

# Genome-Wide Genetic Diversity among Components Does Not Cause Cultivar Blend Responses

S. J. Helland\* and J. B. Holland

## ABSTRACT

Genetically diverse plant populations may be better able to exploit ecological resources and reduce interplant competition than genetically homogeneous populations. Cultivar blends can have greater productivity and yield stability than pure lines; however blend effects are not consistent. The varying levels of genetic diversity represented in blends may confound the interpretations and comparisons of the results of different blend studies. We tested the hypothesis that genetic diversity of blend components is related to blend performance by evaluating blends of a set of five early-maturing and a set of 10 midseason-maturing oat (*Avena sativa* L.) cultivars in two separate experiments at eight Iowa environments. Within each experiment, pure lines and all possible two-way blends were evaluated for grain yield and test weight means and stability and adaptability parameters. The genetic diversity of each blend was estimated by pedigree diversity [ $1 - \text{coefficient of parentage (COP)}$ ], amplified fragment length polymorphism (AFLP)-derived genetic distances ( $1 - \text{Dice coefficient}$ ), and phenotypic diversity (based on height and heading date differences). Blend response was limited in these experiments and was not correlated with any diversity measure, and blend stability parameters were not consistently related to diversity measures across experiments. We also investigated the relationship between pedigree diversity and blend performance in other crops by computing the coefficients of parentage of cultivar pairs used in previous blend studies in maize, soybean, and wheat. Pedigree diversity was correlated with higher blend response only in two of 10 experiment–environment combinations tested. Genome-wide genetic diversity alone does not cause positive crop blend responses.

**S**UPERIORITY OF CULTIVAR BLENDS OVER pure-line cultivars has been observed in numerous crops, including soybean [*Glycine max* (L.) Merr.], oat, wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), sorghum [*Sorghum bicolor* (L.) Moench], cotton (*Gossypium hirsutum* L.), and rice (*Oryza sativa* L.) (Smithson and Lenné, 1996); however, research in oat, soybean, barley, maize (*Zea mays* L.), and wheat has also indicated that the effects of blending cultivars vary. In some cases, blending may result in no significant gain in yield or no reduction in disease damage, and may even have negative effects (Smithson and Lenné, 1996). For example, Frey and Maldonado (1967) found a significant increase in mean blend yields over component pure-line yields in oat only in high-stress environments. Similarly, Helland and Holland (2001) reported that blends of early-maturity, but not midseason-maturity, oat culti-

vars had greater grain yield than their component pure lines on average. Furthermore, they reported that blend response varied among different combinations of cultivars.

Most oat, soybean, and wheat cultivars are pure lines. The available pure-line cultivars represent a wide range of unique, homozygous genotypes often adapted to specific environmental conditions. A genetically variable plant population will often have a greater chance of successful adaptation across a range of environments than a genetically homogeneous population (Allard and Bradshaw, 1964). A cultivar blend capitalizes on this principle by using a mixture of two or more pure-line cultivars grown in the field at the same time in an attempt to achieve greater yield and yield stability.

Ecologists have demonstrated that increasing species diversity contributes to greater ecosystem productivity and stability (Tilman et al., 1997; van der Heijden et al., 1998; Hector et al., 1999). The benefits of genetic diversity within single-species populations have also been demonstrated. Overcompensation, the ability of genetically diverse populations to use resources more efficiently and successfully than monomorphic populations, has been documented in *Drosophila melanogaster* (Peng et al., 1991). Similarly, Cole and Wiernasz (1999) found there was a positive relationship between colony fitness and genetic diversity measured at two isozyme loci in harvester ant [*Pogonomyrmex occidentalis* (Cresson)] colonies. It is not known whether this principle also extends to within-species genetic variation in crops. If it does, we expect that increasing the genetic diversity among blend components should result in greater blend response and yield stability. We hypothesized that a confounding factor contributing to the variability among results of previous blend experiments was the level of genetic diversity among cultivars included in the blends under study. If high levels of genetic diversity between the components of a blend are important for increasing blend response, the response may be variable among blends and among experiments.

The objective of this experiment was to determine the relationship between genetic diversity (as estimated by genetic distance, COP, and phenotypic differences) and blend response, blend stability, and blend adaptability in oat. Additionally, we investigated the relationship between the COP and blend response in other crops by analyzing blend data from previously published experiments in soybean (Gizlice et al., 1989), maize (Hoekstra

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**Abbreviations:** AFLP, amplified fragment length polymorphism; BYDV, *Barley yellow dwarf virus*; COP, coefficient of parentage; GSE, genetic similarity estimate; NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System personal computer program; QTL, quantitative trait loci.

et al., 1985), and wheat (Finckh and Mundt, 1992; Mundt et al., 1995).

## MATERIALS AND METHODS

### Experimental Design and Observations

Two separate experiments were performed to evaluate early- and midseason-maturing cultivars of oat. Entries in the first trial consisted of five early-maturing cultivars (Dane, Don, Horicon, Sheldon, and Starter) grown as pure lines and as all possible two-cultivar blends. In the second trial, 10 midseason-maturing cultivars (Blaze, Burton, Chaps, Jerry, Jim, Newdak, Ogle, Prairie, Premier, and Rodeo) were evaluated as pure lines and in all two-way cultivar blends. Blends were developed by mixing approximately equal numbers of seeds of each line as determined by the weight of a 100-seed sample of each cultivar. Both experiments were grown in 1998 and 1999 at Ames (central Iowa), Nashua (northeastern Iowa), Crawfordsville (southeastern Iowa), and Lewis (southwestern Iowa). Experimental designs were square or rectangular lattice designs with three replications at each environment. Experimental units were four-row, 3.72-m<sup>2</sup> plots sown with 1000 seeds each. Plots were not treated with fungicides or insecticides, and were subject to natural infestations of crown rust (*Puccinia coronata* Corda var. *avenae* W.P. Fraser & Ledingham) and Barley yellow dwarf virus (BYDV).

