

# Divergent Selection for Two Measures of Intake Potential in Smooth Bromegrass

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## ABSTRACT

Voluntary intake is often the greatest limitation to animal performance. Two different laboratory measures of intake potential were used as selection criteria in four smooth bromegrass (*Bromus inermis* Leys) populations. The objective of this study was to measure the direct and correlated responses to one cycle of divergent selection for neutral detergent fiber (NDF) concentration or particle-size reduction index (PSRI). Sixteen progeny populations were created by divergent selection (high or low) for NDF or PSRI of smooth bromegrass leaf blades within four populations. Divergent selection for NDF resulted in changes of 4 to 8 g kg<sup>-1</sup> (0.7–1.3% of the mean) for NDF and –2.2 to –3.0% (6.6–9.4% of the mean) for PSRI. Divergent selection for PSRI resulted in changes of 1.9 to 3.9% (5.4–12.9% of the mean) for PSRI and inconsistent changes for NDF. Although realized heritabilities were low, there was additive genetic variation for both traits within each population. Divergent selection for NDF resulted in strong changes in PSRI, in the opposite direction, and largely consistent across populations, suggestive of possible pleiotropic relationships. Conversely, divergent selection for PSRI resulted in weak and/or inconsistent changes in NDF, suggesting that there are multiple mechanisms by which PSRI is regulated in smooth bromegrass leaves. Decreased NDF concentration uniformly results in increased PSRI, but PSRI can probably be increased by alterations to the cell wall or to changes in cell types without changes to NDF concentration of herbage.

PLANT BREEDING EFFORTS to develop forage crops with improved intake potential are increasing (Casler and Vogel, 1999). While there has been considerable effort at making genetic improvements in digestibility (Casler and Vogel, 1999), most ruminant nutritionists consider voluntary intake to be more important than digestibility in limiting animal performance (Fahey and Hussein, 1999). Up to 70% of the variation in animal production can be attributed to variation in intake, while only 20% can be attributed to variation in digestibility (Crampton et al., 1960).

Physical distension of the rumen is the major factor limiting voluntary intake of high producing ruminants on high-forage diets (Mertens, 1994). For most high-forage diets, intake of fibrous bulk generally causes rumen fill and satiation before the ruminant has maximized its caloric intake, resulting in a reduced plane of nutrition (Van Soest, 1994). Voluntary intake can be increased by reducing bulk volume of the feed, which increases intake prior to satiation, or by increasing fiber clearance from the rumen, which reduces the time required to stimulate appetite.

Forage-plant breeders face two challenges: to identify selection criteria that relate to bulk volume of feeds or to their rate of clearance from the rumen, and to deter-

mine which selection criteria will have the greatest impact on animal performance without detrimental effects on agronomic traits. Plant breeders must evaluate large numbers of individual plants, often with some form of replication, to obtain reliable data upon which to base selection decisions. Thus, plant breeding programs must rely upon indirect assessments of intake, based on inexpensive, rapid, and repeatable laboratory predictors.

Cell wall concentration, estimated as neutral detergent fiber (NDF), provides a measure of fibrous bulk which is negatively associated with voluntary intake (Van Soest, 1994). It is not known whether forages that have inherently high NDF concentration limit ruminant intake by their low caloric density or their high bulk volume, two characteristics that are typically confounded in voluntary intake studies (Van Soest, 1994). Nevertheless, NDF concentration is a heritable trait in several forage species, with genetic progress demonstrated in smooth bromegrass (Casler, 1999a), reed canarygrass, *Phalaris arundinacea* L. (Surprenant et al., 1988), and maize, *Zea mays* L. (Wolf et al., 1993). Rates of gain from selection for reduced NDF concentration of herbage are reported to be as high as 12 g NDF kg<sup>-1</sup> DM year<sup>-1</sup> (Casler and Vogel, 1999).

