Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

Project Manager Name: David D. Biesboer, Ph.D.

Project Manager Email Address: biesboer@umn.edu

Project Title: Genetic Diversity, Conservation and Threats to Wild Rice

Project Number: # 068 - C1+2

1. Abstract. Wild rice is an important semi-domesticated plant in Minnesota. It is recognized as being under threat from changes in hydrology of streams, lakes and rivers, changes in seasonal housing along lakeshores, and competition from both native and exotic aquatic species. However, the most important threat is a loss of genetic diversity as habitat declines, competition increases and global climate change accelerates. This proposed research seeks to utilize polymorphic microsatellite DNA markers (also called simple sequence repeats, or SSRs) and the powerful tools of bioinformatics to study the genetic diversity of wild rice across Minnesota. The genetic information from this study will be used to directly assist natural resource managers in the conservation and restoration of this valuable native species in the State.

2. Background. Wild rice (*Zizania palustris* L.) is an economically important annual, semi-domesticated plant found in the Upper Midwest and extending into Canada. It grows in shallow lakes and slow moving rivers. In addition to *Z. palustris*, at least three other taxa are generally recognized as clearly distinct species in this genus. They include *Z. aquatica* L., a larger statured plant with a more branched panicle and smaller seed size that is found in the Great Lakes region and on the eastern seaboard (Grombacher et al. 1993); *Z. texana* Hitchc. that is an endangered species restricted in distribution to only two populations in the San Marcos River of Texas (Richards et al. 2007); and *Z. latifolia* Griseb., a disjunct species known as Manchurian wild rice because its native habitat is in Eastern Asia. *Z. palustris* and *Z. aquatica* occur sympatrically in some areas of the state (de Wet and Oelke 1978).

Questions remain as to the exact nature of species of wild rice across North America and, of course, the genetic composition of those species. For example, some authors identify wild rice in the Midwest as either *Zizania palustris* or *Z. aquatica* based on their relatively easily identifiable morphological characteristics. However, others still use the older classification system of Dore (1969) who recognized various subspecies of wild rice including *Z. palustris* var. *palustris*, and *var. interior*; and Z. *aquatic* var. *aquatica*, var. *brevis*, and var. *subbrevis*. Experimental hybridizations demonstrated that crosses between *Z. palustris* and *Z. aquatica* and their "varieties" produced some fertile hybrids at a low frequency (Duvall and Biesboer 1988). Morphological characteristics and the fact that interbreeding occurs suggests that these varietal types occur in Minnesota (personal observations by the investigators). They may be only distinguishable by genetic analysis because growing conditions influence the morphology of this very plastic species. For example, both genetics and ecology influence the biomass of seeds per square meter of wild rice populations. Types of water bodies and other factors such as sediment composition appear to account for 71.3% of the variance. Genetic diversity possibility accounts for the rest (Eule-Nashoba 2010).

Many threats have been noted for their impact on natural populations of wild rice in the state of Minnesota. These include changes in hydrology of lakes and streams, changes in seasonal housing on lakes (that has jumped 500% in the last 20 years), and competition from native and exotic species that out-compete wild rice in natural stands. However, the most important threat is a loss of genetic diversity as habitat declines, competition increases and global climate change accelerates (Natural Wild Rice in Minnesota, 2008). A recent report clearly documents the downward environmental spiral that lakes in Minnesota are experiencing and its effect on wild rice populations (MNDNR, Shallow Lakes, 2010). It is noted in this latter report that populations of wild rice have declined over time, especially for many of the populations along margins of smaller lakes and streams.

As populations of wild rice plants are lost from Minnesota, the State will lose the natural genetic diversity found in these populations. The genetic diversity in natural populations is important in maintaining the ability of any individual species to adapt to changing environmental conditions, especially as global climate change looms in the future. Before more losses occur, we propose to carefully examine the genetic diversity of populations of wild rice across the State.

