Figure 1.) These populations were all located within one degree of latitude (45°28' to 46°10') but spanned more than three degrees east to west (88°59' to 92°32'). For each population, we also estimated an index of isolation based on the distance from that site to other populations in the region. (See Table 1.)

At each site, GLIFWC personnel sampled leaves from 30 to 41 plants via canoe between late July and early September 1995. Leaf samples (12.7 to 15.24 cm) were then deposited in labeled plastic bags and placed onto ice until they could be sent via overnight mail to the laboratory in Madison. Voucher specimens from the populations surveyed were deposited with the University of Wisconsin Herbarium (WIS). To reduce the chance that genetic patterns were affected by historical patterns of collecting, transporting, and planting seeds by indigenous people, we chose to sample populations with no known history of replanting and minimal disturbance.

Wild Rice Sites

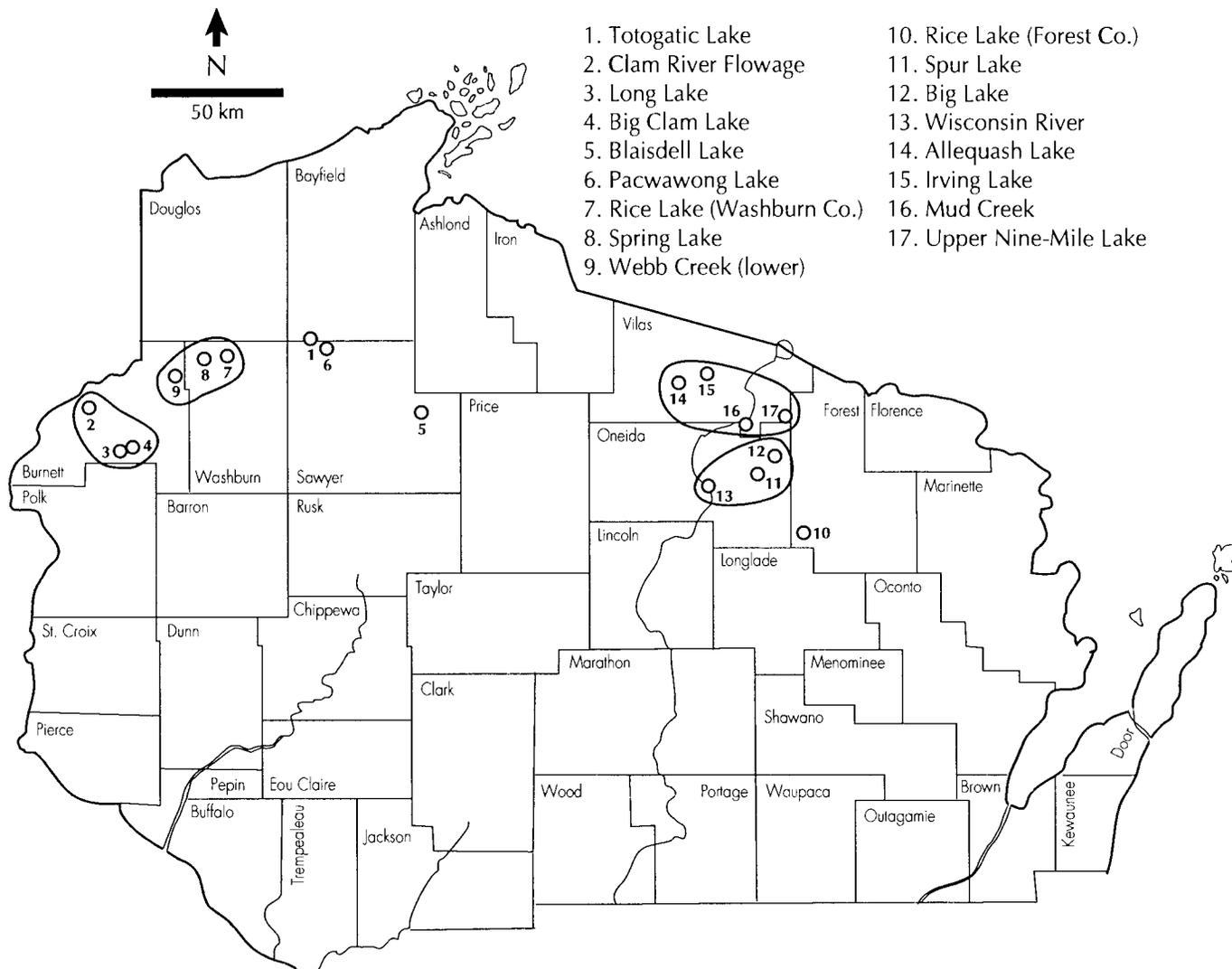


Figure 1. Map of northern Wisconsin showing the geographic distribution of the 17 wild rice populations surveyed and locations of the four groups identified in the results.

