

Agricultural Fitness of Smooth Bromegrass Populations Selected for Divergent Fiber Concentration

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ABSTRACT

Selection for reduced neutral detergent fiber (NDF) concentration has been used as a mechanism of improving intake potential of perennial grasses by ruminant livestock. However, reduced NDF concentration is typically associated with reduced forage yield, although the reasons for this genetic correlation are unknown. The objectives of this study were to determine the relative contributions of pleiotropy, linkage, and drift to the genetic correlation of NDF concentration with four agricultural fitness traits: forage yield, survival, seed yield, and lodging. These traits were measured on four smooth bromegrass (*Bromus inermis* Leyss.) populations that had undergone one cycle of divergent selection for NDF concentration. Selection responses for forage yield were linear and homogeneous, suggesting pleiotropic effects. Growth and expansion of the cell wall appears to be essential for accumulation of forage yield, lending a certain allometry to these two traits. However, natural selection within swards appeared to regulate this response, because forage yield responses disappeared by the third production year. Selection responses for survival and lodging were linear, but nonhomogeneous, suggesting linkage. Selection responses for seed yield appeared to be regulated by all three phenomena, with drift (asymmetry) the most important. Seed yield is highly sensitive to inbreeding depression, which occurs as a result of drift. Short-term divergent selection experiments, analyzed by a factorial ANOVA model, provide a mechanism to identify genetic phenomena responsible for observed genetic correlations.

BREEDING FOR INCREASED intake potential of perennial grasses for ruminant livestock, by selection for reduced NDF concentration, have been plagued by persistent reductions in forage yield (Casler, 1999; Han et al., 2001; Surprenant et al., 1988). Because NDF is approximately equal to cell-wall concentration (Van Soest, 1994), a positive genetic correlation between NDF and forage yield may be a biological necessity. The pertinent questions are how large is this genetic correlation, what genetic phenomenon underlies it, and is it pliable? The three questions are interrelated because the cause will determine the size of the correlation and whether it is static or stochastic.

Selection typically results in changes to traits that were not specific selection criteria or under directed selection pressure, i.e., correlated responses. These correlated responses can occur by one or more of four mechanisms: linkage, pleiotropy, drift, or nonequilibrium allele frequencies. The distinction among these various genetic causes is extremely important, particularly when the cor-

relation is undesirable, as is the case with NDF and forage yield. The consequences, resolution, and/or amelioration of these various factors in agricultural crops are dramatically different.

Undesirable linkages can be alleviated by recombination, if time and resources are sufficient. Multiple cycles and large population sizes may be necessary if loci are tightly linked. Pleiotropy, by definition, cannot be corrected by any methodology known today. Drift, which results in inbreeding, can be corrected by the use of hybrids or strain crosses to restore heterosis, but only if selection was conducted in multiple populations of reasonably divergent pedigrees. Drift can be minimized by increasing effective population size, but this is costly, either in increased effort or decreased selection intensity. Nonequilibrium allele frequencies may cause asymmetrical selection responses in positive vs. negative directions, but is probably a minor factor, as its effect is generally small relative to selection coefficients (Falconer and Mackey, 1996).

Casler (1999) applied the method of orthogonal contrasts to test and estimate two independent effects of divergent phenotypic selection for NDF. Divergence between high and low Cycle-1 populations estimated the direct effect of selection for NDF on the correlated trait, forage yield, which would be due to linkage and pleiotropy together. Because effective population size was identical for both directions, asymmetry of response between the high and low populations (the difference of their mean relative to the mean of the base population) estimated the direct effect of drift combined with the effect of nonequilibrium allele frequencies (Falconer, 1953; Falconer and Mackey, 1996). The causal relationship between drift and asymmetry of selection responses is well documented in animals (Falconer, 1977). In WB-RP₁ smooth bromegrass, approximately half of the variation in forage yield among selection cycles was due to linkage and/or pleiotropy of loci controlling NDF and forage yield and half due to drift and/or nonequilibrium allele frequencies (Casler, 1999).

In a single population or selection line, separation of linkage and pleiotropy is impossible by quantitative genetic approaches and extremely difficult with molecular markers. However, multiple populations or replicated selection within a single population may be used to partially separate these two genetic phenomena (Casler, 2002). The main effect of selection, across populations or replicates, is a partial measure of the effect of pleiotropy. Pleiotropy, common effects of a single locus on multiple traits, would be constant across populations if the same pleiotropic loci are segregating in each population. This assumption may not be necessary if we assume that each trait is controlled by a large number of quantitative trait loci, each with relatively small effects. Quan-

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titative genetic studies (Casler, 2000) and molecular marker studies (Lübberstedt et al., 1997; Smith et al., 1993) suggest that this assumption is valid in the case of forage yield and NDF. In this case, subtle changes in segregating loci across populations probably have little effect on the measure of the pleiotropic effect. This effect includes extremely tight linkages that could not be broken by a relatively small number of selection cycles.