Very little is known about the genetic diversity of natural wild rice. Lu et al. (2005) used isozyme analysis of 17 populations of wild rice across northern Wisconsin, and showed that wild rice genetic diversity was moderate, compared to similar outcrossing grass species. Larger populations of wild rice in larger lakes expressed higher levels of genetic variability and smaller inbreeding coefficients than did smaller or more isolated populations; the study also noted that gene flow was limited between drainages. One important conclusion of this study was that small populations with high levels of diversity might demand special efforts in identification and conservation.

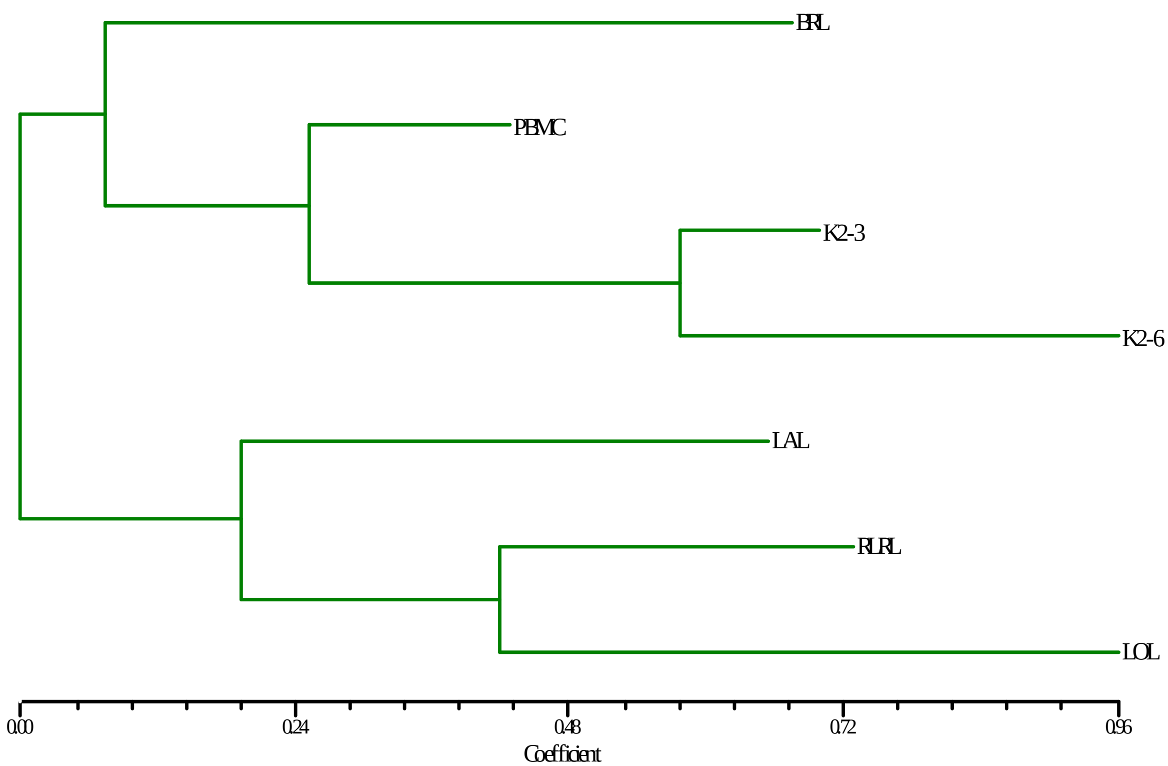
Other work has centered on using polymorphic microsatellite DNA markers (also called simple sequence repeats, or SSRs) to study the genetic diversity of wild rice. SSRs have recently become a very powerful tool to study genetic diversity in plants, and are widely considered to be the most informative molecular markers for studying population-level genetic diversity. They are common, easily identified features of the genome consisting of short, randomly repeated DNA sequences (1-6 base pairs) that are widely and ubiquitously distributed throughout the plant and animal kingdoms. SSRs are highly polymorphic and thus can be used to detail genetic variation both between closely related individuals and widely divergent organisms. The nature of SSRs are that: a) only small quantities of DNA are needed for an analysis of diversity, b) multiple SSR alleles may be detected at a single locus using PCR based screening, c) SSRs are co-dominant, d) SSRs are distributed over the entire genome, and e) analysis can now be semi-automated to save much time and money. SSR markers will be used to explore genetic diversity in wild rice, as our labs have recently developed a large panel of useful SSRs for wild rice genetic diversity studies (Kern, 2009; Kahler, 2010; Kahler et al., in preparation).