Table 1. Location and description of wild rice populations surveyed in 1995.

Population Number	Code	Body of Water	County	Lake area (ac.)	Isolation index*	Population area (ac.)	Population density
1	BDTCL1	Totogatic Lake	Bayfield	537	4	410	Medium-dense
2	BTCRF1	Clam River Flowage	Burnett	359	3	36	Dense
3	BTLGL1	Long Lake	Burnett	318	5	85	Medium-Dense
4	BTBML1	Big Clam Lake	Burnett	1218	5	200	Medium-Dense
5	SRBLL1	Blaisdell Lake	Sawyer	370	1	65	Sparse-Medium
6	SRPGL1	Pacwawong Lake	Sawyer	160	4	125	Medium-Dense
7	WNRCL1	Rice Lake	Washburn	132	4	15	Dense
8	WNSGL1	Spring Lake	Washburn	54	4	19	Medium
9	WNWKCL	Webb Creek (lower)	Burnett	17	5	6	Dense
10	FTRCL1	Rice Lake	Forest	208	2	5**	Medium
11	ODSRL1	Spur Lake	Oneida	113	3	70	Medium-Dense
12	ODTRF1	Big Lake	Oneida	198	3	80	Medium-Dense
13	ODWNR1	Wisconsin River	Oneida	1384	3	150	Medium-Dense
14	VSAHL1	Allequash Lake	Vilas	426	5	75	Medium-Dense
15	VSIGL1	Irving Lake	Vilas	403	5	120	Sparse-Medium
16	VSMDC1	Mud Creek	Vilas	240	5	35	Medium-Dense
17	VSULL1	Upper Nine-Mile Lake	Vilas	91	3	55	Medium-Dense

* 1 is the most isolated; 5 is the least isolated.

** Rice Lake (Forest Co.) had an unusually small coverage of wild rice in 1995 relative to other years (when cover was typically closer to 100 ac.)

The leaf samples were immediately frozen upon arrival in a -80°C freezer. Within the following three weeks, the leaf samples were ground with a cold (5°C) mechanical grinder in a few drops of buffer solution in conical analyzer cups. The buffer consisted of 1 mM EDTA, 10 mM KCl, 10 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2 M Tris-HCl, 0.1 % 2-mercaptoethanol, and 8 g PVPP (40,000 mw) per 200 ml of solution. The homogenized leaf samples were then returned to the -80°C freezer until they were run on gels.

Extracted samples were placed into horizontal starch (11.3%) gels for electrophoresis following the procedures of Marty et al. (1984). We used three types of buffer systems for the 12 enzymes screened: #6 of Soltis et al. (1983) (tris/citric acid system, pH 7.8) for phosphoglucose isomerase (PGI; EC 5.3.1.9), aspartate aminotransferase (AAT; EC 2.6.1.1), alcohol dehydrogenase (ADH; EC 1.1.1.1), and Esterase (EST; EC 3.1.1.-); # 11 of Soltis et al. (1983) (histine/HCl system, pH 7.0) for shikimic acid dehydrogenase (SKDH; EC 1.1.1.25), malic enzyme (ME; EC 1.1.1.40), adenylate kinase (AK; EC 2.7.4.3) and 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44); and buffer AC of Marty et al. (1984) (histine/citric system, pH 6.5) for glutamic-pyruvic transaminase (GPT; EC 2.6.1.2), malate dehydrogenase (MDH; EC 1.1.1.37), isocitric dehydrogenase (IDH; EC 1.1.1.42), and phosphoglucomutase (PGM; EC 2.7.5.1). The 12 isozyme systems yielded 13 interpretable loci, namely: Gpt, Mdh-1, Mdh-2, Mdh-3, Pgm, Skdh, Me, 6Pgd-1, 6Pgd-2, Pgi, Adh, Aat, and Est. We carefully scored stained bands onto data sheets and photographed all gels to make a permanent record of the results.

Data Analyses

Using scored genotypes, we calculated various measures of genetic variability, including the mean number of alleles per polymorphic locus, percent polymorphic loci, and Nei's gene diversity (Nei 1972, 1973, 1987) based on the expected heterozygosity in each population. To better

compare genetic variability across populations, we multiplied the three measures above to produce an overall index of genetic variability. (See Table 2.) We also estimated genetic distances between all pairs of populations based on Nei's D measure (Nei 1972, 1987). This distance reflects time since divergence when differences are due to genetic drift.

