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Genetic variability is correlated with population size and reproduction in American wild-rice (*Zizania palustris* var. *palustris*, Poaceae) populations[1]

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American wild-rice (*Zizania palustris* var. *palustris*) has served as a staple for indigenous North Americans for thousands of years, but has had significant habitat losses in recent centuries. We investigated genetic variability among 17 wild-rice populations in northern Wisconsin using 13 isozyme markers. We then compared these genetic patterns to differences in habitat and population characteristics and phenotypic variation in plant growth and reproduction across sites. Wild-rice's mean genetic diversity (0.15) is moderate compared to wind-pollinated outcrossers but lower than the mean (0.20) reported for the Poaceae. Estimated inbreeding coefficients within populations (f) average 0.12 but vary greatly among the populations (from −0.44–0.52), suggesting heterogeneous population histories. Larger populations in larger lakes express higher levels of genetic variability and smaller inbreeding coefficients than smaller or more isolated populations. The number of panicles per plant is also higher in populations with greater genetic variability. Estimated genetic differentiation among the 17 populations (F_{ST}) was high (0.30), suggesting limited gene flow among drainages. Wild-rice population size and degree of isolation have opposing effects on its genetic variability, and plant performance is positively associated with genetic variability.

Key words: genetic structure; isolation index; life history traits; population size; *Zizania*.

Knowing the manner in which the ecological environment affects population genetic composition and persistence has been a central theme in biological conservation, where much of the connection is constructed through demographic performances of populations. Theoretical results (e.g., Lynch et al., 1995; Lynch, 1996) demonstrate that small populations tend to accumulate deleterious mutational load and lose quantitative genetic variation via drift, and population persistence may be threatened as a result. Some of these effects have been observed in natural populations (e.g., Keller and Waller, 2002). Recent surveys (e.g., Oostermeijer et al., 2003) stress the importance of building connections between the extent of genetic variation present in populations and population performance. The message is clear that we should obtain and attempt to link ecological, demographic, and genetic variables whenever possible in order to assess the status of a species in its habitats. Although we have accumulated considerable information on the genetic structure of terrestrial plant populations (Hamrick and Godt, 1996a), similar data for aquatic plants is far less common (Ruckelshaus, 1998). In fact, aquatic species are in many ways better choices for examining the influence of habitats on populations, as the habitats are easily defined in size.

American wild-rice [*Zizania palustris* var. *palustris* (Fassett) Dore] is a native aquatic annual found in wetlands of north-central and eastern North America (Dore, 1969). It has served as a traditional staple for indigenous peoples for centuries (Johnson, 1969) as well as a specialty commercial crop more recently (Hayes et al., 1989). Despite the booming industry related to wild-rice, habitat loss and disruptions of hydrological regimes have caused natural populations of this species to dwindle drastically over the last century (Rogosin, 1951; Fannucchi et al., 1986; Meeker, 1993). The importance of wild rice to native peoples and its ecological role as a food source for wildlife in wetland ecosystems make the conservation of extant natural populations and their genetic variability a serious concern (Waller et al., 2000).

Environmental conditions, including sediment nutrients (Painchaud and Archibold, 1990) and plant competition (Lee, 2002), change the growth of wild-rice. Fluctuating water levels, in particular, may favor the growth of wild-rice by reducing the cover of competing aquatic plants (Thomas and Stewart, 1969; Meeker, 1993). Little is known, however, about the manner in which the change of habitats alters population genetic composition and how genetic structure, in turn, affects plant performance, although population size, life history, and reproductive system have been shown to significantly influence patterns of genetic variability (Hamrick and Godt, 1990; Akimoto et al., 1998).

Here we report an integrated study of how habitat size and shape, the degree of isolation, and population size affect patterns of genetic variability in American wild-rice in northern Wisconsin. We also compare life history and reproductive characters among populations to see how these reflect (or contribute to) the genetic differences found among populations. By linking genetic components to ecological factors, these results contribute to our ability to improve existing conservation strategies for this important wetland species.