Voluntary intake is also limited by the rate at which rumen digesta can be degraded and cleared from the rumen (Weston, 1996; Wilson and Kennedy, 1996). Most feed particles do not pass from the rumen until they have been reduced to a particle size sufficient to pass a 1-mm sieve for sheep (Reid et al., 1977) or a 1- to 2-mm sieve for cattle (Poppi et al., 1985; Waghorn et al., 1989). Chewing and rumination are the major mechanisms of particle size reduction in ruminants (McLeod and Minson, 1988; Wilson et al., 1989). Indeed, voluntary intake of ruminants can be predicted by laboratory measures of breakdown resistance of forages. Examples include particle size distribution following artificial mastication (Troelson and Bigsby, 1964), energy required to grind forages through a given sieve size (Weston, 1985), energy required to shear or compress forage tissue (Baker et al., 1993), and particle size reduction index (Culvenor and Casler, 1999). Particle size reduction index (PSRI) is the percentage of ball-milled leaf particles that pass through a 1-mm screen in a dry sieve (Casler et al., 1996).

Heritability of PSRI in smooth bromegrass leaves is moderate and rate of genetic gain has averaged 2 to 6% per cycle of selection (Culvenor and Casler, 1999). Divergent selection for PSRI in smooth bromegrass has resulted in fairly consistent changes in NDF concentration (Casler et al., 1996; Culvenor and Casler, 1999). For each percentage unit change in PSRI, NDF concentration changed by –2.1 to –4.1 g kg<sup>-1</sup> (Culvenor and Casler, 1999). This relationship, and its consistency among different populations of smooth bromegrass, in-

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icates the presence of a negative genetic correlation between NDF and PSRI, which has ranged from  $-0.85$  to  $-0.23$  (Casler et al., 2000). Indeed, NDF may be one of the plant traits that controls PSRI. The objectives of this experiment were to estimate the progress that can be achieved by selection for NDF or PSRI in smooth brome grass, to determine mutual correlated selection responses for NDF and PSRI, and to identify genetic factors responsible for these correlated responses.

## MATERIALS AND METHODS

Selection was applied to four smooth brome grass populations: the cultivars Alpha and Lincoln, and the synthetic populations WB19e and WB88S. The four populations were chosen to represent germplasm with differential pedigrees, origins, and (hypothetically) patterns of linkage disequilibria. Lincoln is a land race cultivar that was created as a seed increase of a wild collection from Hungary. Alpha is a contemporary cultivar derived by several cycles of selection from a diverse germplasm pool. WB19e is a Syn-2 strain cross between four diverse populations, including Alpha. WB88S is a Syn-2 strain cross among five wild collections from a 1988 expedition to the Altai Mtns. of southern Siberia (USDA-ARS, 1990).

Three hundred 70-d-old seedlings of each population were transplanted to a field in May 1992. Plants were arranged in 10 blocks of 30 plants with a spacing of 0.9 m between all adjacent plants. The experiment was located at Arlington, WI ( $43^{\circ}20'N$ ,  $89^{\circ}23'W$ ) on a Plano silt loam (fine-silty, mixed, mesic, Typic Argiudoll). Weeds were controlled using a combination of tillage, hand weeding, and application of  $1.12 \text{ kg ha}^{-1}$  alachlor [2-chloro-*N*-2,6-diethylphenyl]-*N*-(methoxymethyl)-acetamide] with  $0.56 \text{ kg ha}^{-1}$  bromoxynil [3,5-dibromo-4-hydroxybenzotrile], and  $0.07 \text{ kg ha}^{-1}$  imazethapyr [( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid]. Plants were fertilized with  $112 \text{ kg N ha}^{-1}$  in late June. Applications of P and K were made to this and all other field sites, in autumn before plot establishment, as recommended for grass hay production. Growth conditions were normal and plants produced new tillers throughout the growing season. Plants were clipped in late June at a 9-cm stubble height.

### Selection for Neutral Detergent Fiber (NDF) Concentration

In mid-August 1992, plants were approximately 20 to 25 cm tall and made up entirely of leaf blades and sheaths (pseudostems). A sample of leaf blades (approximately 50 g dry matter) was clipped from each plant, with a stubble height of 10 cm. Leaf blades were used because they provide uniform samples, increasing heritability and eliminating selection for plant-part ratios (Casler, 1999c). Tissue samples were placed in paper bags and dried at  $60^{\circ}\text{C}$ . Dried samples were ground through a 1-mm screen of a Wiley-type mill and reground through a 1-mm screen of a cyclone mill. Two independent subsets of each sample were scanned on a near-infrared reflectance spectrophotometer (NIRS). Two random plants from each block of each population comprised a stratified random subset of 80 plants that was subjected to wet-laboratory analysis. Concentration of NDF was determined by the procedure of Van Soest et al. (1991), omitting the  $\alpha$ -amylase step. Data on NDF concentration of the 80-plant subset were used to calibrate the NIRS for prediction of the entire set of 2400 scanned samples (four populations  $\times$  300 plants  $\times$  two scanned

subsets). Means over the two scanned subsets of each sample were computed before selection.