We estimated inbreeding coefficients (f) in each population from the deficit in heterozygotes relative to what is expected under random mating, averaging across variable loci. We also calculated Wright's (1951, 1965) hierarchical F-statistics for these populations. Such an analysis partitions the overall deficit of heterozygotes among the set of populations (F_{IT}) into both a within-population component due to localized inbreeding (F_{IS}) and an among-population component due to population subdivision and differentiation (F_{ST}). Our approach involved weighing the analyses by population sizes and the variance in allele frequencies (Weir and Cockerham 1984) and estimating standard errors for these statistics by jackknifing the results over populations and loci. We used Weir's program DIPLOID.FOR to perform these analyses (Weir 1990).

We estimated population sizes at each site by multiplying field estimates of plant density by population area. (See Table 1.) These values were then used along with the isolation index and lake area to test for relationships between genetic variability and population parameters.

RESULTS

Levels of Polymorphism

Totogatic Lake had both the most polymorphic loci (8/13) and the highest gene diversity (0.226; see Table 2). The highest allelic diversity occurred in Big Lake and Pacwawong Lake. In contrast, Blaisdell Lake had both the fewest number of polymorphic loci (2/13) and the lowest mean number of alleles per locus. Average Nei gene diversity was 0.150 (s.d. 0.047) and ranged from 0.063 in Spring Lake to 0.226 in Totogatic Lake. Of

the 13 loci scored, four did not vary across the populations, two (Est and 6-Pgd-2) exhibited low levels of variability, and seven (Skd, Adh, Gpt, Mdh-1, Me, Pgi and Pgm) were highly polymorphic. (See Table 2.) Populations averaged 2.14 alleles per polymorphic locus over the nine variable loci. Locus Est had two alleles in 4 of the 17 populations. Although the population at Upper Nine-Mile Lake showed no variation at most (9/13) of its loci, it did vary at locus 6-Pgd-2, which was monomorphic in all other populations.

Levels of Inbreeding

Inbreeding coefficients (f) averaged across loci ranged from appreciably negative values (-0.44 at Spring Lake and -0.37 at Blaisdell Lake) to large positive values (0.36 at Mud Creek and 0.52 at population 10, the Rice Lake in Forest Co.; see Table 2). This variation in f values does not appear to reflect any obvious geographic gradient. Such a broad range in f values suggests that populations differ considerably in their inbreeding history. Founder effects, population bottlenecks, assortative mating, or self-fertilization could all boost inbreeding coefficients within populations. Negative f values, on the other hand, suggest outcrossing, recent hybridization, disassortative mating, or possibly selection-favoring heterozygotes. The average coefficient of inbreeding over populations was rather low (0.076), suggesting that such averages can hide important among-population variation. Population inbreeding (f) was unrelated to lake area, isolation, and estimated population size, but appears positively correlated with estimated plant density ($r = 0.47$, $p = 0.05$).

Comparisons among Populations

To test how geographic context affects genetic parameters in wild rice, we correlated Nei's gene diversity with four predictor variables: estimated population isolation, lake area, and estimated population area and size (area \times density). Correlations with lake area and isolation were positive but non-significant ($r = 0.18$ and 0.37 , respectively). Correlations with rice population area

($r = 0.51$) and estimated population size ($r = 0.50$), however, are similarly positive and significant ($p < 0.05$), suggesting that larger, more extensive populations support higher levels of gene diversity. As estimated population area and size are themselves highly inter-correlated ($r = .98$), one cannot distinguish their respective effects.

Pairwise Nei genetic distances between all pairs of the 17 populations were weakly positively correlated with the geographic distances separating populations ($n = 120$, $r = 0.181$). This appears to be non-significant, however, due to the small sample size and non-independence among pairs of points. Thus, genetic differences do not appear to closely track geographic distances, but proximity may affect genetic similarity.

Population Differentiation and Gene Flow

We used hierarchical F-statistics to estimate population differentiation across northern Wisconsin. (See Table 3.) Within-population inbreeding (F_{is}) averaged $0.117 \pm .103$, slightly higher than the simple average over populations calculated in Table 2. Genetic differentiation among populations (F_{st}) was appreciable ($0.298 \pm .047$) and contributed more to the total deficit of heterozygotes observed ($F_{it} = 0.383 \pm .098$).