If populations are reasonably diverse in pedigree and have not undergone previous multiple cycles of convergent selection, random mating would cause their patterns of linkage disequilibria to differ across populations (Dudley, 1993). If linkage causes the genetic correlation, the apparent genetic correlation between the selection criterion (e.g., NDF) and the correlated trait (e.g., forage yield) should vary among base populations. Thus, the statistical interaction of base populations with the correlated selection response becomes an independent measure of the effect of linkage or, more specifically, variation in the pattern of linkage disequilibrium. Such an interaction could not be due to pleiotropic loci, unless completely different sets of loci are segregating in each population. It should also be noted that "linkage" disequilibrium in this case also includes the more general phenomenon of gametic phase disequilibrium, in which alleles at different loci in different linkage blocks appear in disequilibrium simply because of restricted population size (Hartl and Clark, 1997). Gametic phase disequilibrium is less serious than linkage disequilibrium, because it has a half-life of one generation of random mating.

Finally, the differences between drift and nonequilibrium allele frequencies can be indirectly inferred, but not directly tested. If drift affects the correlated response criterion, it would largely be due to inbreeding depression, changes in phenotype directly related to level of inbreeding in the population. If the correlated response criterion is susceptible to inbreeding depression, this effect could be reasonably similar across populations and could be measured as the average asymmetry effect across populations. The interaction of selection populations and asymmetry effects, i.e., inconsistency of asymmetry effects across populations, would indicate a more random, or less predictable, phenomenon. A greater response to positive selection would indicate average allele frequency <0.5 , while a greater response to negative selection would indicate average allele frequency >0.5 (for additive loci controlling the response criterion). This interaction may also suggest differential inbreeding depression among populations, an effect likely to be larger than the effect of nonequilibrium allele frequencies. Presence of asymmetry effects in some populations and absence in others would suggest differential inbreeding depression.

The objective of this study was to estimate the independent effects of pleiotropy, linkage, drift, and nonequilibrium allele frequency on the apparent genetic correlation between a selection criterion (NDF) and four potentially correlated traits (forage yield, survival, seed yield, and lodging) in four base populations of smooth brome-grass that have undergone one cycle of divergent phenotypic selection.

MATERIALS AND METHODS

Germplasm and Selection History

Phenotypic selection was applied to four smooth brome-grass populations: the cultivars Alpha and Lincoln and the synthetic populations WB19e and WB88S-Alt (Falkner and Casler, 1998). The four populations have widely different origins, including both meadow (WB88S) and steppe (Alpha, Lincoln, WB19e) climatotypes and wild germplasm (WB88S), a land race (Lincoln), and cultivated germplasm (Alpha and WB19e). Because of their different origins and different levels of synthesis and selection history, these populations should have considerably different patterns of linkage disequilibrium. Cycle-1 high-NDF and low-NDF progeny populations were created in each base population. The selection protocol was described in detail by Casler (2002). Direct selection responses to divergent selection for NDF concentration were highly consistent across populations ($4.1\text{--}7.7\text{ g kg}^{-1}\text{ cycle}^{-1}$), were highly linear, and were all significant at $P < 0.01$ (Casler, 2002). The selection intensity was $p = 10/300 = 0.033$.

Forage Yield Test

The four base populations and the eight selected populations were planted in $0.9\text{--} \times 3.0\text{-m}$ plots at three locations in April 1997. Locations and soil types were: Arlington, WI [$43^{\circ}20' \text{ N}$, $89^{\circ}23' \text{ W}$; Plano silt loam (fine-silty, mixed, mesic Typic Argiudoll)], Marshfield, WI [$44^{\circ}40' \text{ N}$, $90^{\circ}10' \text{ W}$; Withee silt loam (fine-loamy, mixed Aquic Glossoboralf)], and Ashland, WI [$46^{\circ}35' \text{ N}$, $90^{\circ}54' \text{ W}$; Portwing silt loam (fine, mixed, superactive, frigid Oxyaquic Glossudalf)]. The experimental design was a split-plot in randomized complete blocks with four replicates, in which the four base populations were whole plots, and selections (high, original, and low) were subplots. The seeding rate was 21 kg ha^{-1} on a pure-live-seed basis. Germination of each population and cycle was determined according to standardized procedures (Association of Official Seed Analysts, 1998). Plots were clipped twice during the establishment year and fertilized with 56 kg N ha^{-1} .