SSR analysis has been used to study *Zizania texana* (Richards et al. 2004) and *Zizania latifolia* (Quan et al. 2009) with neither study attempting to use their respective data for understanding diversity within the genus *Zizania*. However, Kahler (2010) recently, and very convincingly, showed the efficacy of using SSR markers for studying diversity in *Zizania palustris*.

A set of eleven SSR markers was used to investigate the genetic diversity among five natural wild rice populations and two cultivated wild rice populations. The amount polymorphism was measured using they Polymorphism Information Content (PIC) calculation (Weir, 1996). This set of SSR markers exhibited and an average PIC value of 0.70. Markers with PIC values of 0.6 and above are considered to be highly polymorphic. The PIC value of 0.70 is comparable to the values reported in white rice (*Oryza sativa*) genetic diversity studies.

The SSR marker data were used to calculate the genetic distance among the sampled wild rice populations. The Nei72 genetic distance coefficient (Nei, 1972) was used to obtain genetic distance measurements. Figure 1 shows the first reported wild rice phylogram illustrating the genetic diversity among wild rice populations.

Fig. 1. Genetic diversity phylogram of five natural wild rice populations and two cultivated wild rice populations. BRL = Big Rice Lake, PBMC = Pokegama Bay Main Channel, K2-3 = K2EF-Cycle 3, K2-6 = K2EF=Cycle 6, LAL = Laura Lake, RLRL = Little Rice Lake, LOL = Low's Lake.



The genetic distance measurements indicated that all of the sampled natural wild rice populations were genetically unique and each population contained genetic alleles that were not found in the other natural populations. The two most genetically similar natural populations were Little Rice Lake and Low's Lake. These lakes are not geographically close and they are in separate water drainages. This result indicates that geography alone cannot be used to determine which natural wild rice populations are unique. As expected, the K2-3 and K2-6 cultivated populations were the most genetically similar to one another. However, they were still observed to have genetic variation. This is important for the proposed work because it illustrates the power of SSR markers in differentiating very similar wild rice populations.

One of the Co-PIs on this project (Kern) has been funded through the Wisconsin Sea Grant Institute to investigate the genetic diversity of wild rice populations in the St. Louis River estuary near Duluth, Minnesota. The project proposed here offers considerable synergism with the Sea Grant project, primarily because it expands the Sea Grant work to take a broader, state-wide approach to understanding wild rice genetic diversity. The Sea Grant project is in its final stages, and has already provided important insights into the utility of our SSR markers, typical levels of wild rice genetic diversity, and has shown general levels of genetic distance between different wild rice populations (Kahler, 2010). Additionally, the site-specific nature of this project may provide examples of management strategies aimed at expanding wild rice into suitable habitats (Kern, 2009).

Genetic information can directly assist natural resource managers in the conservation and restoration of native species. Loss of genetic diversity is detrimental to natural populations in that it does not allow the species to adapt to changing environments. Once the extent of diversity in existing wild rice populations is determined, it can be compared with other aquatic or terrestrial plant populations. Further, genetic variation between small isolated populations can be determined and the genetic relationship among wild rice populations can be determined.

Also, studies of genetic diversity can determine the source of plants that have been removed from their natural environments. Certainly this has been the case for wild rice that has been collected and seeded in many different places over time both by people in Native American communities and by European settlers.

Finally, if restorations of lakes or streams must be done in the near or long-term future, genetic profiles of our wild rice populations will dictate the appropriate seed sources for establishing populations with the best genetic profiles for their habitats.

3. Hypothesis.

It is hypothesized that natural populations of wild rice in Minnesota are genetically distant from each other.