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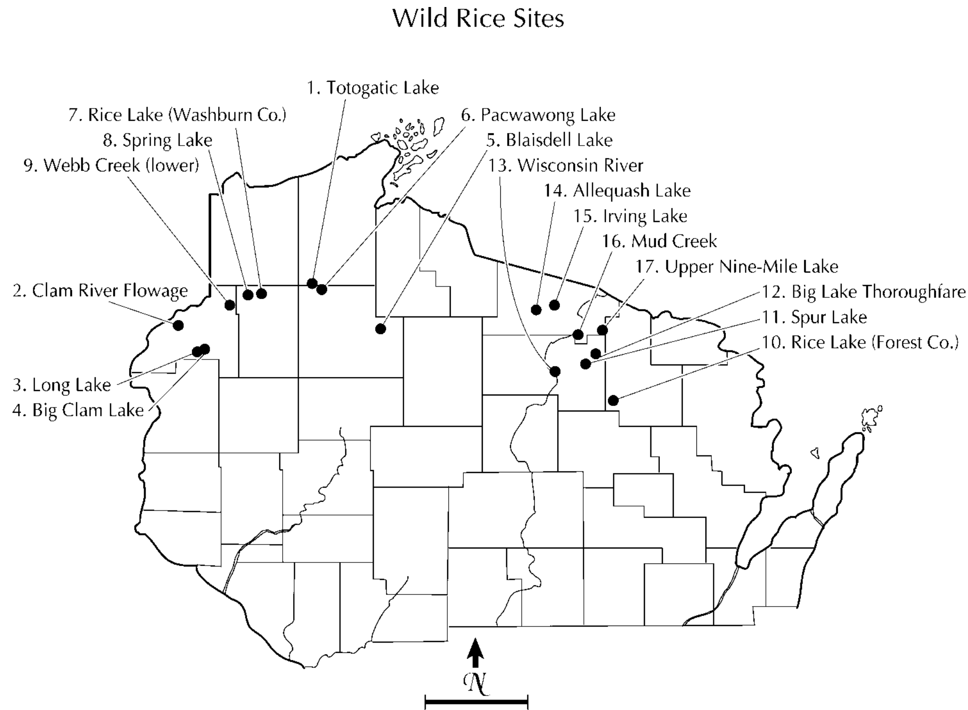

Fig. 1. Map of northern Wisconsin, USA, showing the geographic distribution of the 17 surveyed populations of *Zizania palustris* var. *palustris*.

MATERIALS AND METHODS

The species—Earlier work concerning *Zizania* examined species boundaries (Warwick and Aiken, 1986; Duvall and Biesboer, 1988) and the relationship between phenotypic plasticity and genetic variability across species (Counts, 1993). Two varieties of *Z. palustris* are recognized: *Z. palustris* var. *palustris* (the populations studied here) and *Z. palustris* var. *interior* (Dore and McNeill, 1980; Warwick and Aiken, 1986; Counts, 1993) following Fasset's early study on *Zizania* (1924). The two varieties differ in both vegetative and reproductive traits (Warwick and Aiken, 1986). American wild-rice, *Z. palustris* var. *palustris*, is a diploid annual (Kennard et al., 2000) pollinated mainly by wind. Because the pistillate phase overlaps little with the staminate phase within individual inflorescences (Elliot, 1980; Goldman, 1990), outcrossing is considered the major reproductive mode. Natural seed dispersal occurs by water and possibly occasionally via grain-consuming animals.

Field samples and measurements—The 17 wild-rice populations in northern Wisconsin selected for our survey are within one degree of latitude (45°28′ to 46°10′) but range from 88°59′ to 92°32′ in longitude (Fig. 1). These populations differ in local population history, size, and water area. Knowing the distribution of the wild-rice populations in Wisconsin, we were able to establish an isolation index to reflect qualitatively the average distance of each sampled population from its nearby populations. The index ranged from 1 (least isolated) to 5 (most isolated; Table 1). We also estimated visually the density of each sampled population using a scale of 1–5, representing sequentially low to high densities. We then estimated overall population size at

TABLE 1. Locations and ecological characters of the wild-rice populations surveyed in 1995.

Population	Water body	County	Lake area (hectares)	Isolation index[a]	Population area (hectares)	Density index
1	Totogatic Lake	Bayfield	217	2	166	4
2	Clam River Flowage	Burnett	145	3	15	5
3	Long Lake	Burnett	129	1	34	4
4	Big Clam Lake	Burnett	493	1	81	4
5	Blaisdell Lake	Sawyer	150	5	26	2
6	Pacwawong Lake	Sawyer	65	2	51	4
7	Rice Lake	Washburn	53	2	6	5
8	Spring Lake	Washburn	22	2	8	3
9	Webb Creek (lower)	Burnett	7	1	2.4	5
10	Rice Lake	Forest	84	4	2[b]	3
11	Spur Lake	Oneida	46	3	28	4
12	Big Lake Thoroughfare	Oneida	80	3	32	4
13	Wisconsin River	Oneida	560	3	61	4
14	Allequash Lake	Vilas	172	1	30	4
15	Irving Lake	Vilas	163	1	49	2
16	Mud Creek	Vilas	97	1	14	4
17	Upper Nine-Mile Lake	Vilas	37	3	22	4

[a] 1 is the least isolated, and 5 the most isolated.
[b] In 1995, an unusually small population coverage was observed, relative to other years when the typical coverage was close to 40 ha.

each site in an arbitrary unit using the product of the relative density and the corresponding population area (Table 1).