Plots were harvested with a flail harvester three times per year in 1998 through 2000, generally in early June, early August, and October. Each location was fertilized with 90 kg N ha^{-1} in early spring and following each of the first two harvests of each year. A random 500-g sample was collected from the harvested forage of each plot and dried at 60°C for dry matter determination. Forage yield was summed over harvests within each year before any statistical analysis. Survival of each plot was determined by assessing ground cover immediately following the first harvest of 2000 from two random placements of a 25-cell frequency grid with $15\text{--} \times 15\text{-cm}$ cells (Vogel and Masters, 2001).

Seed Yield Test

Seedlings of each population were raised for 12 wk in the greenhouse before transplanting to the field in May 1997. Seed yield tests were transplanted adjacent to each of the three forage yield tests (Arlington, Marshfield, and Ashland). Each plot consisted of a row of 15 seedlings, spaced 0.3 m apart within rows and 0.9 m apart between rows. The experimental design was identical to that used for the forage yield test, except for the use of three replicates. Plants were clipped and fertilized as described for the forage yield test during the establishment year.

By May of 1998, adjacent plants within rows had tillered sufficiently to form nearly continuous rows at Marshfield. Plots were fertilized with 56 kg N ha^{-1} in early spring 1998 and 1999.

Seed ripening was determined when most peduncles were yellowed to the flag-leaf node, the date of which did not vary among populations or cycles. Each plot was scored for lodging percentage as an average across all plants, taking into account tiller angle with respect to the ground, the number of plants lodged, and the proportion of each plant that was lodged (e.g., 0 = all plants fully vertical; 50 = all tillers of all plants at a 45° angle, half of the plants vertical and half flat on the ground, or some variation thereof; and 100 = all plants flat on the ground). All lodging ratings were made by the same person at all locations in each year. All seed was harvested in 1998 and 1999 in bulk from each plot, dried at 30 to 45°C for several weeks, threshed, cleaned, and weighed. Threshing and cleaning was done by one person to avoid differences due to technique. Seed yield tests were treated with pre-emergence herbicides for weed and volunteer seedling control as described by Falkner and Casler (1998).

The seed yield tests at Arlington and Ashland were allowed an additional year for establishment. Rows were continuous by spring 1999 and seed was harvested in 1999 and 2000.

Statistical Analysis

Forage yield, survival, seed yield, and lodging were analyzed by conventional ANOVA, nearest neighbor analysis (NNA), or trend analysis (Casler, 1999) for each location–year combination. The best model was chosen on the basis of the lowest average variance of a treatment mean (Brownie et al., 1993). The data for each location–year were re-analyzed using only the spatial terms in the best model (excluding all treatment effects) and the residuals were saved. The experiment mean was added to each residual so that they represented the original data adjusted to eliminate the spatial variation accounted for by either the nearest neighbor or trend analysis model. The frequencies with which spatial adjustments were made were as follows (number of location–years in parentheses): none (4), NNA (3), and trend (2) for forage yield; none for ground cover; and none (3), NNA (1), and trend (2) for seed yield and lodging. Relative efficiencies ranged from 98 to 137%, averaging 113% across all locations, years, and variables.

Adjusted values for forage yield, survival, seed yield, and lodging were analyzed by ANOVA using a combined split-plot-in-time model over locations and years (Steel et al., 1996). The model for the combined ANOVA was $Y_{ijkl} = \mu + L_i + \rho_{ij} + Y_k + LY_{ik} + \delta_{ijk} + G_l + GL_{il} + \gamma_{jl} + GY_{kl} + GLY_{ikl} + \epsilon_{ijkl}$, where μ = the grand mean, L_i = the fixed effect of the i th location, ρ_{ij} = the random effect of the j th block within the i th location, Y_k = the fixed effect of the k th year, LY_{ik} = the interaction effect of the i th location with the k th year, δ_{ijk} = the random interaction effect of the j th block with the k th year within the i th location, G_l = the fixed effect of the l th population, GL_{il} = the interaction effect of the i th location with the l th population, γ_{jl} = the random interaction effect of the j th block with the l th population within the i th location, GY_{kl} = the interaction effect of the k th year with the l th population, GLY_{ikl} = the interaction effect the i th location with the k th year and the l th population, and ϵ_{ijkl} = the residual associated with the $ijkl$ th observation. Some degrees of freedom were subtracted from pooled experimental errors in these ANOVAs, according to the number of parameters fit in the spatial models for each location–year combination (Casler, 1999; Smith and Casler, 2004). Experimental error mean squares and all F tests were recomputed in a spreadsheet after adjustment of error degrees of freedom. All effects were fixed, except replicates, which were assumed to be random. These analyses were equivalent to the pre-adjustment-based-on-total-yield method of Smith and Casler (2004) with the exception that the

Table 1. Percentage of the sums of squares attributable to populations, selection effects, and interactions of selection effects with populations for four smooth bromegrass populations subjected to one cycle of phenotypic selection for divergent neutral detergent fiber (NDF) concentration, as measured by forage yield in the first 3 yr of production.