Some field and laboratory variability was removed from the estimates of plant phenotypes by processing samples in numerical order and computing *t*-scores to adjust for differences among block means:  $t_{ij} = (X_{ij} - M_j)/s_j$  where  $t_{ij}$  is the *t*-score for NDF of the *ij*th plant,  $X_{ij}$  is the raw datum for the *ij*th plant,  $M_j$  is the mean of the *j*th block, and  $s_j$  is the standard deviation of the *j*th block (Casler, 1992).

The 10 plants with the highest  $t_{ij}$  values and the 10 plants with the lowest  $t_{ij}$  values for each of the four populations were split into four ramets and transplanted into crossing blocks in July 1993. Each of the eight crossing blocks (four populations  $\times$  two directions of selection) had four replicates of 10 clones arranged in a randomized complete block design. Crossing blocks were isolated from each other by a minimum of 10 m. Fewer than 5% of smooth brome grass pollinations derive from pollen that traveled farther 10 m or greater distance (Knowles and Ghosh, 1968). As an additional precaution against pollen contamination, winter rye (*Secale cereale* L.) was planted between each adjacent crossing block. Crossing blocks were fertilized with  $56 \text{ kg N ha}^{-1}$  in late April 1994. The winter rye crop was approximately 30 cm taller than smooth brome grass at the time of smooth brome grass anthesis. Seed was harvested from each plant in July 1994 and bulked in equal quantities across clones within each progeny population.

### Selection for Particle Size Reduction Index (PSRI)

Selection for divergent PSRI was completed in the same four populations described above for NDF. Details of the PSRI laboratory protocol are in Casler et al. (1996) and Culvenor and Casler (1999). Briefly, the procedure involves the following four steps: ball-milling a 2-g sample of dry leaf blades for 30 s, dry-sieving the ball-milled particles through a 1-mm screen for 60 s, collecting and weighing the particles that passed the 1-mm screen, and computing PSRI as the percentage of leaf dry matter that passed through the 1-mm screen. The initial population sizes for the PSRI selections were similar to those for the NDF selections, but only five plants were selected as parents of each PSRI progeny population. Otherwise, the remaining details of selection for divergent PSRI were identical to those for divergent NDF.

### Evaluation of Progeny Populations

Seed of the four base populations and the 16 selected populations (four base populations  $\times$  two selection directions  $\times$  two selection criteria) were planted in 0.9- by 3.0-m plots at three locations in April 1997. Locations and soil types were: Arlington, WI, Marshfield, WI [ $44^{\circ}40'N$ ,  $90^{\circ}10'W$ ; Withee silt loam (fine-loamy, mixed Aquic Glossoboralf)], and Ashland, WI [ $46^{\circ}35'N$ ,  $90^{\circ}54'W$ ; Portwing silt loam (fine, mixed, superactive, frigid Oxyaquic Glossudalf)]. The experimental design was a split-split-plot in randomized complete blocks with four replicates, in which the two selection criteria (NDF and PSRI) were whole plots, the four base populations were sub-plots, and selections (high, original, and low) were sub-sub-plots. The seeding rate was  $21 \text{ kg ha}^{-1}$  on a pure-live-seed basis. Germination of each population and cycle was determined according to standardized procedures (AOSA, 1998). Plots were clipped twice during the establishment year and fertilized with  $56 \text{ kg N ha}^{-1}$ .