In 1999, plots were rated for severity of crown rust and BYDV within the 2 wk following heading date in four and three environments, respectively, using a nine-point combined scale of incidence and severity (Helland and Holland, 2001). Heading date was recorded at Ames as the number of days after planting when 50% of the panicles in each plot were fully emerged. Heights were measured as the distance between soil level and panicle tips after heading was complete. Plots were machine-harvested, and grain yield (kg ha<sup>-1</sup>) and test weight (kg m<sup>-3</sup>) were measured on every plot. Further details of experimental procedures were given by Helland and Holland (2001).

In the oat experiments, mean blend responses for yield and test weight were estimated as the difference between the mean of a blend and the average of its component pure-line means. Mean blend responses for yield (measured in kg ha<sup>-1</sup> or as a percentage of mean component cultivar yields) were also obtained from previously published experiments in wheat (Finckh and Mundt, 1992; Mundt et al., 1995), maize (Hoekstra et al., 1985), and soybean (Gizlice et al., 1989).

### Genetic Diversity Estimation

Three phenotypic diversity measures were estimated for each oat blend. The absolute values of the mean differences between the components of each blend grown as pure lines were computed for heading date, height, and BYDV score.

Coefficients of parentage were calculated using pedigrees and breeding records and according to the methods of Murphy et al. (1986). Pedigree information and coefficients of parentage for oat cultivars were obtained from Souza and Sorrells (1988), National Germplasm Resources Laboratory (2002), F. L. Kolb (2000, personal communication), and H. F. Kaeppler (2000, personal communication). Coefficients of parentage for soybean cultivars were taken from Carter et al. (1993), and C. Tinius (2000, personal communication) provided additional information. Pedigree information and COPs for the wheat cultivars included in each experiment were obtained from Fox et al. (1997). Maize pedigrees were provided by G. Hoekstra (2000, personal communication) and L. Kannenberg (2000, personal communication). Several commercial hybrids used

in the Hoekstra et al. (1985) study had unknown pedigrees and were therefore excluded from our analysis. Pedigree diversity was defined as  $(1 - \text{COP})$ .

DNA isolation methods were described by Holland et al. (2001). Restriction digestion, primer labeling, polymerase chain reaction, and gel electrophoresis procedures for AFLP analysis followed Vos et al. (1995). Four AFLP primer pairs were selected based on polymorphism revealed in a previous screen of two oat cultivars. Primer pairs used were a combination of *EcoRI* and *MseI* core primers plus three selective bases: *EcoRI* + *ACG/MseI* + CTG, *EcoRI* + *ACG/MseI* + CTT, *EcoRI* + *ACG/MseI* + CTC, and *EcoRI* + *ACC/MseI* + CTC. Polymerase chain reactions for AFLP analysis were performed twice, and autoradiograms were scored independently by two researchers. Polymorphisms that were scored consistently across replicate AFLP analyses were used to estimate genetic diversity among cultivars. Genetic similarity estimates (GSEs) were calculated between all pairs of individuals in all possible combinations using the Dice coefficient of genetic distance (Dice, 1945). According to this coefficient, the GSE is the measure of genetic similarity between a pair of samples,  $i$  and  $j$ :

$$\text{GSE}_{ij} = 2a/(2a + b + c),$$

where  $a$  = the number of bands common to lines  $i$  and  $j$ ,  $b$  = the number of bands present in  $i$  but absent in  $j$ , and  $c$  = the number of bands absent in  $i$  but present in  $j$ . All calculations and analyses were conducted using the Numerical Taxonomy and Multivariate Analysis System personal computer (NTSYS-pc) program (Rohlf, 1992). The AFLP-based genetic diversity estimates were computed as one minus the AFLP-based Dice coefficient of similarity.

### Statistical Analysis of Oat Experiments

Mean squares, estimates of means, and LSDs for yield and test weight were obtained using PROC GLM in SAS (SAS Institute, 1999). Blend responses were declared significant if they exceeded the threshold value of

$$t_{(\alpha = 0.05)} \sqrt{\left(\frac{1}{re} + \frac{1}{2re}\right) \text{MS}_{\text{GE}}},$$

where  $r$  = number of replications per environment, 3;  $e$  = number of environments, 8; and  $\text{MS}_{\text{GE}}$  was the mean square for genotype  $\times$  environment interaction.

Stability was estimated with Shukla's genotype  $\times$  environment interaction variance,  $\sigma_i^2$  (Shukla, 1972), and adaptability was estimated with Lin and Binns' superiority statistic,  $P_i$  (Lin and Binns, 1988), which incorporates the magnitude of yield in its measure of stability. Lower values of these parameters reflect greater stability or adaptability. Blend responses for mean yield and test weight were regressed on diversity measures using PROC REG in SAS (SAS Institute, 1999). Negative values of the stability and adaptability estimates were also regressed on diversity measures. Negative values were used so that positive regression slopes would indicate a positive relationship between increasing diversity and increasing stability or adaptability. To test for parabolic nonlinear responses to diversity, the distribution of blend phenotypes were plotted against diversity measures and inspected. Blend phenotypes were also regressed on second-order polynomials of diversity measures. Correlations among the four diversity measures within each experiment were estimated by Mantel tests (Fortin and Gurevitch, 1993). To estimate the  $P$  values of each correlation coefficient, data were permuted at least 2000 times using NTSYS-pc (Rohlf, 1992).

**Table 1. Genetic and phenotypic diversity estimates, mean yield and test weight blend responses, and mean stabilities of five early-maturing oat cultivars grown as all possible two-way blends in eight Iowa environments.**

Blend	Genetic diversity		Phenotypic diversity		Blend response†		Yield stability		Test weight stability	
	AFLP	Pedigree	Heading date	Height	Grain yield	Test weight	$P_i$ ‡	$\sigma_i^2$ §	$P_i$	$\sigma_i^2$
	1 – Dice	1 – COP¶	d	cm	kg ha <sup>-1</sup>	kg m <sup>-3</sup>	× 10 <sup>4</sup>	× 10 <sup>3</sup>	× 10	(kg ha <sup>-1</sup> ) <sup>2</sup>
Dane/Don	0.47	0.94	1	5	144	3	17	22	148	88
Dane/Horicon	0.08	0.45	6	6	3	4	13	12	150	70
Dane/Sheldon	0.45	0.88	4	6	234*	8#	19	43	162	46
Dane/Starter	0.35	0.89	2	1	114	5	20	66	72	14
Don/Horicon	0.54	0.94	5	11	-43	1	62	63	84	113
Don/Sheldon	0.72	0.96	3	11	146	5	41	34	80	68
Don/Starter	0.60	0.96	1	6	50	-1	60	54	66	389
Horicon/Sheldon	0.47	0.88	2	0	44	6	38	63	96	57
Horicon/Starter	0.43	0.89	4	5	49	6	34	19	19	32
Sheldon/Starter	0.31	0.71	2	5	103	9#	55	57	38	285
Mean of all blends	0.44	0.85	3	6	84*	5*	36	43	92	116
Blend means – pure-line means							-22*	-28*	-54	-64
LSD (0.05)							15	40	38	79

\* Significant at the 0.05 probability level.