Source of variation	df	First year	Second year	Third year
Populations (P)	3	53.8**	43.9**	70.1**
Divergence (D)	1	33.1**	35.6**	2.4
D × P	3	6.3*	0.5	4.2
Asymmetry (A)	1	3.8*	5.2	6.7
A × P	3	3.1	14.9	16.5

* Mean square for effect was significant at $P < 0.05$.

** Mean square for effect was significant at $P < 0.01$.

spatial adjustment method was allowed to vary among locations and years in the current study. Residual analyses were conducted and little evidence was found for violation of the assumptions of normality and uniform variance (Steel et al., 1996).

Degrees of freedom for the 12 populations were partitioned as a complete factorial partition between four populations and three cycles of selection, in which selection cycles was further partitioned into linear (divergence) and nonlinear (asymmetry) effects as follows: base populations (3 df), divergence (1 df), populations × divergence (3 df), asymmetry (1 df), and populations × asymmetry (3 df). The 3 df for populations were ignored and the remaining 8 df were tested by contrasts and their sums of squares were expressed as a percentage of their sum. Divergence was used as a measure of pleiotropy. Populations × divergence was used as a measure of linkage. Asymmetry and populations × asymmetry were used as measures of the effects of drift or nonequilibrium allele frequencies.

RESULTS

Population × location, population × year, and population × location × year interactions were not significant for survival, seed yield, and lodging. Conversely, these interactions were all significant for forage yield ($P < 0.05$). Therefore, the orthogonal contrast analysis (divergence and asymmetry) was applied to forage yield data for each location–year combination. Results were highly consistent across locations, but inconsistent across years. Therefore, all analyses were based on means over locations and years for survival, seed yield, and lodging. Forage yield analyses were based on means over locations.

For forage yield, average divergence between high- and low-NDF cycles was the largest source of variation among selection cycles for the first two production years (Table 1). Divergence accounted for 72 and 63% of the sum of squares among selection cycles for Years 1 and 2, respectively (Table 1). For these two years, divergence was generally consistent across populations, ranging only from 0.14 to 0.73 in Year 1 and 0.38 to 0.53 in Year 2 Mg ha⁻¹ cycle⁻¹ (Table 2; Fig. 1). There was some evidence for inconsistency in Year 1, as indicated by the divergence × population interaction (Table 1) and the low response for Lincoln (Table 2; Fig. 1). There was only slight evidence of drift (asymmetry of selection response) on forage yield, as indicated by the main effect in Year 1 (Table 1) and WB88S in Year 2 (Table 2). This was also indicated by the relatively high amount of

Table 2. Correlated linear responses, proportion of variation due to selection (R^2), and asymmetry of selection responses for forage yield during one cycle of divergent phenotypic selection for neutral detergent fiber (NDF) concentration in four base populations of smooth bromegrass.

Population	Linear response	R^2	Asymmetry
	Mg ha ⁻¹ cycle ⁻¹		Mg ha ⁻¹
First year			
Alpha	0.73**	0.95	0.29
WB19e	0.56**	0.74	0.59*
Lincoln	0.14	0.77	-0.13
WB88S	0.66**	0.84	0.50
Pooled	0.52**	0.82	0.31
Second year			
Alpha	0.45*	0.87	0.31
WB19e	0.53*	0.82	0.42
Lincoln	0.45*	0.73	-0.48
WB88S	0.38*	0.33	0.94*
Pooled	0.45*	0.69	0.30
Third year			
Alpha	-0.01	0.01	-0.17
WB19e	0.07	0.21	0.23
Lincoln	-0.01	0.00	0.48
WB88S	0.16	0.96	0.06
Pooled	0.05	0.29	0.15

* Mean square for effect was significant at $P < 0.05$.

** Mean square for effect was significant at $P < 0.01$.

variance accounted for by the linear selection responses in Years 1 and 2 (Table 2). Forage yield responses to divergent selection for NDF were nonexistent by Year 3 (Tables 1 and 2), an effect that was consistent across locations (data not shown).