Plots were harvested with a flail harvester three times per year in 1998 and 1999, generally in early June, early August, and October. Each location was fertilized with  $90 \text{ kg N ha}^{-1}$  in early spring and following each of the first two harvests of

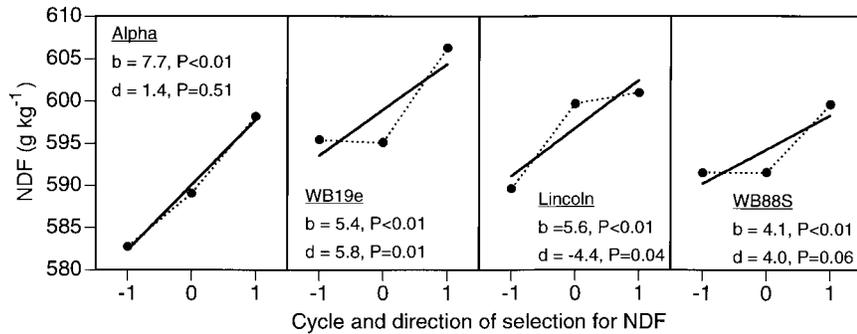


Fig. 1. Direct responses of neutral detergent fiber (NDF) concentration to divergent selection for NDF in four smooth bromegrass populations. Means are over four replicates, three locations, 3 yr, and three harvests. Solid lines represent linear selection responses (b) and dashed lines represent effects of drift (d).  $R^2 = 0.99, 0.73, 0.83,$  and  $0.76$  for Alpha, WB19e, Lincoln, and WB88S, respectively.

each year. A random 500-g sample was collected from the harvested forage of each plot and dried at 60°C. The flail harvester, built at the University of Wisconsin Marshfield Agricultural Research Station, has very little loss in leaf tissue, preserving the plant-part structure of standing forage.

Forage samples were split in two—half was ground to pass a 1-mm screen in a Wiley-type mill and half was reserved for PSRI analysis. Ground samples were scanned on a near-infrared reflectance spectrophotometer and a calibration subset of 72 samples was chosen by cluster analysis of spectral reflectance values (Shenk and Westerhaus, 1991). The calibration samples were analyzed for neutral detergent fiber (NDF) by the procedure of Van Soest et al. (1991) with the exceptions that sodium sulfite and  $\alpha$ -amylase were excluded. Values of NDF were predicted for all samples with a single calibration equation:  $SE_{cal} = 12.3 \text{ g kg}^{-1}$ ,  $R_{cal}^2 = 0.94$ ,  $SE_{val} = 16.7 \text{ g kg}^{-1}$  and  $R_{val}^2 = 0.89$ . Calibration samples for second and third harvests (vegetative growth stage) were analyzed for PSRI in duplicate by the procedures of Culvenor and Casler (1999). Values of PSRI were predicted for all second- and third-harvest samples with a single calibration equation:  $SE_{cal} = 1.03\%$ ,  $R_{cal}^2 = 0.89$ ,  $SE_{val} = 1.34\%$  and  $R_{val}^2 = 0.83$ . The PSRI variable could not be analyzed on first-harvest samples which consisted largely of reproductive tillers.

Data for NDF and PSRI were analyzed by analysis of variance using the split-plot-in-time model (Steel et al., 1996). Populations and selections were assumed to be fixed, while locations, years, and replicates were assumed to be random. Realized heritability for NDF was computed by Method 4 of Culvenor and Casler (1999); the selection differentials were computed from the original selection nursery, while realized gains were computed from the evaluation experiments described above. The genotypic coefficients of variation for each trait were computed as  $100s_G/M$ , where  $s_G$  = the variance component for populations and  $M$  = the experiment mean.

For each correlated response variable, the 11 df for populations and selection cycles were partitioned into populations (3 df), divergence (1 df), population  $\times$  divergence (3 df), asymmetry between high and low selections (1 df), and population  $\times$  asymmetry (3 df). Asymmetry was computed as  $C0 - [(C1H + C1L)/2]$ , where  $C0$  = the original population,  $C1H$  = Cycle 1 high, and  $C1L$  = Cycle 1 low. Casler (1999a) and Falconer (1953) attributed asymmetrical selection responses to drift, but it should be recognized that direction of selection per se has a small effect on selection responses (Falconer and Mackey, 1996). Divergence between high and low selections, consistent across populations, would suggest that some loci are pleiotropic for the selection criterion and response criterion. The population  $\times$  divergence interaction would indicate differential correlated response across popula-

tions, largely because of differential linkages between loci controlling the selection criterion and response criterion.