† Blend response is the difference between the blend mean and the mean of two component pure lines.

‡ Lin and Binn's adaptability parameter ( $P_i$ ); entries with lower values of  $P_i$  have greater adaptability across testing environments.§ Shukla's stability estimate ( $\sigma_i^2$ ); entries with lower values of  $\sigma_i^2$  have greater stability across testing environments.

¶ Coefficient of parentage (COP); entries with lower COPs are less related.

# Significant at the 0.10 probability level.

### Statistical Analysis of Previously Published Experiments

Blend responses within environments reported by Finckh and Mundt (1992) and Hoekstra et al. (1985) were analyzed across environments using SAS Proc Mixed (SAS Institute, 1999), considering blends to be fixed effects and environments to be random. Significance of individual and overall blend responses from these experiments was tested with *estimate* statements in Proc Mixed. Mundt et al. (1995) reported significance tests for individual blend responses in their study, and we constructed significance tests for overall blend responses by adjusting the least significant difference for pairwise comparisons reported in their paper. Significance tests for individual blend responses and overall blend responses for data from Gizlice et al. (1989) were constructed based on the genotype × environment MS they reported for their experiment. Linear and quadratic regressions of percentage blend responses on pedigree diversity were tested for each experiment.

Finckh and Mundt (1992) tested wheat blends and pure lines both in plots inoculated with *Puccinia striiformis* Westend., the causal agent of stripe rust, and in plots protected from stripe rust by fungicide treatment. Similarly, Mundt et al. (1995) tested wheat blends and pure lines in plots with low or high levels of severity of septoria blotch (*Septoria tritici* Roberge in Desmaz.) and eyespot [*Psuedocercospora herpotrichoides* (Fron) Deighton]. For these two experiments, therefore, we separately regressed blend responses from plots with high disease pressure, responses from plots with low disease pressure, and responses averaged across disease treatments on pedigree diversity. Similarly, Hoekstra et al. (1985) reported that mean yields and blend responses differed dramatically across the 2 yr of their experiment, so we separately regressed blend responses from each year as well as mean responses across years on pedigree diversity from that study.

## RESULTS

### Cultivar Blends in Oat

Thirty-four repeatable AFLP bands were scored across all oat cultivars. The AFLP-derived genetic diver-

sities of cultivar pairs ranged from 0.08 to 0.72 in the early-maturing oat cultivar blends and from 0.06 to 0.55 in the midseason-maturing blends (Tables 1, 2). Pedigree diversities of cultivar pairs ranged from 0.45 to 0.96 in the early-maturity experiment and from 0.23 to 0.96 in the midseason-maturity experiment (Tables 1, 2). Heading date differences between cultivars ranged from 1 to 6 d and from 0 to 4 d in the early- and midseason-maturity experiments, respectively (Tables 1, 2). The ranges of height diversity for each experiment were 0 to 11 cm among the early-maturing cultivars and 0 to 12 cm among the midseason-maturing cultivars (Tables 1, 2). Variation in these diversity measures should be sufficient to detect correlations between diversity and blend response that would be of practical value. Genetic and pedigree diversity measures were significantly correlated in both the early ( $r = 0.89$ ,  $P = 0.03$ ) and midseason-maturity ( $r = 0.48$ ,  $P = 0.004$ ) experiments. In addition, pedigree diversity and height differences were correlated in the midseason-maturity experiment ( $r = 0.56$ ,  $P = 0.0005$ ).

Mean blend response was significantly positive for grain yield, test weight, and adaptability ( $P_i$ ) for grain yield in the early-maturity experiment (Table 1). Individually, the blend response for grain yield of the blend of Dane and Sheldon was positive. Using a more liberal threshold for significance ( $\alpha = 0.10$ ), test weight blend response was significantly positive for Dane/Sheldon and Sheldon/Starter. In the early-maturity experiment, only one of the 10 blends (10%) had a numerically negative blend response for either grain yield or test weight (Table 1), which was a significant deviation from the expected number of negative responses under the hypothesis that all blend responses are zero ( $\chi^2 = 6.4$ , 1 df,  $P < 0.05$ ). In contrast, the overall blend responses for yield and test weight in the midseason-maturity experiment were not significant (Table 2). Furthermore,

**Table 2. Genetic and phenotypic diversity estimates, mean yield and test weight blend responses, and mean stabilities of 10 midseason-maturing oat cultivars grown as all possible two-way blends in eight Iowa environments.**