The extent of wild rice genetic diversity is unknown. Genetic diversity will be measured using the most modern and powerful techniques of molecular biology and bioinformatics available at this time. A better understanding of wild rice genetic diversity in Minnesota will result in the dual benefits of conserving and restoring wild rice populations in the State.

4. Methodology.

<u>Experimental Design- Sampling</u>. In 2008, a completed inventory of lakes by the MNDNR noted that 1292 lakes or river/stream segments historically supported wild rice. Of these, 777 have information on natural wild rice coverage. Sampling will be limited to two lakes representing unique water drainages from each Minnesota county listed in the MNDNR

report. Additional collections will be made in lakes with small populations of rice in southern, western and extreme northeastern Minnesota that have not been well inventoried or explored for their wild rice populations. According to Lu et al. (2005), these smaller populations will likely harbor unique genotypes not present in large contiguous lake systems, and may serve as critical reservoirs for locally-adapted wild rice genotypes, and serve as important seed resources for restoration efforts. Sampling of wild rice from lakes and streams will occur as wild rice approaches maturity beginning in late July and can continue until a week or two prior to senescence in early September.

Leaves will be collected from 50 individuals per lake population. Plants will be sampled from the densest populations at each site on a transect through the population by kayak, collecting one plant at 10 m points on a 500 m transect. At the center of each transect, a GPS location will be recorded. The upper two most leaves will be collected from each plant, individually bagged and stored on ice up to 3 days until freezing at -80 °C in the laboratory.

Experimental Design -Laboratory Protocols. The following techniques are used in our laboratories on a routine basis for the genetic analysis of *Zizania*.

DNA will be isolated from leaf samples using a Qiagen BioSprint 96 automated DNA isolation instrument. This machine, while seemingly expensive, is state-of-the-art for studies of this type. Its benefits include: a) isolation of DNA in ca. 6 days verses 80 days of expensive manual labor; b) outsourcing of DNA isolation would cost approximately twice as much as the original cost of the machine; and c) the machine would be made available for future work in wild rice.

Isolated DNA will be quantified in 96-well plates using UV absorbance at 260 nm on a BioTek Synergy 2 machine. PCR will be carried out using a set of 15 polymorphic wild rice SSR markers (Kahler, 2010). The reverse primer for each marker will be labeled on the 5' end with a fluorescent tag from Applied Biosystems, Inc. PCR reactions will be carried out in 10 ul reaction volumes using Qiagen HotStar Taq Master Mix. Automated capillary-based electrophoresis of the PCR product will be carried out on an ABI 3100 genetic analyzer by Biogenetic Services, Inc. Allele fragment sizes will be reported using the GeneScan software package from ABI.

The NTSysPC 2.1 software package (Exeter) will be used to calculate allele frequency and genetic distance data using the reported allele sizes. Allele frequency data will be used to calculate the polymorphism information content (PIC) values for the marker set among the sampled wild rice populations. A genetic distance phylogram will be constructed using NTSysPC 2.1 for reporting the genetic distance among the Minnesota populations.

Expected Results. It is expected that as a result of the proposed project, the level of genetic diversity among naturally occurring populations of wild rice in Minnesota will be determined. The proposed methods outlined earlier in this proposal have been used previously to study genetic diversity in most major food crops including rice, corn, soybean, wheat, barley and wild rice as well as, animals, fungi and bacteria.

The small-scale wild rice genetic diversity works reported to date (Lu et al. 2005; Kahler 2010) indicated that wild rice harbors genetic diversity but that small, isolated populations may contribute more to the overall level of diversity than do larger populations. It has also been reported that SSR markers are an ideal genetic marker for this study. Wild rice populations included in the proposed study that are genetically unique will be identified to the appropriate resource managers. The genetic diversity information will be made publicly available for use in selecting appropriate wild rice seed sources for reestablishing historic wild rice populations.