## RESULTS AND DISCUSSION

Population means were significantly different for NDF and PSRI averaged over all locations and years ( $P < 0.01$ ). Interactions of populations with location, year, and harvest were all nonsignificant ( $P > 0.05$ ). This is consistent with results from previous experiments (Casler and Vogel, 1999). All results are presented as means over locations, years, and harvests.

### Divergent Selection for NDF

Direct selection responses were significant ( $P < 0.01$ ) for NDF of all four populations (Fig. 1). Direct responses to selection for NDF averaged 0.7 to 1.3% of the population mean. Responses were highly linear, accounting for 73 to 99% of the variation among cycles (Fig. 1), and were homogeneous ( $P = 0.22$  for population  $\times$  divergence interaction). Asymmetry of selection responses was significant only for WB19e and Lincoln, with marginal significance for WB88S. The inconsistency in asymmetry responses suggests that they are more likely due to expected differences between directions of selection (Falconer and Mackey, 1996) and not to drift. Drift would most likely result in similar profiles (positive vs. negative), depending on the effect of inbreeding on NDF concentration, which is largely unknown. Conversely, a greater response for NDF in the positive direction (e.g., WB19e and WB88S) suggests a mean allele frequency  $< 0.5$ , or a mean allele frequency  $> 0.5$  for Lincoln. Rates of gain were similar to that observed for NDF in reed canarygrass (Surprenant et al., 1988) and a different smooth bromegrass population (Casler, 1999b).

Realized heritability for NDF concentration was low relative to other selection experiments based on NDF or other measures of forage nutritional value, ranging from 0.09 for WB88S to 0.20 for Lincoln. Realized heritability of NDF in a different smooth bromegrass population averaged 0.31 (Casler, 1999a). Additive genetic variation exists within each population for NDF concentration, and progress was achieved in all four populations. Genotype  $\times$  environment interaction, on a large

**Table 1. Mean squares, *P*-values, and sum of squares percentages (SS%) for analysis of variance of particle size reduction index (PSRI) measured on four smooth brome grass base populations and eight selected populations created by divergent selection for neutral detergent fiber (NDF) concentration.**

Source of variation	df	MS	<i>P</i>	SS%
Populations	3	50	0.10	5.6
Divergence (average)	1	2445	<0.01	90.5
Populations × divergence	3	12	0.42	1.4
Asymmetry	1	46	0.06	1.7
Populations × asymmetry	3	7	0.66	0.8

scale, was not an impediment to progress. Selection for reduced NDF concentration, based on a single measurement of vegetative herbage (leaf blades) harvested from a single plant, was successful, regardless of the location, year, or harvest upon which progress was evaluated. Low realized heritabilities suggest the need for large population sizes and high selection intensities in future selection experiments. Because genotype × environment interaction was generally not important, replication of selection units is not expected to improve the efficiency of selection (England, 1977).

Divergent selection for NDF led to consistent and significant changes in PSRI (Table 1, Fig. 2). Linear correlated responses of PSRI to divergent selection for NDF averaged 6.6 to 9.4% of the original population means, approximately 10 times greater than the direct responses for NDF (Fig. 1 vs. Fig. 2). Furthermore, the genotypic coefficient of variation (based on the genotypic variance for all populations) was 7% for NDF and 56% for PSRI. These results suggest that there is relatively more genetic variation in these populations for PSRI than for NDF. If NDF is a factor determining PSRI, then there are probably additional factors that regulate PSRI, so that genetic variation for PSRI reflects accumulated genetic variation for numerous plant traits that may be either biochemical or structural in nature (Casler et al., 2000).