Blend	Genetic diversity		Phenotypic diversity		Blend response†		Yield stability		Test weight stability	
	AFLP	Pedigree	Heading date	Height	Grain yield	Test weight	$P_i$ ‡	$\sigma_i^2$ §	$P_i$	$\sigma_i^2$
	1 - Dice	1 - COP	d	cm	kg ha <sup>-1</sup>	kg m <sup>-3</sup>	× 10 <sup>4</sup>	× 10 <sup>3</sup>	× 10	
								(kg ha <sup>-1</sup> ) <sup>2</sup>		
Blaze/Burton	0.27	0.87	2	2	-142	-2	84	113	191	592
Blaze/Chaps	0.33	0.69	0	3	18	1	15	33	152	141
Blaze/Jerry	0.33	0.92	0	10	138	1	43	196	42	457
Blaze/Jim	0.30	0.84	0	2	73	-2	14	39	122	151
Blaze/Newdak	0.34	0.45	1	4	49	-3	70	116	232	161
Blaze/Ogle	0.33	0.67	1	4	108	1	22	36	223	72
Blaze/Prairie	0.17	0.81	1	1	-54	1	38	59	282	276
Blaze/Premier	0.27	0.83	1	1	46	2	31	68	59	139
Blaze/Rodeo	0.33	0.70	4	5	133	3	6	23	171	352
Burton/Chaps	0.41	0.89	2	5	-43	-1	54	60	285	156
Burton/Jerry	0.35	0.95	2	12	75	-1	89	57	78	126
Burton/Jim	0.46	0.90	2	4	-109	-1	66	40	223	222
Burton/Newdak	0.47	0.91	1	6	87	6	104	56	278	304
Burton/Ogle	0.55	0.87	1	6	15	0	76	34	385	268
Burton/Prairie	0.47	0.93	1	3	-154#	-1	88	50	475	585
Burton/Premier	0.42	0.82	1	1	-163#	-2	110	25	171	192
Burton/Rodeo	0.41	0.89	2	7	-88	2	58	31	308	646
Chaps/Jerry	0.20	0.94	0	7	62	4	36	23	60	62
Chaps/Jim	0.40	0.73	0	1	184#	7	11	122	153	105
Chaps/Newdak	0.33	0.57	1	1	78	6	53	38	276	87
Chaps/Ogle	0.16	0.23	1	1	67	4	28	88	340	237
Chaps/Prairie	0.33	0.59	1	2	-111	-4	33	47	507	266
Chaps/Premier	0.48	0.87	1	4	43	3	27	29	127	94
Chaps/Rodeo	0.06	0.35	4	2	-43	-7	15	58	390	124
Jerry/Jim	0.36	0.94	0	8	82	3	44	53	50	344
Jerry/Newdak	0.39	0.90	1	6	78	5	115	85	92	264
Jerry/Ogle	0.30	0.93	1	6	152	-5	58	50	183	297
Jerry/Prairie	0.39	0.96	1	9	-37	0	76	32	177	18
Jerry/Premier	0.46	0.95	1	11	54	0	77	61	42	377
Jerry/Rodeo	0.23	0.94	4	5	-47	-4	62	69	129	185
Jim/Newdak	0.45	0.80	1	2	168#	1	54	177	251	112
Jim/Ogle	0.33	0.68	1	2	59	4	29	52	296	416
Jim/Prairie	0.41	0.83	1	1	59	2	24	132	362	474
Jim/Premier	0.18	0.92	1	3	7	1	33	41	122	305
Jim/Rodeo	0.37	0.74	4	3	63	2	12	30	226	130
Newdak/Ogle	0.41	0.47	0	0	82	1	85	62	418	38
Newdak/Prairie	0.31	0.73	0	3	50	0	73	69	516	111
Newdak/Premier	0.48	0.90	0	5	-45	-1	126	80	217	171
Newdak/Rodeo	0.40	0.57	3	1	56	0	54	22	370	143
Ogle/Prairie	0.26	0.49	0	3	63	-5	37	150	674	513
Ogle/Premier	0.43	0.88	0	5	90	-6	47	83	326	352
Ogle/Rodeo	0.23	0.23	3	1	7	-8	35	59	519	81
Prairie/Premier	0.49	0.93	0	2	-118	-7	67	67	380	265
Prairie/Rodeo	0.33	0.59	3	4	-98	4	37	76	492	1034
Premier/Rodeo	0.48	0.88	3	6	-2	-9	31	42	259	93
Mean of all blends	0.35	0.77	1	4	22	0	52	65	256	209
Blend means - pure-line means							-7	-10	-21	31
LSD (0.05)							17	40	71	181

† Blend response is the difference between the blend mean and the mean of two component pure lines.

‡ Lin and Binn's adaptability parameter ( $P_i$ ); entries with lower values of  $P_i$  have greater adaptability across testing environments.§ Shukla's stability estimate ( $\sigma_i^2$ ); entries with lower values of  $\sigma_i^2$  have greater stability across testing environments.

|| Coefficient of parentage (COP); entries with lower COPs are less related.

# Significant at the 0.10 probability level.

no individual blend responses were significant at  $\alpha = 0.05$ . At  $\alpha = 0.10$ , two blends had significantly positive blend responses and two blends had significantly negative blend responses for yield (Table 2). As in the early-maturity oat study, a significantly greater number (30) of numerically positive yield blend responses were observed than expected (22.5) under the hypothesis that all blend responses are zero ( $\chi^2 = 5.0$ , 1 df,  $P < 0.05$ ).

The difference in heading date between a blend's component cultivars was not linearly related to blend response for yield, test weight, or stability in the early- or midseason-maturity experiments. There was a weak nonlinear relationship between heading date difference and blend yield adaptability in the midseason study only

( $P$  value of quadratic regression coefficient = 0.044, model  $R^2 = 0.13$ ), in which adaptability was lowest for blends with intermediate levels of heading date differences (Fig. 1). The linear regression of negative values of the adaptability estimate ( $P_i$ ) for the test weight of each blend on the height difference between component cultivars was significant in the midseason-maturity experiment ( $r^2 = 0.21$ ,  $P = 0.001$ ; Fig. 2). As height differences increased, so did  $-P_i$  ( $b = 24.2 \times 10^4 \text{ kg}^2 \text{ m}^{-6}$ ), indicating that test weight adaptability increased with increasing differences in height (Fig. 2). Height difference was not related to other blend performance measures, however.