For survival, divergence between high- and low-NDF populations was highly inconsistent across populations, divergence \times population being the largest source of variation (Table 3). One population had a significant correlated response for survival, with Alpha demonstrating a strong negative genetic correlation between NDF

Table 3. Percentage of the sums of squares attributable to populations, selection effects, and interactions of selection effects with populations for four smooth bromegrass populations subjected to one cycle of phenotypic selection for divergent neutral detergent fiber (NDF) concentration, as measured by three correlated response variables.

Source of variation	df	Survival	Seed yield	Lodging
		%		
Populations (P)	3	77.5**	19.5*	52.2**
Divergence (D)	1	0.6	7.6*	0.5
D \times P	3	16.3*	27.3**	34.8**
Asymmetry (A)	1	1.4	17.4**	1.1
A \times P	3	4.2	28.2**	11.3

* Mean square for effect was significant at $P < 0.05$.

** Mean square for effect was significant at $P < 0.01$.

and survival (Table 4; Fig. 2). Effects of drift were not significant for survival, either within populations or pooled across populations.

For seed yield, all sources of variation were significant, with the average effect of divergence making up the smallest share of the sum of squares (Table 3). While the average selection response was significant, indicating an average negative genetic correlation between NDF and seed yield, selection responses were highly variable among populations (Table 5). The range in linear selection responses among populations was more than five times greater than the average linear selection response. However, the asymmetry effects were the most important responses for seed yield. Asymmetry effects explained most of the response for Alpha and WB19e, but only a small portion of the response for Lincoln and WB88S (Table 5; Fig. 2). Furthermore, averaged across populations, the asymmetry effect was nearly three times larger than the linear selection effect for seed yield.

The pattern of effects for lodging followed the pattern

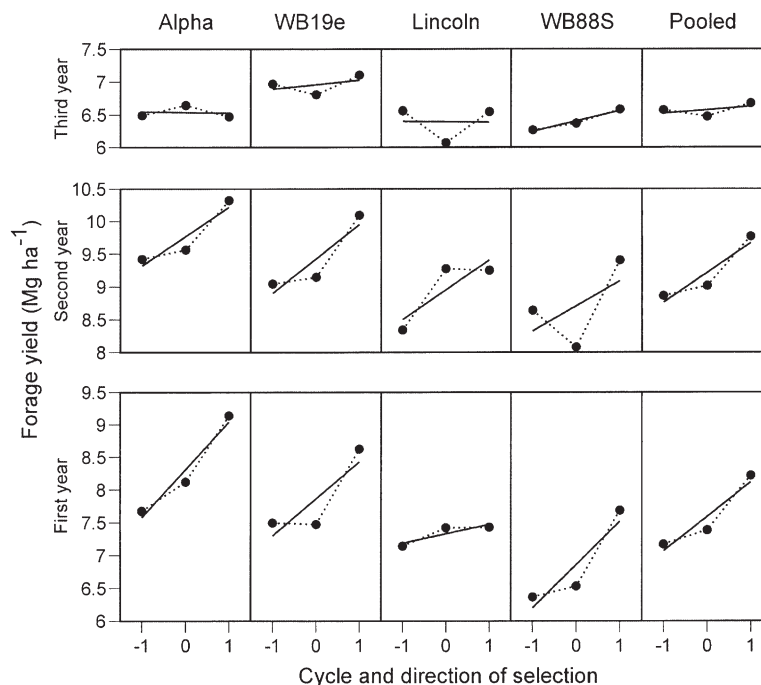


Fig. 1. Correlated linear selection responses (solid lines) and asymmetry effects (dotted lines) for forage yield in the first three production years measured on four smooth bromegrass populations subjected to one cycle of divergent phenotypic selection for divergent neutral detergent fiber (NDF) concentration. Each data point represents an average over four replicates and three locations.

Table 4. Correlated linear responses, proportion of variation due to selection (R^2), and asymmetry of selection responses for survival during one cycle of divergent phenotypic selection for neutral detergent fiber (NDF) concentration in four base populations of smooth brome grass.

Population	Linear response % cycle ⁻¹	R^2	Asymmetry %
Alpha	-6.4**	0.99	-1.0
WB19e	2.2	0.38	-4.8
Lincoln	1.0	0.16	-3.8
WB88S	0.6	0.12	2.8
Pooled	-0.7	0.41	1.7

** Mean square for effect was significant at $P < 0.01$.

observed for survival (Table 3). However, unlike survival, three populations demonstrated significant linear selection responses, although the responses were highly variable (Table 6; Fig. 2). The range in linear selection responses among populations was more than nine times greater than the average linear selection response. Asymmetry effects were not significant for lodging, either within populations or pooled across populations. The observed linear selection responses, which accounted for most of the observed variation, suggested both positive and negative genetic correlations between NDF and lodging, depending on population.