Table 3. Hierarchical F statistics among the 1995 populations of *Zinania palustris* var. *palustris* in northern Wisconsin. Estimation follows the procedures of Weir and Cockerham (1984). Standard deviation (s.d.) is derived via jackknifing.

	Fit	Fst	Fis
mean	0.383	0.298	0.117
s.d.	0.098	0.047	0.103

We also used our estimate of F_{st} to estimate average rates of gene flow among the populations surveyed. Assuming the "island model" of population structure where each population is equally likely to contribute migrants to, or receive migrants from, other populations (Wright 1951), the mean number

Table 2. Numbers of alleles at each locus, mean number of alleles per polymorphic locus, percent polymorphic loci, gene diversity, index of genetic variability, and inbreeding coefficient for 17 populations in 1995.

Population No.	Locus														Ave. # of alleles per locus	Percent polymorph. loci	Nei's Gene diversity	Index of genetic variability	Inbreeding coefficient (f)
	Aat	Skd	Adh	Gpt	Mdh-1	Me	Pgi	Pgm	Est	Mdh2	Mdh3	6Pgd-1	6Pgd-2						
1	1	2	2	2	2	2	2	2	2	1	1	1	1	2.00	0.62	0.23	0.28	0.18	
2	1	2	1	2	2	2	2	2	2	1	1	1	1	2.00	0.54	0.16	0.17	0.15	
3	1	1	2	1	2	2	2	2	1	1	1	1	1	2.00	0.38	0.12	0.09	0.02	
4	1	3	2	2	2	3	2	--	1	1	1	1	1	2.33	0.50	0.17	0.19	0.31	
5	1	2	1	1	1	1	1	2	1	1	1	1	1	2.00	0.15	0.08	0.02	-0.37	
6	1	4	2	2	2	2	2	2	1	1	1	1	1	2.29	0.54	0.20	0.25	-0.02	
7	1	3	1	2	2	3	2	2	1	1	1	1	1	2.33	0.46	0.19	0.21	0.20	
8	1	2	1	2	2	1	1	1	1	1	1	1	1	2.00	0.23	0.06	0.03	-0.44	
9	1	2	2	2	2	2	2	2	1	1	1	1	1	2.00	0.54	0.18	0.20	0.21	
10	1	2	2	2	2	2	2	1	1	1	1	1	1	2.00	0.46	0.13	0.12	0.52	
11	1	3	2	2	2	1	2	2	2	1	1	1	1	2.14	0.54	0.14	0.17	0.12	
12	1	3	2	2	2	1	2	3	2	1	1	1	1	2.29	0.54	0.16	0.20	0.04	
13	1	2	2	2	1	1	2	1	1	1	1	1	1	2.00	0.31	0.15	0.09	0.05	
14	1	3	2	2	2	1	2	2	1	1	1	1	1	2.17	0.46	0.19	0.19	-0.01	
15	1	4	2	2	2	1	2	2	1	1	1	1	1	2.33	0.46	0.20	0.21	0.01	
16	1	2	2	2	1	1	2	2	1	1	1	1	1	2.00	0.38	0.09	0.07	0.36	
17	1	4	1	2	1	1	2	1	1	1	1	1	2	2.50	0.31	0.11	0.09	-0.01	
														Mean	2.14	0.44	0.15	0.15	0.08
														s.d.	0.17	0.13	0.05	0.07	0.23

of successful migrants per generation is estimated by:

$$Nm = ((1/F_{st}) - 1) / 4$$

This formula suggests that, on average, these wild rice populations exchange about 0.6 migrant individuals per generation. This rate is neither so high as to genetically homogenize populations ($Nm \gg 1$) nor so low that populations become isolated ($Nm \ll 1$). It is, however, appreciably lower than the average for outcrossed wind-pollinated plants generally (2.3; Hamrick and Godt 1990) and low enough to allow a moderate degree of differentiation among populations.