Pedigree diversity was negatively related to  $-P_i$  for

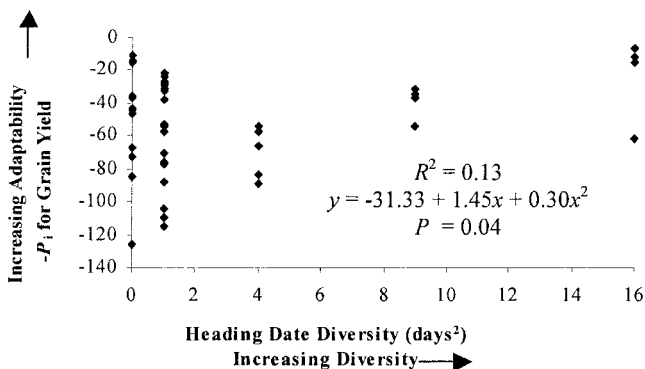


Fig. 1. Quadratic regression of negative values of Lin and Binns' superiority measure ( $-P_i$ ) for grain yield on the heading date difference between component pure-line midseason-maturity oat cultivars.

grain yield in the midseason-maturity experiment ( $b = -52.4 \times 10^4 \text{ kg}^2 \text{ ha}^{-2}$ ,  $r^2 = 0.12$ ,  $P = 0.02$ ; Fig. 3). Conversely, as pedigree diversity increased (cultivars were less related), the adaptability of test weights increased in the midseason-maturity experiment ( $b = 430.2 \times 10 \text{ kg}^2 \text{ m}^{-6}$ ,  $r^2 = 0.32$ ,  $P = 0.0001$ ; Fig. 4). AFLP-based genetic diversity was also negatively related to adaptability for grain yield in the midseason oat experiment ( $b = -126.4 \times 10^4 \text{ kg}^2 \text{ ha}^{-2}$ ,  $r^2 = 0.19$ ,  $P = 0.003$ ; Fig. 5).

A nonlinear relationship was observed between pedigree diversity and blend response for test weight in the early maturity oat experiment, with the greatest response observed for the blend with pedigree diversity of 0.71 (Sheldon/Starter), and responses decreasing for blends with lower and higher pedigree diversities (Fig. 6). Both linear and quadratic regression coefficients were significant at  $P = 0.01$ , with model  $R^2 = 0.66$ ; however, the relationship was highly dependent on one observation (Dane/Horicon) with pedigree diversity lower than Sheldon/Starter. A similar trend was observed in the midseason-maturity oat experiment, where the greatest blend response for test weight observed for Chaps/Jim (pedigree diversity of 0.73) and blend responses decreased at both lower and higher levels of pedigree diversity, but the linear and quadratic coefficients had  $P$  values of 0.06 and 0.07, respectively, and the model  $R^2$  was only 0.09.

Variability in crown rust resistance likely did not contribute to blend response in this study because signifi-

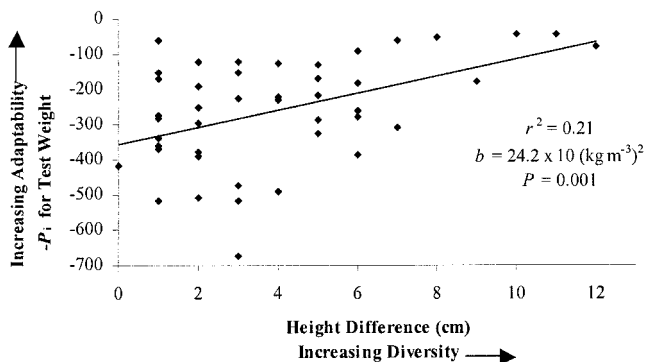


Fig. 2. Regression of negative values of Lin and Binns' superiority measure ( $-P_i$ ) for test weight on the height difference between component pure-line midseason-maturity oat cultivars.

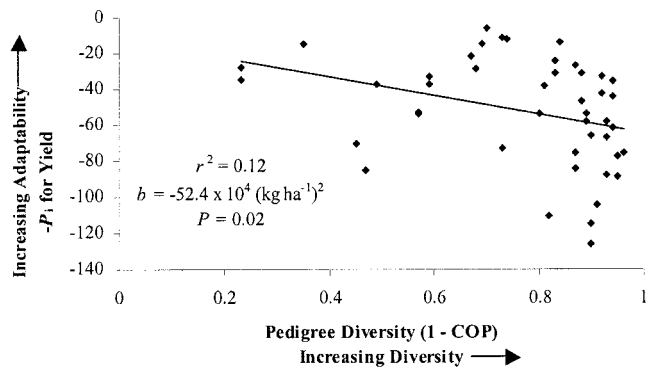


Fig. 3. Regression of negative values of Lin and Binns' superiority measure ( $-P_i$ ) for grain yield on the pedigree diversity (1-coefficient of parentage) between component pure-line midseason-maturity oat cultivars.

cant differences for crown rust resistance scores were not observed among the pure-line cultivars in the environments studied (Helland and Holland, 2001). Significant differences were observed for mean BYDV resistance scores among pure lines in the early maturity experiment, but no relationship between the diversity of component BYDV resistance scores and blend traits was observed. Genetic diversity was not otherwise related to blend performance.

### Cultivar Blends in Other Species

Pedigree diversity for cultivar pairs used to form blends in the wheat experiments ranged from 0.13 to 0.34 in one experiment (Table 3; Finckh and Mundt, 1992) and from 0.50 to 1.00 in the other (Table 4; Mundt et al., 1995). Pedigree diversity ranged from 0.75 to 1.00 in the maize experiment (Table 5; Hoekstra et al., 1985), and from 0.31 to 0.91 in the soybean experiment (Table 6; Gizlice et al., 1989). Of the four non-oat-blend experiments analyzed, blend responses were significantly related to pedigree diversity in only two cases. Soybean yield blend response (Gizlice et al., 1989) was significantly related to pedigree diversity ( $r^2 = 0.17$ ,  $P < 0.05$ ). The slope of this regression line was positive ( $b = 275.1 \text{ kg ha}^{-1}$ ); thus, soybean blends developed from more genetically diverse cultivars tended to have greater blend responses (Fig. 7). Wheat blends from plots inoculated with stripe rust in the experiment of Finckh and

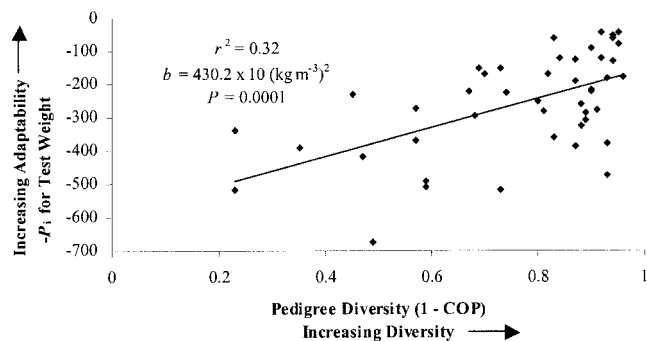


Fig. 4. Regression of negative values of Lin and Binns' superiority measure ( $-P_i$ ) for test weight on the pedigree diversity (1-coefficient of parentage) between component pure-line midseason-maturity oat cultivars.