DISCUSSION

There is little agreement in the literature as to the relative importance of linkage or pleiotropy as the principal phenomenon causing genetic correlations. Falconer and Mackey (1996) and Simmonds and Smartt (1999) argue that pleiotropy is more important, while Mather and Jinks (1982) argue that linkage is more important. Linkage alone can maintain a large genetic correlation only when linkages are tight and/or the population is highly inbred (Lande, 1984). Mutations to loci with overlapping phenotypic specificities are capable of maintain-

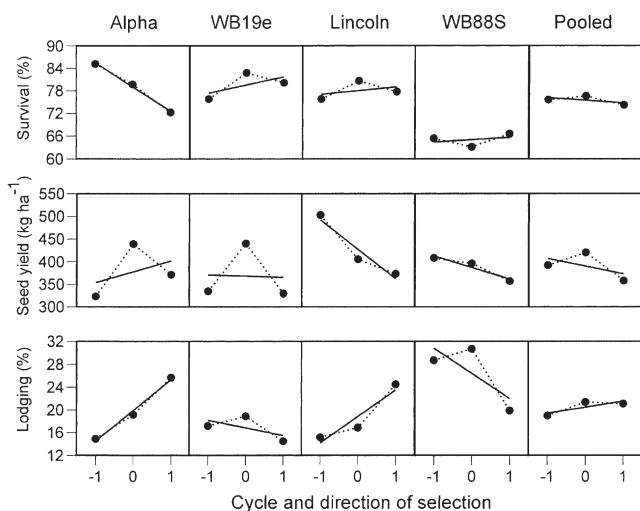


Fig. 2. Correlated linear selection responses (solid lines) and asymmetry effects (dotted lines) for survival, seed yield, and lodging measured on four smooth brome grass populations subjected to one cycle of divergent phenotypic selection for divergent neutral detergent fiber (NDF) concentration. Each data point represents an average over four replicates, three locations, and (for seed yield and lodging) 3 yr.

Table 5. Correlated linear responses, proportion of variation due to selection (R^2), and asymmetry of selection responses for seed yield during one cycle of divergent phenotypic selection for neutral detergent fiber (NDF) concentration in four base populations of smooth brome grass.

Population	Linear response kg ha ⁻¹ cycle ⁻¹	R^2	Asymmetry kg ha ⁻¹
Alpha	24	0.17	-92**
WB19e	-3	0.00	-108**
Lincoln	-65**	0.92	33
WB88S	-26	0.92	-13
Pooled	-17*	0.50	-45**

* Mean square for effect was significant at $P < 0.05$.

** Mean square for effect was significant at $P < 0.01$.

ing genetic correlations in populations under mild selection pressure (Lande, 1980; Russell et al., 1963; Sprague et al., 1960).

Jinks et al. (1985) used dihaploidy and single seed descent to separate the effects of linkage and pleiotropy for selection under self-fertilization. They found genetic correlations between pairs of traits that fell into one of four categories: (i) no linkage or pleiotropy, (ii) pleiotropy only, (iii) linkage only, or (iv) linkage and pleiotropy together. Trait-pairs that fell into the second category were largely allometric traits, some measure of organ size or yield. Simmonds and Smartt (1999) were clearly considering allometric traits when they argued that the complexity of living organisms and potential autocorrelations between growth stages might be caused, directly or indirectly, by pleiotropic loci with overlapping phenotypic specificities.

Genetic correlations were observed between NDF concentration and each of the agricultural fitness traits. However, the pattern to these correlations differed among the traits, showing a high degree of consistency for forage yield in the first 2 yr, no response in Year 3, highly inconsistent results for survival and lodging, and a high degree of asymmetry for seed yield.

The highly consistent and positive genetic correlation between NDF and forage yield in Years 1 and 2 is consistent with numerous previous studies (Casler, 1999; Han et al., 2001; Hovin et al., 1976; Marum et al., 1979; Surprenant et al., 1988). Across harvests, locations, and years, NDF concentration averaged 477 to 687 g kg⁻¹, averaging 593 g kg⁻¹ for the entire study (Casler, 2002). These values largely represent cell-wall concentration of herbage, with the exception of some pectin and cell-wall proteins that are neutral-soluble (Van Soest, 1994).