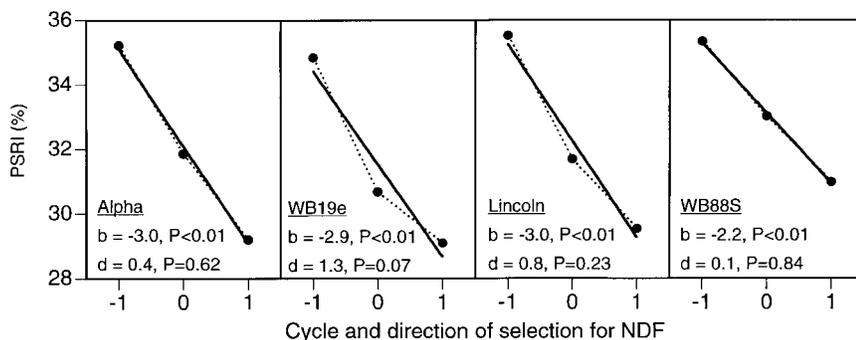
The single degree of freedom for divergence, averaged over populations, accounted for 90.5% of the PSRI sum of squares for populations and cycles (created by divergent selection for NDF) and 95.6% of the PSRI sum of squares for cycles within populations (Table 1). Correlated responses of PSRI to selection for NDF were highly linear with  $R^2$  ranging from 0.94 to 0.99, indicating no evidence of asymmetrical correlated selection re-

sponses. The phenotypic correlation between NDF and PSRI for these 12 populations and cycles was  $-0.77$  ( $P < 0.01$ ).

Previous reports have indicated relatively high negative genotypic correlations between NDF and PSRI (Casler et al., 2000; Culvenor and Casler, 1999). Observed genotypic correlations may be due to pleiotropy, linkage disequilibrium, or drift. Because pleiotropy is not influenced by linkage patterns or random assortment of alleles at different loci, its effects should be relatively constant across different populations. Therefore, its effects can be measured by the common correlated response across populations. Conversely, linkage disequilibrium is expected to vary among populations, particularly if they are of diverse origin as in this study. Therefore, its effects can be measured as differences in correlated responses among populations.

These results suggest that most of the correlated response of PSRI to selection for NDF was due to pleiotropic effects of loci, or to very tight linkages between loci controlling the two traits, a phenomenon that cannot be distinguished from pleiotropy without very large populations and/or several cycles of recombination. Linkages between loci with relatively large map distances would have been partially broken during recombination, resulting in a partial breakdown of the genetic correlation between NDF and PSRI. Large genetic correlations can be maintained in random mating populations only by tight linkages in the absence of pleiotropy (Lande, 1984). Thus, linkage does not appear to be an important factor regulating the correlated response of PSRI to divergent selection for NDF. The consistency of PSRI responses to divergent selection for NDF implicates NDF concentration as a biological determinant of PSRI; selection for low NDF appears to be a mechanism to obtain plants with high PSRI. Rapid and thorough particle size breakdown of smooth brome grass leaves during ball-milling appears to be facilitated by low fiber concentration.

Thus, selection for low NDF concentration has potentially two distinct benefits for voluntary intake by ruminants. First, reduced NDF concentration decreases bulk volume and/or increases caloric density of the forage, allowing the ruminant to consume a greater amount of forage before satiation (Van Soest, 1994), thus increas-



**Fig. 2. Indirect responses of particle size reduction index (PSRI) to divergent selection for neutral detergent fiber (NDF) concentration in four smooth brome grass populations. Means are over four replicates, three locations, 3 yr, and two harvests. Solid lines represent linear selection responses (b) and dashed lines represent effects of drift (d).  $R^2 = 0.99, 0.94, 0.97,$  and  $0.99$  for Alpha, WB19e, Lincoln, and WB88S, respectively.**