This project has significant potential to benefit wild rice management and restoration in Minnesota. Due to human activities and exotic species invasion, wild rice has suffered precipitous declines in Minnesota, and is a major target of restoration activities across its range due to its ecological, cultural, and economic importance. In Minnesota, considerable interest exists among natural resource professionals in adopting genetically-sound restoration techniques for wild rice, particularly with regard to identifying appropriate seed sources. This approach not only increases the likelihood of successful restoration, but also may help protect unique, locally-adapted genotypes from competition and gene introgression—an interest which is especially common among Native American groups and others who seek to maintain the genetic "purity" of local rice beds.

It is important to note that the majority of restoration professionals will agree that whenever possible, access to genetic data is critical in guiding restoration efforts. In Minnesota, this approach has been used to understand restoration principles in everything from Lake Superior dune grasses to the prairie chicken to several species of sport fish, including a major effort to restore walleye in Red Lake. Our preliminary data show that wild rice populations have considerable genetic distinctiveness from each other, thus we propose that large-scale access to the genetic diversity results will be quickly utilized by wild rice restoration professionals and other natural resource managers and, where appropriate, may also provide important evidence to convey additional protections for its habitat as well.

From a basic scientific standpoint, genetic distance and allele frequency data will be useful for future studies to determine the exact speciation of wild rice populations that occur in Minnesota. From an applied scientific standpoint, it could benefit the wild rice breeding program of the University of Minnesota. The genetic data will be made publicly available and wild rice breeders will be able to compare the alleles currently present in the breeding germplasm against all of the alleles observed in the diversity study.

Finally, as an additional note, we recognize the misunderstandings commonly associated with the use of molecular genetic technologies, and how the considerable economic, cultural, and ecological importance of wild rice emphasizes the need for outreach to diverse stakeholder groups, including the Native American community. In recent months, we have sought input from each of the Tribes in Minnesota regarding this proposal, and will continue to seek their advice on certain aspects of this study. It is our intention to actively work to keep lines of communication open between us, natural resource professionals, and the general public, including Minnesota's tribes, in an effort to overcome any lack of understanding or concern associated with our research. We will also invite members of various stakeholder groups to presentations and seminars when our work is being presented, and maintain an open offer to any group to discuss our research.

5. Results and Deliverables.

Results and Deliverables	**Outcomes**
Wild rice allele frequency data	Identify unique populations
Wild rice genetic distance data	Group MN populations by similarity
Polymorphic set of SSR markers	Useful for future comparative studies

6. Timetable. This project and timetable are straightforward.

Begin and End Dates	**Results and Deliverable**
1 July 2011 to 15 September 2011	Collect and store collected wild rice leaves from 30 lake/stream populations; progress report on collections
16 September 2011 to 30 June 2012	SSR analysis from 1^{st} 30 lakes; first analysis of data; update website with interim progress; interim report to LCCMR by 30 June on first analysis
1 July 2012 to 15 September 2012	Collect and store wild rice leaves from and additional 50 lake/stream populations; add collections from other researchers in Canada or other states; progress report on collections
16 September 2012 to 30 June 2013	SSR analysis from 2^{nd} lake group; final report to LCCMR; preparation of final scientific publications

7. Budget.

2011-2012 Detailed Project Budget

IV. TOTAL TRUST FUND REQUEST BUDGET: 2 years

BUDGET ITEM *(See list of Eligible & Non-Eligible Costs, p. 13)*	**AMOUNT**
Dr. Anthony Kern (one month summer salary x 2 summers) - $9850 Graduate student, MS (salary + tuition + fringe rate (24 months) - $76034 Undergraduate students (isolate DNA; $9/hr + 8.16% FICA x 594 hrs) - $5785	$92,000
Equipment/Tools/Supplies: Supplies - DNA isolation chemicals, mortars, collecting supplies (2 years) - $6,000 SSR genetic analysis (3000 plants x ca. $5/sample x 2 years) - $41,000 Canoe or kayak (plastic, car top for wild rice collections in shallow, rice choked lakes and rivers; this unit will be heavily used; it must be an easily portable unit; the canoe will be used after the study for similar future wild rice research or studies similar to this ENRTF study and kept at the U of M Itasca Biology Station for rice research) - $1000 Qiagen BioSprint 96 automated DNA isolation instrument - This machine rapidly isolates and purifies DNA in a very efficient and cost effective manner. It will save many hundreds of hours of hand labor and is state-of-the art in genetic diversity studies. This machine will be part of undergrad and grad training. It will remain at the U of M & used in future genetic diversity studies similar to this ENRTF study for any researcher - $45,000	$93,000
Travel: Vehicle rental (Fleet services; U of M; cargo van; pickup) 6 wks x $265/wk - $1590 Fuel prices: ESTIMATED (7850 miles/yr x 2 years) - $5260 Lodging (U of M per diem) $70 per day x 40 days - $2800	$10,000
	$195,000