Table 6. Correlated linear responses, proportion of variation due to selection (R^2), and asymmetry of selection responses for lodging during one cycle of divergent phenotypic selection for neutral detergent fiber (NDF) concentration in four base populations of smooth brome grass.

Population	Linear response % cycle ⁻¹	R^2	Asymmetry %
Alpha	5.4**	0.99	1.1
WB19e	-1.4	0.38	-3.0
Lincoln	4.7*	0.88	2.9
WB88S	-4.4*	0.58	-6.4
Pooled	1.1	0.71	-1.4

* Mean square for effect was significant at $P < 0.05$.

** Mean square for effect was significant at $P < 0.01$.

The plant cell wall represents a physical frame on which numerous plant functions and processes are built. Cell walls are responsible for the retention of upright growth as tillers grow taller, larger, and heavier. Cell walls also function in the transport of nutrients, photosynthate, and water through the vascular system of a tiller. Older phytomers have higher NDF concentrations (Kephart et al., 1990), suggesting an evolutionary adaptation to maintain upright tillers in the grass canopy. These functions of cell walls all allow the plant to continue accumulating dry matter, assuming that no other physiological functions becoming limiting.

The genetic correlations between NDF and forage yield appear to be physiological in origin, caused largely by overlapping genic specificities, i.e., pleiotropy. The consistency of linear selection responses across populations, and their strong linearity per se, provide statistical evidence for this conclusion. Alleles that cause reductions in NDF concentration, no matter how favorable for ruminant animal performance, also cause reductions in forage yield. Cell walls are an essential component of a herbage crop and little of the variation in cell-wall concentration is independent of forage yield. Casler (2000) proposed two solutions to break this genetic correlation, one based on linkage and one based on drift. The results of this study suggest that these solutions are unlikely to result in large changes to the natural genetic correlation between NDF concentration and forage yield. Previous estimates of this genetic correlation range from 0.53 to 0.89 (Casler et al., 1990; Hovin et al., 1976; Marum et al., 1979) and these results suggest that this genetic correlation is not likely malleable by selection.

The forage yield results for Year 3 are at odds with those from Years 1 and 2. Taken alone, the results from Year 3 provide no evidence for a genetic correlation between forage yield and NDF. Indeed, we might even conclude that there is no genetic variation for forage yield in these four populations. However, previous research has demonstrated considerable genetic variation for forage yield in WB19e and WB88S (Casler, 1998a) and this is likely true for Alpha and Lincoln as well. Thus, the unique aspect of the Year-3 results is likely due to (i) some environmental factor(s) acting to suppress expression of genes regulating forage yield or (ii) natural selection within sward plots.

There are few environmental factors that are in common between the three locations used in this study. The locations range from southern to northern Wisconsin, a total distance of 380 km. The three locations differ widely in soil type, rainfall pattern, snow cover, and temperature. Thus, it seems likely that the changes in results from Year 1 to 3 (reduction in average selection response, reduction in R^2 , increase in P value—slight reduction in Year 2 and large reduction in Year 3), which were highly consistent across the three locations, were due to an age effect rather than a climatic or edaphic effect. Smooth brome grass can become sodbound with age, a condition in which rhizome and tiller production are restricted and forage yields are significantly reduced, likely reducing genetic variability for forage yield. However, the rates of N fertilization (270 kg N ha^{-1}) should have been suffi-

cient to eliminate sodbinding as a factor (Casler and Carlson, 1995).

This leaves the question of natural selection in sward plots. Natural selection can occur rapidly in monoculture stands of forage grasses and can eventually lead to dominance of a relatively small number of clones (Casler, 1998b; Casler et al., 1996; McLellan et al., 1997). Natural selection may be manifested as a form of stabilizing selection, eliminating the most extreme high-yield plants from the high-NDF selections and the most extreme low-yield plants from the low-NDF selections, resulting in gradual loss of genetic differences between divergent selection lines. In effect, divergent populations would converge over time under this scenario. Interestingly, the loss of genetic divergence for forage yield was not accompanied by loss of genetic divergence for NDF (Casler, 2002). Therefore, if natural selection is responsible for the age-related changes in genetic correlation between forage yield and NDF, plants that begin to dominate in low-NDF population swards after 3 yr may represent useful germplasm for breaking the genetic correlation between forage yield and NDF.

Finally, it should be noted that these results are specific to the conservation harvest management used in this study. Smooth brome grass produces culmed regrowth, so that each harvest consisted of elongated stems. This characteristic creates the potential for a greater physiological dependence of forage yield on cell-wall development than for a grass that does not produce culmed regrowth or for smooth brome grass that is managed, by frequent cutting or grazing, to minimize stem production. The relationships and genetic phenomena observed herein may not apply to grass crops without culmed growth.