Despite the lack of a strong association between geographic and genetic distance, we generally expect gene flow to decrease with distance, making nearby populations more genetically similar to each other. Such a pattern may exist in that we observed four distinct groups of populations across northern Wisconsin. We used three criteria to identify such groups: 1) geographic proximity, or physical interconnections among the habitats; 2) patterns of variation where presumed ancestral populations contain more alleles while presumed derived populations display only a subset of these alleles; and 3) derived populations, which tend to have lower gene diversity than their ancestral populations. Group I in the northeast contains four populations: Irving Lake, Allequash Lake, Upper Nine-Mine Lake and Mud Creek. These populations share common alleles at most of their loci, perhaps in response to local gene flow. The population of Irving Lake was the most diverse (see Table 2). Whereas, the population at Mud Creek, occupying a small body of water, was the least variable and contained a subset of alleles present at Irving Lake. This cluster also resembles the second cluster located in the southeastern part of the survey region, including populations at Spur Lake, Big Lake, and the Wisconsin River. The population at Big Lake was most diverse, while the Wisconsin River population contained only a sub-portion of Group II's total allelic diversity. Group III is located in the southwestern part of the survey region and includes

populations in the Clam River Flowage, Big Clam Lake, and Long Lake. Again, we found that the population growing in the smallest body of water (Long Lake) possessed few alleles and the lowest gene diversity and percent polymorphism. Group IV in the northwest is made up of populations in the Rice Lake of Washburn Co., Webb Creek (lower), and Spring Lake, again with this smallest population displaying the least diversity.

DISCUSSION

Overall levels of polymorphism in wild rice resemble those found in isozyme studies of similar plant species. Among the 190 annuals tabulated by Hamrick and Godt (1990), there were an average of 2.07 alleles per polymorphic locus, 51% polymorphic loci, and a Nei gene diversity (D) of 0.16 compared to 2.14 alleles, 44% polymorphic loci, and a D of 0.15 in wild rice. (See Table 4.) This D value is also quite close to the average value (0.148) for wind outcrossing species (mostly continental) and resembles those found in analyses of regional-scale genetic variation in other species (Hamrick and Godt 1990). However, this level of gene diversity is only moderate compared with the aquatic outcrossing species, *Carex lasiocarpa* ($D = 0.266$; McClintock and Waterway 1993).

In addition, wind-pollinated species as a group tend to average higher levels of polymorphism than wild rice, possibly because this group includes many woody species (e.g., pines) known for their high levels of polymorphism (Mitton et al. 1977). Thus, wind pollination generally appears to be effective in promoting outcrossing and maintaining genetic variation in wild rice.

While average levels of gene diversity resemble those found in other plant species, more of this diversity occurs among, rather than within, populations in wild rice. Wild rice populations are somewhat differentiated as evidenced by an F_{st} value of 0.3, suggesting that gene flow may be limited among these populations. Estimated gene flow between populations averages only 0.6 migrant individuals per generation. (While this value could

Table 4. Comparison between *Zizania* population genetic variation and data reported from other isozyme studies in plants. Source of comparative information: Hamrick & Godt 1990 for number of loci, etc. and Brown 1979 for f values.

		Number of Loci	Ave. # of alleles per locus	Percent polymorph. loci	Gene diversity	Inbreeding coefficient (f)
<i>Zizania</i> (this study)	Mean s.d.	13	2.14 0.17	43.7 12.5	0.15 0.05	0.076 0.234
Other Annuals (N=190)		14.9	2.07	50.7	0.16	0.220
Geographic range:						
	Narrow (N=101)	16.9	1.83	45.1	0.14	
	Regional (N=193)	16.7	1.94	52.9	0.15	
	Widespread (N=10)	14.6	2.29	58.9	0.20	
Other wind pollinated species (N = 105)		16.7	2.40	66.1	0.16	

reflect, in part, artificial transplantation of wild rice seeds among sites, we attempted to include only populations without any history of such movements.) This rate of gene flow is lower than that found generally in outcrossed wind-pollinated plants and low enough to allow some differentiation among populations. Since G_{st} tends to underestimate N_m when N_m is large (Slatkin and Barton 1989), the average N_m for wind-pollinated outcrossing plants could be even larger. It is also lower than N_m values observed in the marine aquatic grass, eelgrass (1.1-2.8; Ruckelshaus 1998). Such differentiation is reflected in the apparent loss of genetic diversity from several of the smaller populations and the correlations among groups of populations in geographic proximity.

The positive correlation of Nei's gene diversity with estimated population size suggests that larger populations harbor greater gene diversity. This tendency could reflect either the tendency for heterogeneous environments to favor more genotypes or the ability of large populations to better sustain new or rare alleles. In either case, the result suggests that larger populations serve as more effective reservoirs of genetic diversity. Such populations are also more likely to act as a source for emigrants to colonize new rice beds.