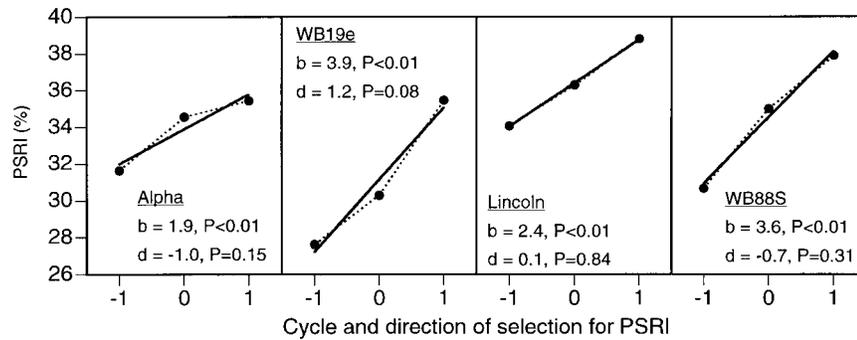


Fig. 3. Direct responses of particle size reduction index (PSRI) concentration to divergent selection for PSRI in four smooth bromegrass populations. Means are over four replicates, three locations, 3 yr, and two harvests. Solid lines represent linear selection responses (b) and dashed lines represent effects of drift (d).  $R^2 = 0.91, 0.97, 0.99, \text{ and } 0.99$  for Alpha, WB19e, Lincoln, and WB88S, respectively.

ing voluntary intake of dry matter per unit of time. Second, the increased particle size breakdown of low-NDF plants may lead to an increased rate of passage through the rumen. Forage particles are largely broken down by chewing and rumination (McLeod and Minson, 1988; Wilson et al., 1989). Several methods of predicting the energy required to shear, compress, mill, or otherwise break down particle size are highly correlated with voluntary intake (Baker et al., 1993; Troelson and Bigsby, 1964; Weston, 1985). More rapid particle-size breakdown leads to more rapid clearing of small particles from the rumen, which decreases the time required to stimulate appetite (Van Soest, 1994), also increasing voluntary intake of dry matter per unit of time.

### Divergent Selection for PSRI

Direct selection responses were significant ( $P < 0.01$ ) for PSRI of all four populations (Fig. 3). Direct responses to selection for PSRI averaged 5.4 to 12.9% of the population mean. These responses were approximately half those observed when these populations were evaluated under spaced-planted conditions (Culvenor and Casler, 1999), suggesting that spaced plants may inflate estimates of genetic responses to selection for PSRI. Responses were highly linear, accounting for 91 to 99% of the variation among cycles (Fig. 3) and were highly heterogeneous ( $P < 0.01$  for population  $\times$  divergence interaction). Asymmetry of selection was not significant. These observations confirm those of Culvenor and Casler (1999) that there is a large amount of additive

genetic variation for PSRI in each of these four populations. Genotype  $\times$  environment interactions were relatively unimportant in detecting genetic gains, as indicated by analysis of variance in this study and that of Culvenor and Casler (1999).

Correlated responses of NDF to selection for PSRI were significant only within Lincoln (Fig. 4). The change in NDF due to selection for PSRI in Lincoln was slightly less than the direct response to selection for NDF in Lincoln, but was characterized by a large asymmetric effect, suggesting that the average frequency of alleles for PSRI was  $<0.5$  in Lincoln (Falconer and Mackey, 1996). Despite the statistical significance of NDF divergence following divergent selection for PSRI (Table 2), observed responses for the other populations were all very small, inconsistent, and nonsignificant. Divergence accounted for only 14.3% of the variation among 11 populations and cycles and only 33.0% of the variation among cycles within populations. Asymmetry accounted for 46.7% of the variation among cycles within populations, suggesting that random assortment (or loose linkages) of alleles was responsible for about half of the correlated responses of NDF to divergent selection for PSRI. Although these results do not agree with the opinions of Falconer and Mackey (1996) or Simmonds and Smartt (1999), or the computations of Lande (1984), they support empirical observations of Jinks et al. (1985). Using dihaploids and single seed descent during selfing, the latter authors showed that either linkage disequilibrium, pleiotropy, or both factors may cause a