In August 1994, we measured morphological characters on approximately 20 plants per population in 14 of the 17 populations. The characters included plant height (measured from the top of the primary root to the tip of the tallest stem), length of the longest leaf (leaf length), the diameter of the thickest stem (stem width), number of panicles per plant, and number of seed scars per panicle. Between late July and early September 1995, we sampled 30–41 plants from each of the 17 populations for genetic analysis. We collected two 12–15-cm segments of leaves from each plant and placed these into individually labeled plastic bags. Samples were sent on ice via overnight mail to the laboratory at the University of Wisconsin at Madison for further processing. Voucher specimens from the populations surveyed are deposited in the University of Wisconsin Herbarium (WIS).

Isozyme study—The leaf samples were immediately frozen in a −80°C freezer upon arrival. Within the following 3 weeks, a 1–2-cm^2 piece of each leaf sample was ground in 3–4 drops of cold buffer solution (pH ~6.5) in a conical 1.5-mL tube using a cold (<5°C) mechanical grinder. The buffer solution consisted of 1 mM EDTA, 10 mM KCl, 10 mM $MgCl_2 \cdot 6H_20$, 0.2 M Tris-HCl, 0.1% 2-mercaptoethanol, and 8 g polyvinylpolypyrrolidone (PVPP, molecular mass 40 000) per 200 mL of solution. The homogenized leaf samples were placed in the −80°C freezer until use.

We performed horizontal gel electrophoresis on the samples following the procedures of Marty et al. (1984). We used three buffer systems in gels of 11.3% starch to assay 12 enzymes. We used buffer number 6 of Soltis et al. (1983) (Tris/citric acid system, pH 7.8) for glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), aspartate transaminase (ATT; EC 2.6.1.1), alcohol dehydrogenase (ADH; EC 1.1.1.1), esterase (EST; EC 3.1.1.-). We used buffer number 11 (Soltis et al. 1983; histine/HCl system, pH 7.0) for shikimate 5-dehydrogenase (SKDH; EC 1.1.1.25), malate dehydrogenase (MDH-NAD+ [or MDH1]; EC 1.1.1.40), adenylate kinase (AK; EC 2.7.4.3), and phosphoglucose dehydrogenase (PGDH; EC 1.1.1.44). We used buffer AC of Marty et al. (1983) (histine/citric system, pH 6.5) for alanine transaminase (ANT; EC 2.6.1.2), malate dehydrogenase (MDH-NADP+ [or MDH2]; EC 1.1.1.37), isocitrate dehydrogenase (IDH; EC 1.1.1.42), and phosphoglucomutase (PGM; EC 2.7.5.1).

Estimating the genetic variability, population structure, and gene flow—We used the genotypes scored at these isozyme loci to estimate percentage of polymorphic loci, mean number of alleles at each polymorphic loci, and Nei's gene diversity (Nei, 1972, 1987). Because sampled wild-rice populations reside in disjunct habitats, with potentially limited gene exchange, we used Wright's island model (Wright, 1951, 1965) to calculate hierarchical F statistics. Assuming neutrality of marker alleles and equilibrium of allelic frequency, this method partitions the overall deficit of heterozygotes among populations (F_{IT}) into an average within-population component due to localized inbreeding (F_{IS}) and an among-population component due to population subdivision and differentiation (F_{ST}). The statistics were weighted by population sizes and variances in allele frequencies (Weir and Cockerham, 1984), jackknifing the results over populations and loci to obtain standard errors. The procedures were carried out using Weir's program DIPLOID.FOR (Weir, 1990). Using the same island model and by further assuming a linear effect of migration rate on allelic frequency (Wright, 1965), we also inferred average gene flow among populations. The mean number of successful migrants per generation (= Nm, the population size times the rate of successful migration) was estimated using Wright's formula $Nm \approx ([1/F_{ST}] - 1)/4$. We also estimated inbreeding coefficients (f) within each population using the average of only polymorphic loci (Weir, 1990, pp. 54–55).

Statistical tests—We used nonparametric and conservative statistical tests to identify significant associations between genetic parameters of populations and their biotic and physical environments. We compared population inbreeding and genetic diversity between lake and riverine habitats using the Wilcoxon rank sum test (Sokal and Rohlf, 1995). We also evaluated relationships among ecological factors (lake area, population size, and isolation), the morphological traits, and genetic variables (f and gene diversity) using Kendall's rank correlations. To control for experiment-wise error rate (type I error), we also conducted sequential Bonferroni tests (Rice, 1988) for comparisons between each measure of genetic variability and ecological parameters (Table 4). No correction was made though for Table 6 because the growth characters are highly correlated and following the Bonferroni correction would substantially inflate type II error.

We further assessed relationships among these ecological, morphological, and genetic variables using principal components analysis by S-Plus (version 6.1, Insightful Corporation, http://www.insightful.com/products/splus/). This method identifies those linear combinations of variables that vary the most among the populations and the distribution of populations over these principal components. Finally, we compared the matrix of Nei's genetic distances (reflecting dissimilarities in allele frequency) to geographic distances using a Mantel test (in Arlequin 2.0; Schneider et al., 2000). This analysis uses a reference distribution generated by random permutations to test the null hypothesis of independence between the two matrices.