Conservation biologists and geneticists often argue that the presence of adequate and appropriate levels of genetic viability may reduce the risks of local extinction. For example, some level of genetic diversity may be essential to allow populations to respond to short-term shifts in selection and, ultimately, long-term evolutionary challenges (Wilcox and Murphy 1985; Ledig 1986; Hamrick et al. 1991; Milligan et al. 1994), and populations of plants in the field can suffer reduced fitness and persistence when the genetically effective population size is small (Newman and Pilson 1997). Small populations, in contrast, are at risk of losing this genetic diversity via inbreeding or random genetic drift. Recent models further suggest that small and isolated populations may be subject to "mutational meltdown" where deleterious mutations accumulate faster than they can be eliminated from the

population by selection (Gabriel et al. 1993; Frankham 1995; Lande 1995).

Historical processes appear likely to have affected the genetic structure of these wild rice populations. In general, we expect smaller populations to express higher levels of inbreeding and to include subsets of the allelic variation present in nearby larger populations, suggesting colonization from similar sources followed by a decay of genetic variation in the smaller populations. However, some populations (e.g., Rice Lake, Forest Co.) experienced a high level of inbreeding without losing much genetic variability. Smaller populations could also harbor unique alleles, as with 6-Gpd-2 at Upper Nine-Mile Lake. Conversely, populations increasing after a bottleneck may still lack variation. For example, although the Blaisdell Lake population occurs near the geographic center of our survey area and is moderate in size, it shows the lowest genetic variability, while a nearby population in Pacwawong Lake possesses a very high level of genetic variation. (See Table 2.) These patterns of genetic differentiation among populations suggest that conserving wild rice may require sustaining both big populations that harbor most of the genetic variation and smaller populations that may allow for continued genetic exchange and the conservation of particular genotypes.

In contrast to gene diversity, levels of inbreeding appear unrelated to population size in wild rice. This may reflect the ability of wind pollination to ensure outcrossing even in "small" populations. Inbreeding did, however, vary widely among populations and increased in sparse populations. This suggests that wind pollination may fail to ensure effective outcrossing when plants are spaced far apart, allowing more self pollen to fertilize the ovules. This hypothesis could be tested by analyzing arrays of progeny for the frequency of selfing in populations that vary in density. Selfing occurs most commonly in annuals and could provide a potential "fail-safe" mechanism for setting seeds in years when cross pollination is difficult.

Several processes could increase inbreeding in some

populations, including founder effects (new populations being colonized by only a few potentially related individuals), population bottlenecks (reflecting past population crashes), assortative mating, or variable levels of self-fertilization. The patchy nature of aquatic habitats generally, and rice beds in particular, make wild rice inherently susceptible to founder effects. The fact that wild rice is an annual prone to "good" and "bad" years also causes its populations to be prone to suffering intermittent bottlenecks that could restrict variation and increase inbreeding. The large among-population variation in inbreeding evident in Table 2 makes it unlikely that this difference is significant.

These wild rice populations cover a broad geographic range and occurred in water bodies of various sizes. Such a broad range could be covered by either a single phenotypically flexible "general purpose" genotype or a number of geographically and genetically distinct sub-populations, each specialized to different conditions. Weedy annuals are often thought to be generalists with versatile genotypes rather than being specialized to particular conditions (Stebbins 1950). High local genetic diversity is sometimes associated with conditions of relative habitat stability and longevity. Because wild rice is an annual that occupies dynamic aquatic habitats that must be recolonized every year, we do not expect that the population differentiation we observed is adaptive. In addition, the regional genetic groups we identified suggest that genetic differentiation is historical and not adaptive.

If we are to protect and maintain genetic variation in these wild rice populations, we need to protect the quality of the aquatic habitats that sustain extensive populations. Doing so may require that we protect both water quality and the natural cycles of water level fluctuation (Meeker 1993). Our results suggest that smaller populations tend to suffer reductions in genetic diversity and that sparse populations experience increases in inbreeding. It would, therefore, be of some interest to monitor population levels to know when reductions in population size and density due to habitat degradation may threaten

genetic variation or increase levels of inbreeding. Because the larger populations occurring in extensive wetlands appear to maintain the most genetic variability and may act as sources to reestablish or replenish smaller and more ephemeral populations, it is essential that these be maintained. However, it also appears that smaller and/or more ephemeral populations may be genetically distinct and could act as critical stepping stones to knit populations together demographically and genetically. Thus, we advocate an extensive approach to conserving wild rice populations that ensures the protection of populations of various sizes distributed throughout the landscapes that have historically supported this valued species.

ACKNOWLEDGMENTS

We thank Kathi Borgmann for lab assistance and the GLIFWC staff for help with field collections.

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