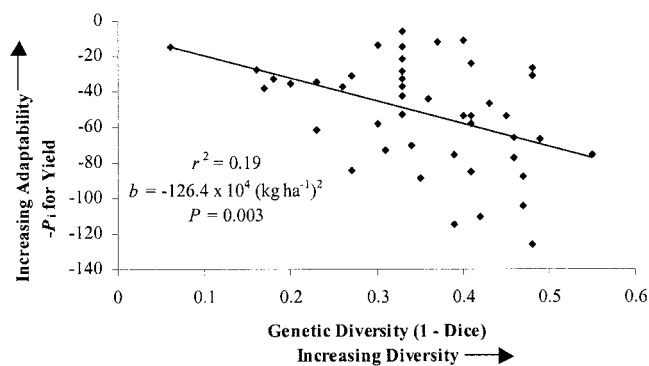


Fig. 5. Regression of negative values of Lin and Binns' superiority measure ( $-P_i$ ) for grain yield on the AFLP-based genetic diversity (1-Dice coefficient) between component pure-line midseason-maturity cultivars.

Mundt (1992) also exhibited a significantly positive relationship with pedigree diversity, with blend responses increasing 85% for each unit of pedigree diversity (Fig. 8;  $P < 0.05$ ,  $r^2 = 0.41$ ). However, no relationship was observed for yield measured in rust-free plots, nor for yields averaged across rust treatments from the same experiment.

## DISCUSSION

This is the first comprehensive report of the limited relationship between molecular genetic, pedigree, and phenotypic diversity and crop blend superiority. We hypothesized that the genetic variation represented by a blend could influence blend response, but the results of our experiments demonstrated that neither genetic diversity, as estimated by AFLPs or COPs, nor phenotypic diversity, as estimated by height and heading date, was consistently related to blend response. Furthermore, among all combinations of experiments, traits, and diversity measures, the only positive linear relationship between genetic diversity and blend performance in oat existed between test weight adaptability and pedigree diversity. Among the other crops evaluated, genetic diversity and blend response were significantly related

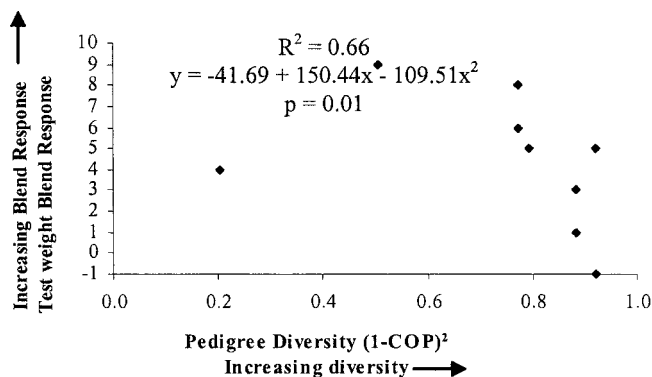


Fig. 6. Quadratic regression of the test weight blend response on the square of the pedigree diversity (1 - coefficient of parentage) between component pure-line early-maturity oat cultivars.

only in soybean and in one wheat experiment only under high disease pressure. A few weak nonlinear relationships were also observed between diversity measures and blend phenotypes, but these were not consistent across experiments or traits, suggesting that they are not of general importance.

Several studies have shown that the relationship between morphological or phenotypic diversity and blend response is variable. Schweitzer et al. (1986) reported that greater diversity in plant height and date of maturity among components of soybean cultivar blends was related to greater yields. In contrast, Gizlice et al. (1989) and Patterson et al. (1963) found that differences in maturity did not result in greater blend response in soybean and oat, respectively. The conflicting results in our oat experiments and among previous experiments in other species (Mumaw and Weber, 1957; Patterson et al., 1963; Allard and Adams, 1969; Luedders, 1979; Schweitzer et al., 1986; Gizlice et al., 1989) demonstrate that the relationship between diversity and blend performance may vary according to the species investigated, the sample of cultivars, or the environments in which they were tested.

There are several possible explanations for the lack of relationship observed between genetic diversity and

Table 3. Pedigree diversities (1 - COP $\dagger$ ) and mean percentage blend responses for grain yield of 10 winter wheat cultivar blends across three environments in the presence or absence of stripe rust or averaged across disease treatments (Finckh and Mundt, 1992).

Blend	Pedigree diversity	Blend response $\ddagger$		
		Fungicide-treated	Stripe rust-inoculated	Average across disease treatments
%				
Faro/Jacmar	0.18	3	-9	-3
Faro/Moro	0.17	9	-7	1
Faro/Tres	0.26	-6	16*	5
Faro/Tyee	0.33	-2	9	3
Jacmar/Moro	0.13	0	-2	-1
Jacmar/Tres	0.19	-1	10	5
Jacmar/Tyee	0.23	4	15*	10*
Moro/Tres	0.19	8	13	11*
Moro/Tyee	0.30	1	12	7
Tres/Tyee	0.34	-3	17*	7
Mean blend response		1	7***	4**

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

$\dagger$  Coefficient of parentage (COP); entries with lower COPs are less related.

$\ddagger$  Percentage blend response measured as the difference between the blend mean and the mean of two-component pure lines divided by the pure-line mean.