To distinguish between plant size and genetic effects on plant reproductive output, we also applied path analysis (Li, 1975). Genetic diversity can affect reproductive output both directly and indirectly, via its effect on plant size (Fig. 4). These two effects may be separately estimated from the correlation coefficients observed in the data by the direct path coefficient p_d and p_s, respectively, and $p_{d,s}$ (the path coefficient from genetic diversity to plant size, estimated to be 0.3 from Table 6). The combined effects of both genetic diversity and plant size can then be estimated in the form of ($p_d^2 + p_s^2 + 2p_d p_s p_{d,s}$).

We also used path analysis to examine how population size and isolation index affects genetic diversity and the number of polymorphic alleles, where p_p represents the path coefficient from population size to either genetic measure and p_i is the path coefficient from the isolation index (Fig. 5). Because our data indicate that the correlation coefficient between population size and isolation is not significantly different from zero, the combined effect of two environmental factors (plant population size and isolation index) on genetic diversity and number of polymorphic allele was estimated by ($p_i^2 + p_p^2$).

RESULTS

Genetic patterns—The 17 populations surveyed inhabited water bodies of various sizes, degrees of isolation, and population densities (Table 1). The 12 isozyme systems yielded 13 interpretable loci, namely: *Ant, Mdh2-1, Mdh2-2, Mdh2-3, Pgm, Skdh, Mdh1, Pgdh-1, Pgdh-2, Gpi, Adh, Aat,* and *Est.* Among the 13 loci scored, seven (*Skdh, Adh, Gpt, Mdh1, Mdh2, Gpi,* and *Pgm*) were highly polymorphic with at least two alleles in most of the populations (Table 2). In contrast, locus *Est* had two alleles in four of the 17 populations, and locus *Pgdh-2* varied only within the Upper Nine-Mile Lake population. Four loci (*Aat, Mdh2-2, Mdh2-3,* and *Pgdh-1*) were monomorphic across all populations. Totogatic Lake had both the largest population area and the highest genetic variability among the 17 populations. The Blaisdell Lake and Spring Lake populations had the lowest levels of genetic variation, but had high levels of observed heterozygosity (Table 2). The most inbred population was the Rice Lake population in Forest County (estimated $f = 0.52$).

Genetic differentiation and gene flow—Despite low inbreeding within populations ($F_{IS} = 0.076$), overall fixation among populations was high ($F_{IT} = 0.383$, Table 3). This reflects the case that most populations were genetically distinct, often containing uniquely polymorphic gene markers (Table 2). This differentiation is evident in the high F_{ST} value (0.30), demonstrating that most of the total deficit of heterozygotes observed reflects among-population differentiation and fixation (Table 3). Using Wright's formula, this degree of differ-

TABLE 2. Genetic features of the 17 wild-rice (*Zizania palustris* var. *palustris*) populations in 1995, including percentage of polymorphic loci (% polymorphic loci), mean number of alleles per polymorphic locus (polymorphic alleles), gene diversity, and inbreeding coefficient (f). The mean and its standard error (1 SE) are also listed for each measure. Maxima and minima appear in boldface type. The locus *Mdh1* refers to the NAD+ form enzyme while loci *Mdh2*-1,-2, and -3 refer to the NADP+ form enzymes, respectively.

Population	*Aat*	*Skdh*	*Adh*	*Ant*	*Mdh2* -1	*Mdh1*	*Gpi*	*Pgm*	*Est*	*Mdh2* -2	*Mdh2* -3	*Pgdh* -1	*Pgdh* -2	% Polymorphic loci	Polymorphic alleles	Gene diversity	*f*
1	1	2	2	2	2	2	2	2	2	1	1	1	1	**0.615**	**2.000**	**0.226**	0.175
2	1	2	1	2	2	2	2	2	2	1	1	1	1	0.538	**2.000**	0.158	0.151
3	1	1	2	1	2	2	2	2	1	1	1	1	1	0.385	**2.000**	0.122	0.017
4	1	3	2	2	2	3	2	—	1	1	1	1	1	0.500	2.333	0.166	0.307
5	1	2	1	1	1	1	1	2	1	1	1	1	1	**0.154**	**2.000**	0.076	−0.370
6	1	4	2	2	2	2	2	2	1	1	1	1	1	0.538	2.286	0.199	−0.025
7	1	3	1	2	2	3	2	2	1	1	1	1	1	0.462	2.333	0.193	0.197
8	1	2	1	2	2	1	1	1	1	1	1	1	1	0.231	**2.000**	**0.063**	**−0.436**
9	1	2	2	2	2	2	2	2	1	1	1	1	1	0.538	**2.000**	0.181	0.206
10	1	2	2	2	2	2	2	1	1	1	1	1	1	0.462	**2.000**	0.129	**0.520**
11	1	3	2	2	2	1	2	2	2	1	1	1	1	0.538	2.143	0.145	0.116
12	1	3	2	2	2	1	2	3	2	1	1	1	1	0.538	2.286	0.162	0.041
13	1	2	2	2	1	1	2	1	1	1	1	1	1	0.308	**2.000**	0.153	0.051
14	1	3	2	2	2	1	2	2	1	1	1	1	1	0.462	2.167	0.189	−0.014
15	1	4	2	2	2	1	2	2	1	1	1	1	1	0.462	2.333	0.196	0.012
16	1	2	2	2	1	1	2	2	1	1	1	1	1	0.385	**2.000**	0.088	0.362
17	1	4	1	2	1	1	2	1	1	1	1	1	2	0.308	**2.500**	0.112	−0.010
Mean														0.437	2.140	0.150	0.076
SE														0.030	0.041	0.012	0.057