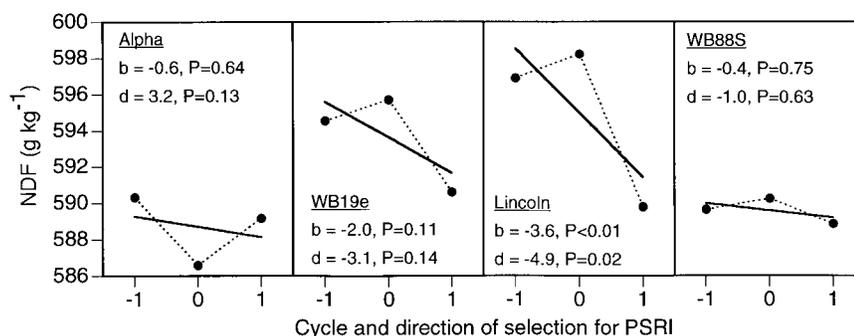


Fig. 4. Indirect responses of neutral detergent fiber (NDF) concentration to divergent selection for particle size reduction index (PSRI) in four smooth bromegrass populations. Means are over four replicates, three locations, 3 yr, and three harvests. Solid lines represent linear selection responses (b) and dashed lines represent effects of drift (d).  $R^2 = 0.09, 0.54, 0.61, \text{ and } 0.32$  for Alpha, WB19e, Lincoln, and WB88S, respectively.

**Table 2. Mean squares, *P*-values, and sum of squares percentages (SS%) for analysis of variance of neutral detergent fiber (NDF) concentration measured on four smooth bromegrass base populations and eight selected populations created by divergent selection for particle size reduction index (PSRI).**

Source of variation	df	MS	<i>P</i>	SS%
Populations	3	2001	<0.01	56.6
Divergence (average)	1	1514	<0.01	14.3
Populations × divergence	3	310	0.23	8.8
Asymmetry	1	410	0.17	3.9
Populations × asymmetry	3	578	0.04	16.4

genetic correlation, depending on the traits. In general, genetic correlations between allometric traits tended to be more frequently regulated by pleiotropy, because of the cascading effect of individual loci on life history traits that are influenced by processes both early and late in the plant's growth cycle or life.

The inconsistent and/or lack of correlated changes of NDF resulting from selection for PSRI may have two possible explanations. First, the relatively low amount of genetic variation for NDF, compared with PSRI (Fig. 1 vs. Fig. 2; genotypic coefficients of variation), may have limited the potential for correlated selection responses. Second, the changes in PSRI resulting from direct selection pressure for PSRI may have resulted from alternative biological mechanisms controlling PSRI. Decreased lignification, decreased ferulate cross-linking between lignin and arabinoxylan, and decreased frequency of highly lignified structural cells are all potential mechanisms for genetically increasing PSRI (Casler et al., 2000). Direct selection for PSRI may have acted upon loci that regulate cell-wall composition and/or cell structure and function without large effects on NDF concentration. Furthermore, these alternative explanations are not mutually exclusive; genetic changes in PSRI may have been largely independent of NDF because of the relatively low amount of genetic variation for NDF. This is supported by the fact that Lincoln, with the only significant NDF response to divergent selection for PSRI, had the highest realized heritability for NDF.

Finally, the differential correlated selection responses observed for selection based on NDF vs. PSRI lead to a different type of asymmetry. The uniformity of PSRI responses to divergent selection for NDF suggested either pleiotropy or tight linkages as genetic factors. Conversely, the lack of uniformity of NDF responses to divergent selection for PSRI suggested loose linkage or random assortment between loci controlling NDF and PSRI. Deviations of average allele frequencies from 0.5 and/or loci that have negative pleiotropic effects on two traits will generally cause asymmetry between the two selection criteria (Bohren et al., 1966), i.e., changes in PSRI due to selection for NDF will be different than changes in NDF due to selection for PSRI, as observed in this study. Differential heritabilities of the two traits, as suggested by Siegel (1962), are not a necessary condition for this type of asymmetry (Bohren et al., 1966). Bohren et al. (1966) also point out that divergence of allele frequencies from 0.5 and/or negative pleiotropic effects will have little effect on asymmetry of correlated

responses between the two selection criteria in the short term, with each cycle of selection showing only  $1/n$  of the long-term difference in correlated responses, where  $n$  = the number of loci. Thus, if many loci control NDF and PSRI, which seems highly likely given the complexity of each trait, the asymmetry observed between NDF and PSRI as correlated response criteria is probably due to multiple genetic phenomena. There are probably pleiotropic loci that control both traits, linkage blocks that contain loci controlling both traits independently (appearing pleiotropic in the short term), and unlinked loci that control each trait. Multiple mechanisms for genetic control of PSRI suggest that PSRI is controlled by unlinked loci to a greater extent than NDF. Further research will be required to elucidate these alternative mechanisms for genetic control of PSRI.

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