**Table 4. Pedigree diversities (1 – COP<sup>†</sup>) and mean percentage blend responses for grain yield of six winter wheat cultivar blends grown under low and high disease pressure caused by Septoria blotch and eyespot across three environments (Mundt et al., 1995).**

Blend	Pedigree diversity	Blend response‡		
		Low disease pressure	High disease pressure	Average across disease pressures
		%		
Madsen/Malcolm	0.78	2	–2	0
Madsen/Gene	1.00	3	4	3
Madsen/Stephens	0.89	–3	1	–1
Malcolm/Gene	1.00	8*	6	7**
Malcolm/Stephens	0.50	–2	–1	–1
Gene/Stephens	1.00	0	1	0
Mean blend response		1	2	1

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

† Coefficient of parentage (COP); entries with lower COPs are less related.

‡ Blend response measured as the difference between the blend mean and the mean of two component pure lines divided by the pure-line mean.

blend response in our experiments, including: (i) the limited blend response in our studies, (ii) a true lack of any underlying relationship between genetic diversity and blend response in crops adapted to production in monoculture, and (iii) a requirement for diversity at a subset of loci, rather than across the whole genome, to cause blend response.

### Limited Blend Response

One factor that may have limited our ability to detect a consistent relationship between blend response and genetic diversity was the limited blend responses observed in our studies. Among the oat experiments, overall blend responses were significant only in the smaller, early-maturity oat experiment. A trend of small blend responses for yield was observed in both oat experiments (based on the significantly greater number of numerically positive blend responses than expected under the hypothesis of zero response for all blends), and mean blend responses were significant in fewer than half of the experiment–environment combinations from previously published studies that we tested (Tables 3–6).

However, significant effects of pedigree diversity were identified in one case where mean blend response was significant (stripe rust-inoculated wheat, Table 3) and in one case where it was not (soybean, Table 6). Taken together, these results suggest that a significant

**Table 5. Pedigree diversities (1 – COP<sup>†</sup>) and mean percentage blend responses for grain yield of six maize cultivar blends averaged across two planting densities within each of 2 yr and averaged across 2 yr (Hoekstra et al., 1985).**

Blend	Pedigree diversity	Blend Response‡		
		1978	1979	2-yr average
		%		
Coop S26/PAG SX111	1.00	3	–2	0
Coop S25/United 106	0.75	8	–2	2
Coop S27/Warwick W777	0.75	13	0	5
PAG SX111/United 106	1.00	–4	–6	–2
PAG SX111/Warwick W777	0.98	19**	4	11
United 106/Warwick W777	0.75	–1	–9	–6
Mean blend response		6*	–1	2

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

† Coefficient of parentage (COP); entries with lower COPs are less related.

‡ Blend response measured as the difference between the blend mean and the mean of two component hybrids divided by the mean of the component hybrids.

mean blend response is neither necessary nor sufficient to detect a relationship between genetic diversity and blend response. Furthermore, a reasonable range of variation in genetic diversity was sampled among the blends and experiments tested, so our results suggest that genetic diversity is not sufficient to cause blend responses.

### No Effect of Genetic Diversity on Blend Response?

Our inability to demonstrate a consistent effect of genetic diversity on blend response may simply be due to the fact that no such effect exists. It may be possible that selection for pure-line performance in oat and other

**Table 6. Pedigree diversities (1 – COP<sup>†</sup>) and mean blend responses for grain yield of 28 soybean cultivar blends across eight environments (Gizlice et al., 1989).**

Blend	Pedigree diversity	Blend Response‡
		kg ha <sup>–1</sup>
Ra604/N77114	0.78	78
Ra604/Forrest	0.31	–50
Ra604/DPI105	0.78	18
Ra604/Ransom	0.82	–25
Ra604/Coker	0.55	22
Ra604/Bragg	0.40	–80
Ra604/GaSoy	0.62	48
N77114/Forrest	0.74	3
N77114/DPI105	0.61	120
N77114/Ransom	0.55	110
N77114/Coker	0.91	162
N77114/Bragg	0.79	–157
N77114/GaSoy	0.82	148
Forrest/DPI105	0.74	–223
Forrest/Ransom	0.79	70
Forrest/Coker	0.70	45
Forrest/Bragg	0.37	–45
Forrest/GaSoy	0.64	80
DPI105/Ransom	0.79	67
DPI105/Coker	0.83	129
DPI105/Bragg	0.82	54
DPI105/GaSoy	0.81	161
Ransom/Coker	0.86	38
Ransom/Bragg	0.81	222
Ransom/GaSoy	0.85	200
Coker/Bragg	0.58	23
Coker/GaSoy	0.69	117
Bragg/GaSoy	0.44	–96
Mean blend response		44*

\* Significant at the 0.05 probability level.

† Coefficient of parentage (COP); entries with lower COPs are less related.

‡ Blend response measured as the difference between the blend mean and the mean of two component pure lines.

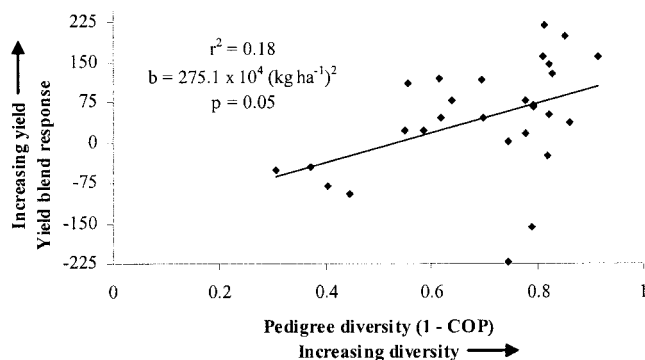


Fig. 7. Regression of the grain yield blend response of 28 soybean cultivar blends on the coefficient of parentage of the cultivars used in the blends (Gizlice et al., 1989).

crops has minimized their capacity to interact positively with other genotypes in the same stand (Antonovics, 1978; Turkington, 1996; Hill, 1996; Fasoula and Fasoula, 1997). If so, the ability to use genetic diversity to increase blend response could be present in genotypes that are adapted to cultivation in genotypically mixed stands, but absent in genotypes adapted to pure-line cultivation, such as those used in the experiments described here.