entiation translates into an estimated rate of gene flow among these 17 populations of 0.59 migrants per generation. Nei's genetic distance is only weakly correlated ($r = 0.18$, Mantel's test, 10 000 permutations, $P = 0.03$) with the geographic distance between populations.

Relationships between ecological characters and measures of genetic variability—The populations surveyed differ mostly in lake area (i.e., habitat size), population area (positively associated with population size), plant height, and seed scars per panicle (seedscars), as indicated by the major loadings in the principal components analysis (Fig. 2). Component (comp.) 1 and comp. 2 may explain approximately 93% of the total variance. The major loadings are from lake area (0.802 on comp. 1, −0.441 on comp. 2), population area (0.485 on comp. 1, −0.866 on comp. 2), plant height (0.306 on comp. 1, 0.734 on comp. 2), and seed scars (0.164 on comp. 1, 0.500 on comp. 2).

Among the ecological variables, lake areas significantly affected population sizes ($r = 0.46$, $P < 0.01$), which, in turn, appear to be associated with the levels of genetic variability ($r = 0.35$–0.40, Table 4). Larger water bodies tend to support larger population areas with higher genetic variability and lower inbreeding levels (but population size per se was not significantly associated with the level of inbreeding). In addition, more isolated populations tend to support lower levels of genetic variability ($r = -0.5$, $P < 0.01$). The isolation index showed no significant correlations with other genetic and ecological measures, nor did lake and riverine habitats differ consistently in their patterns of genetic variability (Fig. 3).

Interestingly, many of the morphological traits measured in the parental generation appear to be positively associated with estimated levels of gene diversity (Table 5). Such traits include

TABLE 3. Hierarchical F statistics among the populations of *Zizania palustris* var. *palustris* in northern Wisconsin in 1995. Estimation follows the procedures of Weirand Cockerham (1984). The standard deviation (SD) is derived via jackknifing.

Statistics	F_{IT}	F_{ST}	F_{IS}
Mean	0.383	0.298	0.117
SD	0.098	0.047	0.103

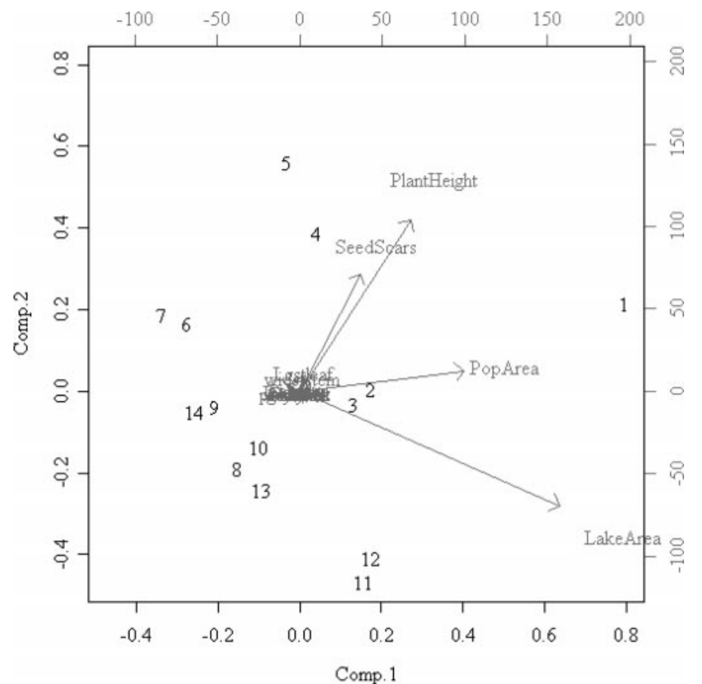

Fig. 2. Biplot of 14 populations of *Zizania palustris* var. *palustris* (listed in Table 5) according to the principal components analysis, showing the major variables with their loadings on components 1 (comp. 1) and 2 (comp. 2). Variables included are lake area, population area, population density, isolation index, percentage of polymorphic loci, number of alleles per polymorphic locus, gene diversity (f), plant height, widest stem diameter, longest leaf length, number of seed scars per panicle, and number of panicles per plant.