V. OTHER FUNDS

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ Being Applied to Project During Project Period:	NA	
Other State $ Being Applied to Project During Project Period:	NA	
In-kind Services During Project Period: Professor Biesboer is on an 11 month appointment at the U of M and ineligible for salary; he will work at a non-mandatory cost share as indicated.	$6,774	
Remaining $ from Current ENRTF Appropriation (if applicable):	NA	
Funding History: *Indicate funding secured prior to July 1, 2011 for activities directly relevant to this specific funding request. State specific source(s) of funds.*	NA	

8. Credentials. A one page CV is presented for each of the following personnel: Dr. D. Biesboer (project manager); Dr. A. Kahler (co-PI); and Dr. A. Kern (co-PI). These researchers have all researched wild rice and are familiar with the plant and its distribution in the Upper Midwest.

Project manager Qualifications and Organization Description

David D. Biesboer, Ph.D.

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Director, Itasca Biological Station and Laboratories
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Department of Plant Biology, 250 Bio Sci, University of Minnesota, St. Paul, Minnesota 55108

EDUCATION

- •Post-doctoral in Organic Chemistry, Indiana University, 1979-80.
- •Ph.D. in Plant Biology, Indiana University, 1975-79.
- •M.S. in Plant Systematics, Illinois State University, 1975.
- •B.S. in Biology-Chemistry, Calvin College, 1973.

PROFESSIONAL EXPERIENCE

- •Director, Itasca Biological Station and Laboratories, 1990 to present.
- •Professor of Plant Biology, University of Minnesota, 1980 to present.
- •Morse-Alumni Distinguished Professor of Teaching, 1995.

AWARDS AND HONORS

- •Fulbright Research/Lectureship to Brazil, 2001
- •Research Partnership Award, Center for Transportation Studies, Minnesota Department of Transportation, 2000.
- •Appointed to the President's "Academy of Distinguished Teachers", 1999.
- •Award of Merit, The Honor Society of Agriculture, 1999.
- •Minnesota Erosion Control Association. Research Award for "Outstanding Contribution Advancing the State of the Art of Erosion Control, 1998.
- •Morse-Alumni Award for Outstanding Contributions to Undergraduate Education; Distinguished Professor of Undergraduate Teaching, 1995.

EXPERTISE

- •Ecosystem biology especially in wetlands biology with experience in the U.S., Bolivia, Brazil, South Africa, and Russia.
- • Biology of wild rice

PUBLICATIONS (a few pertinent to this request out of 70+ scientific publications)

Duvall, M. and D.D. Biesboer. 1988. Nonreciprocal hybridization failure in crosses between annual species of wild-rice (*Zizania palustris* L. x *Zizania aquatica* L. : Poaceae). Systematic Botany 13:229-334.
Duvall, M. and D.D. Biesboer. 1989. Comparisons of electrophoretic seed protein profiles among North American populations of *Zizania*.. Biochemical Systematics and Ecology 17: 39-43.
Cregan, J. 2004. Aspects of seeds storage, pollen travel, and populations dynamics of wild rice (Zizania palustris). M.S. Thesis, U of M,.
Eule-Natosha, A. 2010. Seed size of wild rice in riverine vs. lacustrine environments in Minnesota. M.S. study in progress.