Allard and Adams (1969) presented evidence for this hypothesis by comparing blending ability among four high-yielding commercial barley cultivars and among eight lines from a barley composite cross population that had been subject to natural selection as a mixed population for 18 generations. Blends yielded more than pure lines on average for both sets of genotypes; however, the blend response among lines from the composite cross population study was much greater than that found among the commercial cultivars. Allard and Adams (1969) suggested that because natural selection acted on the composite cross population while it was advanced, the eight lines selected from it were chosen for fitness expressed in competition.

The performance of oat blends composed of more than two or three pure lines may be further evidence for selection against blending ability in pure-line cultivars. Studies of naturally evolved pasture plant communities demonstrated that increasing genotype and species diversity was related to an increase in forage yields (reviewed by Turkington, 1996). It should follow that increased numbers of genetically diverse components in an oat blend will improve blend performance. Previous research has shown, however, that 3- to 10-component blends have no advantage over 2-component blends (Clay and Allard, 1969; Frey and Maldonado, 1967; Helland and Holland, 2001). Perhaps this increased diversity has no effect because the ability to blend well has been bred out of modern pure-line oat cultivars.

Hill (1996) suggests that breeders should select cultivars for performance in blends by evaluating them in mixed stands throughout the selection process. Allard and Adams (1969) and Gizlice et al. (1989) suggest ecological combining ability as a measure of a cultivar's blending ability. Ecological combining ability measures the ability of one genotype to be a good competitor in a blend without negatively affecting the other component

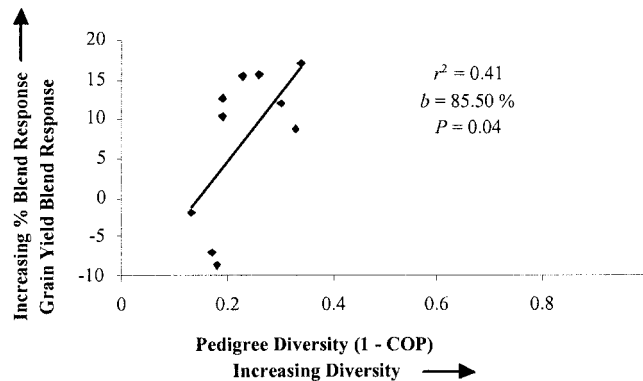


Fig. 8. Regression of the grain yield blend response of stripe-rust-inoculated wheat cultivar blends on the coefficient of parentage of the cultivars used in the blends (Finckh and Mundt, 1992).

genotypes (Allard and Adams, 1969). Choosing blend components by selecting cultivars that have performed well in mixed stands might produce greater blend response than choosing components based on the genetic diversity of pure-line cultivars.

### Diversity at a Subset of Loci Required for Blend Response?

A blend will perform better than its pure-line components only if a reduction in interplant competition for resources occurs. Loci affecting root and leaf structure, plant developmental patterns, nutrient uptake and use, disease resistance, and other phenological and physiological traits likely define a genotype's agro-ecological niche and determine the level of competition between genotypes. It is possible that the key to reduced competition and improved blend performance lies in allelic diversity at these loci, rather than diversity across the whole genome.

Some blend responses in the midseason-maturity oat experiment were negative (at  $P < 0.10$ ). When one individual in a blend is more efficient and vigorous in its use of a common resource, asymmetric competition may occur (Ricklefs, 1993). Since productivity likely is not linearly related to all resource levels, a decrease in the availability of a resource to one plant may reduce its yield more than the yield of a competing plant is increased by its garnering a greater share of the resource (Weiner, 1988). The hyperbolic yield-competition curve resulting from asymmetric competition has been demonstrated by Freckleton and Watkinson (2001). It is possible that the level of asymmetric competition that occurred in some of our oat blends resulted in decreased yield, test weight, stability, or adaptability. The fact that the occurrence of this asymmetric competition in our blends did not vary consistently with overall genetic diversity supports the theory that blend response is less a function of overall genetic diversity than compatibility between specific traits of the blend components.

Although the COP and genetic distance likely predict genome-wide genetic diversity, they may misrepresent the level of diversity found in the subset of genes or quantitative trait loci (QTL) controlling blend response. If allelic diversity at specific loci are critical for minimiz-



ing niche overlap, and hence for maximizing blend response, genome-wide diversity estimates such as COP and the Dice coefficient will tend to be poorly related to blend response. This is analogous to the difficulty in predicting hybrid performance based on DNA-marker-based, genome-wide genetic diversity estimates. Bernardo (1992) demonstrated that including marker loci that are not linked to yield QTL when computing marker-based diversity estimates will reduce the correlation between diversity measures and hybrid yield.

We found one possible example of this in the wheat blend experiments, where yield was measured both on plots inoculated with *P. striiformis* and on plots maintained free of stripe rust by application of fungicide (Finckh and Mundt, 1992). Pedigree diversity was positively related to blend response in diseased plots but not in disease-free plots. Blends with complementary resistances to the two races of stripe rust that were used to inoculate the disease plots, such as Jacmar/Tyee and Moro/Tyee (Finckh and Mundt, 1992), tended to have positive blend responses in the diseased plots (Table 3). In contrast, blends composed of cultivars that were both resistant to one race and susceptible to the second race, such as Jacmar/Moro (Finckh and Mundt, 1992), tended to have negative responses in the diseased plots (Table 3). In this case, genome-wide pedigree diversity was related to diversity at stripe rust resistance loci by chance, and this probably resulted in the positive relationship between pedigree diversity and blend yield response. In the rust-free plots, however, complementary rust resistance loci did not contribute to yield improvement, eliminating the relationship between pedigree diversity and blend response.

The difficulty we face is determining which traits or loci contribute to niche definition and blend response. Variation in the phenotypic measures studied in this experiment, height and heading date, had little effect on blend performance. Variation in disease resistance may be related to blend response under diseased conditions, but this does not carry over to environments with little or no disease. Further research is needed to identify those traits for which variation among the components of a blend contribute to favorable blend response.

Cultivar blends in oat and other crops were seldom superior to pure-line cultivars for yield and yield stability. Genetic diversity, as measured by COP and the Dice coefficient, was not consistently related to blend response. The challenge remains to develop cultivar pairs adapted to growth in mixtures and to identify those physiological or phenotypic components that are involved in both positive and negative intergenotypic interactions in cultivar blends.

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