The correlated responses of survival and lodging, following selection for NDF, are largely indicative of linkage disequilibria between pairs of loci controlling NDF and the response criterion.

Because the populations vary in pedigree, origin, and level of synthesis (number of generations of random mating), patterns of linkage disequilibria will vary among populations, causing differential genetic correlations between traits. The observed significance levels suggest that this variation is not random, but is characteristic of each population. This can only be caused by linkage between loci controlling NDF and the response criterion. Thus, these genetic correlations are ephemeral, subject to the effects of recombination and changes in linkage disequilibria with each cycle of selection or random mating.

While high cell-wall concentrations are necessary for high forage yield, they are not necessary for lodging resistance. Genetic increases in stem or stalk strength, which translate to lodging resistance, have been associated with decreased cell-wall concentration (Ookawa and Ishikara, 1993) or no change in cell-wall concentration (Undersander et al., 1977). Greater stem flexibility, allowing stems to bend without breaking, may be an important factor in lodging resistance (Travis et al., 1996). While cell-wall structure may be important in regulating stem flexibility, cell-wall concentration per se is of ques-

tionable importance. There are no empirical or theoretical reports of genetic correlations between lodging and cell-wall concentration, or NDF.

The genetic relationship between NDF and seed yield appears to be regulated by multiple genetic phenomena. A small part of the linear response (divergence) was consistent across populations, suggesting a relatively few loci that are pleiotropic for NDF and seed yield. The physical infrastructure created by cell walls, so important for forage production, may be partially detrimental to seed production. Structural and soluble sugar pools are in a constant state of flux, varying diurnally (Smith et al., 2001) and seasonally (Smith et al., 1986). Soluble sugars are converted to structural sugars in the cell-wall biosynthesis process, possibly reducing the size of the soluble sugar pool that can be transported to the developing seed in a high-NDF plant.

Despite this possible physiological relationship between NDF and seed yield, linkage and drift are more important than pleiotropy for the NDF-seed yield genetic correlation. The relative importance of drift was expected for seed yield because of extreme sensitivity of seed yield to any form of inbreeding. Perennial grasses, such as smooth brome grass, are highly sensitive to inbreeding and seed yield is typically the most obvious measure of inbreeding depression (Hanson and Carnahan, 1956). All of the significant asymmetry effects were negative, indicating lower seed yield in the mean of the selected populations compared with the unselected population. Assuming the original populations to be non-inbred, average inbreeding coefficients were $F = 0.05$ for Cycle 1 and 0.10 for Cycle 2 (Han and Casler, 1999). While drift is a random process with respect to the specific alleles lost during recombination, it is not necessarily random with respect to specific phenotypic changes.

Drift is the more likely explanation for the differential asymmetry in seed yield across populations. Alpha and WB19e were highly susceptible to inbreeding depression caused by drift, with an average 23% reduction in seed yield. Lincoln and WB88S may not be resistant to inbreeding depression in the long term, but there is substantial evidence that genetic variation exists for susceptibility to inbreeding depression in perennial grasses (Hanson and Carnahan, 1956). Because drift is a random phenomenon, alleles favorable for seed production may have increased in the Lincoln and WB88S selections in this selection cycle. Furthermore, because drift is a phenomenon of restricted sample size, all populations are not expected to respond in a similar manner (Hartl and Clark, 1997). Variation among populations in the number of effective alleles per locus may affect the observed rate of inbreeding depression.

Finally, the use of multiple germplasm pools to estimate linkage disequilibrium raises the question of inferential space. If the populations were selected at random from some defined population, the linkage inference is a random effect of the metapopulation. If the populations were chosen for their specific intrinsic value, as in the current study, the linkage inference is a fixed effect of the chosen populations. This linkage inference could have been developed for a single population, using replicated

selection within the population, which is common in animal selection experiments (Falconer and Mackey, 1996) but rare in plant selection experiments. Furthermore, because each replicated selection line would represent a random sample from the base population, several cycles of selection would be required before linkage disequilibrium patterns could be expected to diverge sufficiently to detect this effect (Dudley, 1993). Should linkage disequilibrium be detected in early generations, it would likely be due to sampling variation rather than to the actual effect of linkage disequilibrium on the genetic correlation within the population. Because genetic correlations are notoriously high in sampling variance, this is to be expected (Toms et al., 1994). Thus, the use of multiple populations, as utilized in the current study, holds the advantages of potentially broad inference space and unambiguous resolution of different genetic phenomena with one or more selection cycles.

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