Organization Description: University of Minnesota, Department of Plant Biology, St. Paul Campus Itasca Biological Station and Laboratories, located in Itasca State Park.

Curriculum Vitae

Alexander L. Kahler
Department of Agronomy and Plant Genetics
University of Minnesota
1991 Upper Buford Circle
411 Borlaug Hall
612-624-3749
kahl0041@umn.edu

EDUCATION:

Ph. D. 2010 University of Minnesota, Applied Plant Science
Dissertation: Genome Organization and Genetic Diversity of Wildrice (*Zizania palustris* L.)
M.S. 2007 University of Minnesota, Applied Plant Science
Thesis: Adapting Rice (*Oryza sativa*) Simple Sequence Repeat (SSR) Markers for Linkage Mapping and Marker-Assisted Selection of Wild Rice (*Zizania palustris*)
B.S. 2000 South Dakota State University, Microbiology
Minor degree in Chemistry, South Dakota State University
B.A. 2000 South Dakota State University, Music Performance
Minor degree in German, South Dakota State University

EMPLOYMENT:

February 2010 – Present, University of Minnesota, Research Associate, St. Paul, Minnesota
September 1998 – August 2000, Biogenetic Services, Inc., Technician, Brookings, South Dakota
May 1992 – August 1998 Identity Genetics, Inc., Technician, Brookings, South Dakota

RELAVENT ABSTRACTS AND PUBLICATIONS:

Kahler, A.L., R.A. Porter and R.L. Phillips. 2007. Marker-assisted Selection for Shattering Resistance in the K2EF Wildrice Breeding Population. University of Minnesota North Central Research and Outreach Center Annual Research Report.

Invited Oral Presentation, “Use of Solid Phase Matrix DNA Archiving and Purification in Plant Genome Analysis”, Plant and Animal Genome XV Conference. San Diego, CA. January 2007.

Poster presentation, “Expanding the Molecular Genetic Map Of American Wildrice (*Zizania palustris*) Through Addition of SSR Markers From Rice (*Oryza sativa*)”, Plant and Animal Genome XIV Conference. San Diego, CA. January 2006.

Phillips, R.L., W.E. Odland and **A.L. Kahler**. 2005. Rice as a reference genome and more. Manuscript. Fifth International Rice Genetics Conference. Manila, Philippines.

Oral presentation, “Adapting Rice Simple Sequence Repeat Markers For Integration into the RFLP-based Linkage Map of American Wildrice (*Zizania palustris*)”, Crop Science Society of America International Meetings. Seattle, WA. November 2004

Kahler, A.L., R.A. Porter and R.L. Phillips. 2001. Major Advances in the Genetics of Wild Rice. Minnesota Cultivated Wild Rice Council Annual Report.

Curriculum Vitae

ANTHONY KERN

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Northland College
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Education

Ph.D. 2002 Montana State University, Plant Genetics
Dissertation: Molecular studies of dicamba-resistant *Kochia scoparia* L.: Physiology of osmoregulant accumulation and molecular characterization of auxin-induced changes in gene expression.

M.S. 1995 Montana State University, Agronomy
Thesis: Physiology of triallate and difenzoquat resistance in wild oats (*Avena fatua* L.).

B.S. 1992 University of South Dakota, Biology (Botany emphasis)

Professional Experience

2009-present President, Northland College Faculty Council
2008-present Associate Professor of Biology, Northland College, Ashland, WI
2008-2009 Interim Chair, Department of Environmental Sciences
2008-2009 Vice-President, Northland College Faculty Council
2002-2008 Assistant Professor of Biology, Northland College, Ashland, WI
2006-present Visiting Scientist, Department of Agronomy and Plant Genetics, University of Minnesota
2003-2005 Director, Northland College Sustainable Agriculture Program

Relevant Grant Proposals Funded

Kern, A.J. and Phillips, R.L. 2008-2011. Utilizing molecular genetic markers to develop wild rice restoration and management guidelines for Great Lakes coastal habitats. Wisconsin Sea Grant Institute. $143,938.