TABLE 4. Results of significant Kendall's tests (one-tailed, $N = 17$) on the correlation coefficients between some of population genetic variability characters and ecological indices for the wild-rice populations (*Zizania palustris* var. *palustris*) in 1995.

Character pair	Kendall coefficient of rank correlation	*P*
Lake area - population area	0.515	0.002
Inbreeding coefficient - population area	−0.279	0.064
Gene diversity - population area	0.353	0.026
Gene diversity - population size	0.353	0.026
Gene diversity - isolation index	−0.473	0.005
% Polymorphic loci - population area	0.309	0.046[a]
% Polymorphic loci - population size	0.353	0.026[a]
Polymorphic alleles - population area	0.445	0.007
Polymorphic alleles - population size	0.400	0.014
Polymorphic alleles - isolation index	−0.503	0.004

[a] Not significant if applied with the sequential Bonferroni correction (number of comparisons = 3) at the experiment-wise error rate of 0.06.

widest stem diameter, longest leaf length, panicle number per plant, and seed scar number per panicle (Table 6). These measures are significantly correlated (Kendall's correlation coefficients range from 0.52–0.78). Path analysis (Fig. 4) reveals that genetic diversity can have both direct ($p_d = 0.261$) and indirect effect ($p_s \times p_{d,s} = 0.339 \times 0.3 = 0.101$) on plant reproductive output. Of the total variance of plant reproductive output, 24% may be explained by the combined effects of genetic diversity and plant size. A similar calculation reveals that the combined effects of population size and isolation index may explain 35% of the variance of the gene diversity and 41% of the variance of the polymorphic allele numbers among populations (Fig. 5).

DISCUSSION

This study provides the first detailed analysis of genetic variability in *Zizania palustris* var. *palustris* and one of the few analyses of any aquatic angiosperm. Among aquatic species, the gene diversity (0.15) of American wild-rice is lower than that of the perennial *Carex lasiocarpa* (0.27; McClintock and Waterway, 1993), but similar to that of Asian annual *Oryza rufipogon* (0.14; Morishima and Barbier, 1990) and higher than that of South American annual *O. glumaepatula* (0.003; Buso et al., 1998). At the species level, the percentage of polymorphic loci (62%) and the population differentiation ($F_{ST} = 0.30$) of American wild-rice resemble corresponding means (62% and 0.28) for the family Poaceae. When compared to the terrestrial system, the mean gene diversity in wild-rice (0.150) is also close to the population average (0.148) reported by Hamrick and Godt (1990) for wind outcrossers, though significantly lower than the mean reported for the Poaceae (0.20; Hamrick and Godt, 1996a).

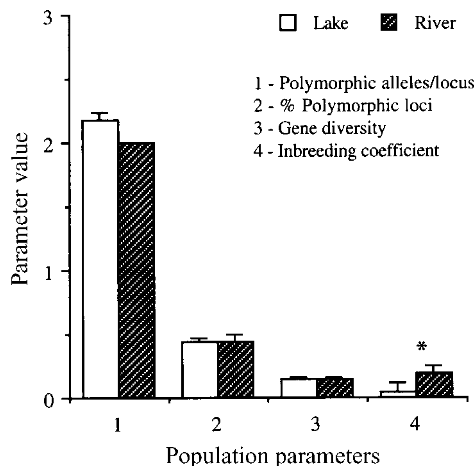

Fig. 3. Comparisons of population parameters (means + 1 SE) between lake habitat (sample size = 13) and riverine habitat (sample size = 4) among 17 populations of *Zizania palustris* var. *palustris* (Wilcoxon rank sum test, one-tailed, * $P = 0.065$). The numbers on the *x*-axis are arbitrary, referring to the compared parameters only.

TABLE 5. Morphological traits (means) of the wild-rice (*Zizania palustris* var. *palustris*) populations in 1994. The population numbers correspond to those in Table 1.

Population	Plant height (cm)	Longest leaf length (cm)	Widest stem (mm)	Number of panicles/plant	Number of seed scars/panicle
1	268.68	25.14	14.36	4.27	98.93
2	215.96	24.70	14.00	3.04	54.16
3	194.89	28.46	13.39	3.16	51.70
6	237.33	25.87	15.30	4.04	79.59
7	261.78	34.13	16.79	3.73	102.35
8	175.44	26.11	12.90	3.50	53.32
9	176.78	23.60	9.86	2.30	45.92
10	149.45	15.88	8.27	2.44	24.32
11	159.00	24.85	9.64	2.23	22.34
12	141.97	21.95	9.51	2.57	44.64
14	137.48	21.08	7.18	1.87	19.26
15	145.16	22.48	8.82	2.47	17.11
16	151.53	15.27	8.42	1.88	12.75
17	151.53	15.27	8.42	1.88	23.02
Mean	183.36	23.20	11.20	2.81	46.39
SD	45.31	5.24	3.12	0.82	29.70

Genetic variability—Both the percentage of polymorphic loci and Nei's gene diversity vary considerably over these 17 wild-rice populations in northern Wisconsin (Table 2). Our analysis suggests that population area/size and degree of isolation are major factors affecting this pattern as their path coefficients are significant and jointly account for 35% and 41% of the variance in genetic diversity and number of polymorphic alleles, respectively.