Kern, A.J. 2008-2010. Investigating patterns of genetic diversity in wild rice populations at Rice Lake National Wildlife Refuge and Tamarac National Wildlife Refuge. U.S. Fish and Wildlife Service Challenge Grants Program. $41,700.

Kern, A.J. Developing molecular genetic tools for use in understanding wild rice genetic diversity. 2006-2008. Sigurd Olson Environmental Institute. $15,000.

Fishbach, J., Demchik, M., Kern, A.J., and McCown, B. 2010-2013. Improving bush-type hazelnuts for commercial production through cooperative regional breeding and evaluation. Wisconsin Specialty Crop Block Grant Program, Wisconsin Department of Agriculture, Trade, and Consumer Protection. $99,960.

9. Dissemination and Use. Results of these studies will be disseminated via a website, public presentations in our respective academic institutions or at state or national workshops or professional meetings, and ultimately as peer-reviewed scientific publications. We will develop a page on an existing website at the Itasca Biological Station and Laboratories to publish all interim data and reports to the public (www.cbs.umn.edu/itasca/). All data we collect will become public and disseminated to assist land managers and conservationists in the preservation and conservation of wild rice in the Upper Midwest.

10. References.

De Wet J.M.J. and E. A. Oelde. 1978. Domestication of American wild rice (*Zizania aquatica* L. Gramineae). J. d'Agric.Trad.Bot. Appl. 25: 67-84.

Duvall, M.R. and D.D. Biesboer. 1988. Nonreciprocal hydridization failure in crosses between annual wild rice species (*Zizania palustri* X *Z. aquatica*: Poaceae). Systematic Botany 13(2): 229-234.

Eule-Nashoba, A. 2010. Seed size in lacustrine and riverine populations of wild rice (*Zizania palustris* L.). MS thesis, University of Minnesota, unpublished.

Grombacher, A.W., R.A. Porter, and L.A. Everett. 1997. Breeding Wild Rice. Plant Breeding Reviews 14: 237-265.

Kahler, A.L., Porter, R., Phillips, R.L., and Kern, A.J. 201_. Development of polymorphic microsatellite markers for use in American wild rice (*Zizania palustris* L.). In final preparation.

Kahler, A.L. 2010. Genome organization and genetic diversity of wild rice (*Zizania palustris* L.). Ph.D. dissertation, University of Minnesota, unpublished.

Kern, A.J. 2009. Developing microsatellite markers to understand the genetic diversity of wild rice in Lake Superior coastal habitats. Proc. Western Great Lakes Res. Conf. 7:35.

Lu, Y., D.M. Waller, and P. David. 2005. Genetic variability is correlated with population size and reproduction in American wild-rice (Zizania palustri var. palustris, Poaceae) populations. American Journal of Botany 92(6): 990-997.

MNDNR, Shallow Lakes, 2010. http://www.dnr.state.mn.us/wildlife/shallowlakes/index.html

Natural Wild Rice in Minnesota, 2008. dnr.state.mn.us/fish_wildlife/legislativereports/20080215_wildricestudy.pdf

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106: 283-292.

Richards, CM., A. Reilley, D.H. Touchell, M.F. Antolin, and C. Walters. 200. Microsatellite primers for Texas wild rice (Zizania texana) and a preliminary test of the impact of cryogenic storage on the allele frequency at these loci. Conservation Genetics 5: 853-859.

Richards, C.M., M.F. Antolin, A. Reilley, J. Poole, and C. Walters. 2007. Capturing genetic diversity of wild populations for *ex situ* conservation: Texas wild rice (*Zizania texana*) as a model. Genetic Resources for Crop Evolution. 54: 837-848.

Quan, Z., L. Pan, W. Ke, Y. Liu, and Y. Ding. 2009. Sixteen polymorphic microsatellite markers from *Zizania latifolia* Turcz. (Poaceae). Molecular Ecology Resources 9(3): 887-

Weir, B.S. 1996. Genetic Data Analysis II. Sunderland, MA: Sinauer Associates, Inc.