Genetic variability is positively correlated with population area and to a lesser extent population size (Table 4). Three major processes may initiate this pattern. First, a larger population might receive more migrant pollen, increasing its genetic variation. Second, a larger water body is often associated with a heterogeneous growth environment (Meeker, 1993). Because additive genetic variance exists for several fitness-related

TABLE 6. Correlation coefficients between genetic variability measures and traits of plant growth and reproduction for *Zizania palustris* var. *palustris*. Significant values are indicated in boldface type (Kendall's tests, one-tailed, $N = 14$, $P < 0.08$).

Morphological traits	Gene diversity	% Polymorphic loci	Polymorphic alleles
Plant height	0.275	0.165	−0.011
Longest leaf length	**0.297**	0.275	0.169
Maximum stem width	**0.319**	0.253	0.011
Number of panicles/plant	**0.363**	0.253	0.006
Number of seed scars/plant	**0.297**	0.165	0.023

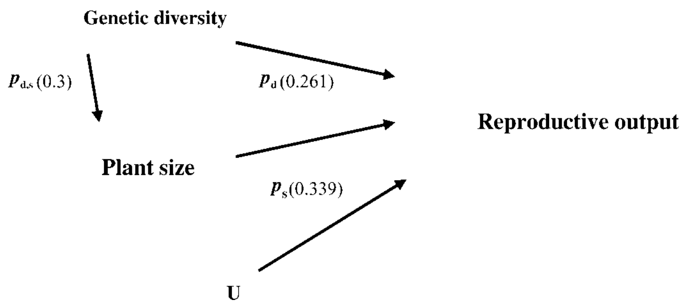

Fig. 4. Path diagram showing the effect of genetic diversity on plant reproductive output (panicle number/plant seed scar number/panicle). Path coefficients are indicated for sources concerned, respectively, with p_d for genetic diversity, p_s for plant size, $p_{d,s}$ for effect of genetic diversity on plant size, and U for the rest of all sources.

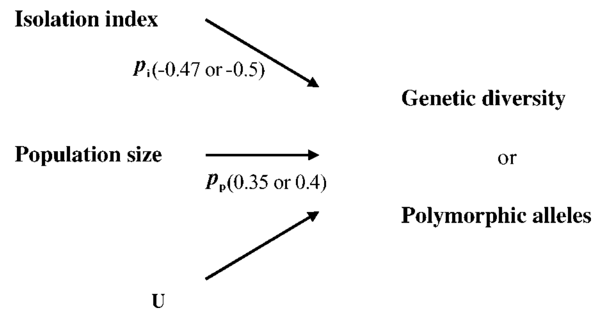

Fig. 5. Path diagram showing the effects of two environmental factors on population genetic viability components of *Zizania palustris* var. *palustris* in 1995. Path coefficients are listed for isolation index (p_i), population size (p_p) and the rest of all sources (U).

traits in wild-rice (Foster and Rutger, 1980), selection in different environments could favor different genotypes, allowing genetic variability to increase. Third, the buffering capacity of a large population to catastrophic events is higher than that of a small population, making drift less likely to erode local genetic variability. As these conditions hold for many species, we suspect the positive relationship between population size/area and genetic variability to be common in aquatic species. Cases showing similar associations have been reported in terrestrial species, such as *Salvia pratensis* and *Scabiosa columbaria* (Treuren et al., 1991), *Lychnis viscaria* (Lammi et al., 1999), *Ranunculus reptans* (Fischer et al., 2000), and *Cochlearia bavarica* (Paschke et al., 2002). Events such as frequent changes in habitat area, human selection, or other perturbations could obscure these relationships, making the associations undetectable in some cases. The populations we surveyed, however, appear relatively stable in these regards.

Wild-rice's genetic variability is affected strongly by the degree of population isolation (Fig. 5) because the highest coefficient of correlation ($r = -0.5$) was found between population isolation index and percentage of polymorphic loci (Table 4). Although a short population history and a strong selection incurred by human activity or habitat disturbance could both lead to a low genetic variability for a population, our knowledge of the distribution and habitats of the surveyed populations suggests that lack of gene flow may have contributed substantially to the pattern seen here.

Correlations between genetic variability and plant growth and reproduction—These patterns of genetic variation would bear little influence on wild-rice conservation had we not observed the positive associations between the gene diversity of a wild-rice population and certain growth and reproductive traits of the parental generation (Table 6). The traits correlated with gene diversity are fitness-related, including plant size (the maximum stem width and the longest leaf length) and reproductive output (panicle number per plant and the average seed scar number per panicle). Although positive correlations between genetic diversity and fitness within species have been rarely reported (e.g., Lienert et al., 2002), a combined analysis across multiple species revealed such a positive correlation (Reed and Frankham, 2003). Higher levels of genetic variability may translate into improved population persistence for wild-rice in natural environments.

Gene flow and population differentiation—Estimated F_{ST} is rather high among these wild-rice populations (0.30), suggesting a correspondingly low rate of gene exchange among populations (estimated $Nm = 0.59$). The migration estimate is necessarily crude due to the assumptions involved in the island model (e.g., Slatkin and Barton, 1989; Whitlock and McCauley, 1998). Nevertheless, even a large margin of error still paints a picture of restricted migration among populations. Although wind pollination appears effective in carrying pollen long distances, most pollination still occurs locally. For instance, for the wind-pollinated seagrass *Thalassia testudinum*, the most effective pollination takes place within 4 km (Schlueter and Guttman, 1998). Marine eelgrass (*Zostera marina*) appears similarly geographically structured with F_{ST} ranging above 0.20 ($Nm \sim 1.1$–2.8) and no correlation between geographic and genetic distance (Ruckelshaus, 1998). Although not strictly comparable, the populations surveyed here span a total of 250 km. Thus, local gene flow among nearby populations must be much higher than between distant populations. As the average gene flow is influenced by the geographic distribution of the populations surveyed, the best comparisons of gene flow among species would include information on the geographical distribution of the populations compared and population sizes.

The estimated low level of gene flow among the 17 populations is consistent with the observed weak correlation between genetic distance and geographic distance by Mantel's test. A similar low correlation has been found in the aquatic species *Eichhornia paniculata* (Barrett et al., 1993) and some terrestrial plant (e.g., Schiemann et al., 2000) and animal species (e.g., Baker et al., 2001). Conversely, a high level of gene flow among the populations could also homogenize the genetic composition of the populations, diminishing correlations between geographic and genetic distance and leading to a low level of F_{ST} as seen in species with continuous distributions in *Pinus* (Hamrick and Godt, 1996b).

Inbreeding coefficient and recent population history—Unlike patterns of genetic variability, which frequently reflect the deeper part of a population history, the inbreeding coefficient (f) responds more to recent events. The large variation observed in estimated f (-0.44–0.52) among the wild-rice beds suggests their rather heterogeneous recent histories, including possible changes in outcrossing rate, habitat disturbance, and direct human influence on the populations. The outcrossing rates are yet to be investigated among *Z. palustris* populations,

but large variations in outcrossing rate have been observed in *Oryza perennis* (Oka and Morishima, 1967) and *O. rufipogon* (Morishima and Barbier, 1990). One source of variation for wild-rice is the percentage of hermaphrodite florets on panicles, which varied from 0.1–0.6 among some Minnesota populations (Liu et al., 1998). Plant density and duration of anthesis between and among individuals may all bring changes to plant outcrossing rate. As outcrossing rate varies among populations and between years, inbreeding coefficient may fluctuate accordingly.

Wisconsin wild-rice populations differ greatly in their recent histories. Both the Blaisdell Lake and Spring Lake wild-rice populations had low levels of genetic variability, but heterozygosity levels were approximately 40% higher than expected (Table 2), suggesting strong selection. In contrast, the Rice Lake population (Forest County) had relatively high genetic variability and also the highest level of inbreeding among the populations surveyed, suggesting a recent history of population inbreeding, possibly due to some disturbance. The Mud Creek population exhibited high inbreeding and low genetic variability, possibly reflecting a recent population bottleneck.

Implications for wild-rice management—A certain level of genetic diversity is vital to allow populations to respond to short-term shifts in selection and, ultimately, long-term evolutionary challenges (Wilcox and Murphy, 1985; Hamrick et al., 1991; Milligan et al., 1994). If larger populations serve as more effective reservoirs of genetic diversity, protecting water bodies that support such populations represents an important step in managing these populations. However, wild-rice population size or area may fluctuate drastically from year to year (or even through a season), making it important to understand how such processes affect genetic variability and the persistence of unique alleles. Small populations that support high levels of genetic diversity demand special attention, as small populations are often at risk of losing genetic diversity via random genetic drift (Franklin, 1980; Frankham, 1995). Isolation index, on the other hand, is another important and knowable factor to consider in conservation plans. Our analysis shows that well-connected populations may preserve more genetic diversity.

Aquatic species rely on specific habitats and dispersal mechanisms, which likely make them subject to different ecological constraints and evolutionary processes than terrestrial plants (Les, 1988; Barrett et al., 1993; Laushman, 1993). In American wild-rice, however, we find levels of genetic variability and population genetic structure similar to those of terrestrial species with similar life histories and breeding systems. Because the growth habit of wild-rice is emergent, a sensible comparison of an aquatic system to terrestrial systems should also include parallel studies on submergent, floating species, as well as on more emergent aquatic species.

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