Relationships among Biomass Yield Components within and between Subspecies of Alfalfa

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ABSTRACT

Crosses between *Medicago sativa* subsp. *sativa* and subsp. *falcata* show a heterotic pattern for total biomass yield, with inter-subspecific crosses outperforming intra-subspecific crosses. Little is known about relationships among agronomic traits in sativa-falcata hybrids. The objective of this study was to examine correlations among twenty traits including total biomass yield, total biomass yield heterosis, agronomic traits and forage nutritive value, and genetic distance and test the hypothesis that they differ in intra versus inter-specific crosses. We crossed nine sativa and five falcata genotypes in a diallel mating design. Progeny means of the 91 crosses were calculated based on field data collected in 1998 and 1999 at two locations in Iowa. Most correlations were the same in inter and intra-subspecific crosses, and generally support previous research. Several correlations, however, differed between inter-subspecific and intra-subspecific groupings. Cases where intra-subspecific correlations radically differ from one another may suggest independent suites of alleles in each subspecies controlling traits of interest, especially if no inter-subspecific correlation is present.

Alfalfa (*Medicago sativa* L.) is a widely cultivated forage crop in the North America. Primary breeding concerns in alfalfa are disease/insect resistance, total forage biomass yield (hereafter "yield"), persistence, and forage nutritive value. Yield improvement will be difficult to achieve if breeders are simultaneously maintaining or improving many other traits; this is one explanation offered for current yield stagnation (Hill et al., 1988; Riday and Brummer, 2002a). The utilization of heterosis found between particular germplasm populations (heterotic groups) may be a means to improve alfalfa yield (Brummer, 1999; Riday and Brummer, 2002a).

Medicago sativa subsp. *falcata* (hereafter, falcata), one of the nine germplasm groups initially introduced into the United Sates (Barnes et al., 1977), is geographically distributed throughout the northern latitudes of Eurasia (Lesins and Lesins, 1979) and may represent a potential heterotic group with the commonly cultivated, purple flowered *M. sativa* subsp. s*ativa* (hereafter "sativa") grown in the upper Midwestern U.S. Crosses between sativa and falcata tend to show heterosis for yield (Westgate, 1910; Waldron 1920; Sriwatanapongse and Wilsie, 1968; Riday and Brummer, 2002a). However, while falcata tends to have good winter hardiness (Barnes et al., 1977), it has a number of agronomic limitations, including its decumbent growth habit, low seed set, and slow regrowth (Riday and Brummer, 2002b), and would likely need to be considerably improved before it could be used in some type of hybrid or semi-hybrid breeding scheme. The existence of adverse correlations among these and other agronomically important traits may present problems for breeders.

Phenotypic correlations in alfalfa, specifically sativa, generally indicate higher yielding plants will be taller, more mature, and have reduced nutritive value (Elliot et al., 1972). Although erect plants with late fall dormancy are often assumed to be more susceptible to winter injury and poorer spring recovery, a recent study found no genotypic correlation between autumn plant height and winter injury in an F_1 population derived from a sativa by falcata cross (Brummer et al., 2000). Most studies, including some that evaluated falcata germplasm, show that stem forage quality traits are highly correlated with each other (e.g. increases in fiber concentration are correlated with decreased digestibility)(Julier et al., 1996; Jung et al., 1997).

No research has been conducted to determine if the hybrids between morphologically divergent germplasm, such as falcata and sativa, have different trait correlations than the progeny of crosses made within the individual germplasm groups. One early study looked specifically at correlations of traits measured on the progeny of a Hairy Peruvian (sativa) by falcata cross, but most of the correlations were based on traits measured in the greenhouse and no comparisons with intra-subspecific crosses were made (Burton, 1937). Understanding the relationships among yield, nutritive value, and agronomic traits will provide a better idea about the ramifications of using falcata germplasm in breeding programs, and in particular, in a hybrid breeding program. Additionally, investigating these correlations could help provide a better understanding of the mechanisms controlling the yield heterosis seen in inter-subspecies hybrids.

Our objective was to determine correlations among a suite of traits measured on the intra- and intersubspecific progeny of nine sativa and five falcata genotypes. Using these correlations, we evaluated the hypothesis that the relationships among traits differed in the progeny resulting from sativa by sativa crosses (SSC), sativa by falcata crosses (SFC), and falcata by falcata crosses (FFC).

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Abbreviations: sativa by sativa crosses, SSC; sativa by falcata crosses, SFC; falcata by falcata crosses, FFC; in vitro dry matter digestibility, IVDMD; neutral detergent fiber, NDF; acid detergent fiber, ADF; acid detergent lignin, ADL; crude protein, CP; percent mid-parent heterosis, MP-heterosis; amplified fragment length polymorphism, AFLP; simple sequence repeat, SSR; general combining ability, GCA; specific combining ability percentage, SCA%

MATERIALS AND METHODS

Plant Material

Fourteen genotypes (nine sativa and five falcata) were used as parents in this experiment. The nine elite sativa genotypes included ABI408, ABI311, ABI419, and ABI314 from ABI Alfalfa, Inc. (12351 W. 96 Terrace, Suite 101, Lenexa, KS 66215); C96-514, C96-673, and C96-513 from Forage Genetics (N5292 S. Gills Coulee Road, West Salem, WI 54669); and FW-92-118 and RP-93-377 from Pioneer Hi-bred International (400 Locust Street, Suite 800, PO BOX 14453, Des Moines, IA 50306). The five falcata genotypes included WISFAL-4 and WISFAL-6 from the semi-improved falcata population, WISFAL (PI560333; Bingham, 1993); C25-6, a semi-improved falcata population developed in Colorado (PI578248; Townsend, 1995); and two genotypes visually selected for vigor from plant introductions that had been planted in the field near Ames, IA: PI214218-1, derived from an accession collected in Denmark in 1954 and PI502453-1, derived from the Russian cultivar Pavlovskaya.

Crossing and Field Design

The fourteen selected parental genotypes were crossed in the greenhouse during autumn 1997 in a half diallel mating design, without reciprocals. Florets were hand emasculated to limit accidental self-pollination. In April 1998, seed from the 91 crosses were planted in the greenhouse. Stem cuttings of the fourteen parents were made at the same time. A total of 105 entries was included in this experiment (91 crosses and 14 parental clones). Plants grown in the greenhouse were transplanted to the field in May 1998 at the Agronomy and Agricultural Engineering Research Farm west of Ames, IA (42ºN, 94ºW) and at the Northeast Research Farm south of Nashua, IA (43ºN, 92ºW). The plot design was a quadruple α-lattice (Patterson and Williams, 1976). Ten plants per plot were planted 30 cm apart within rows spaced 90 cm apart. Plots were separated by 60 cm within rows (Riday and Brummer, 2002a).

Trait Measurements

Complete methods used to measure the traits were presented previously (Riday and Brummer, 2002a,b) and are summarized briefly below. Total yearly yield on a per plant basis was calculated for 1998 and 1999 at each location based on biomass yield measurements taken in August and October 1998 and June, July, and September 1999. Winter injury, scored after new stems had emerged in April 1999, measured crown health and evenness of regrowth on a 1 = least damaged to 5 = most damaged scale (McCaslin and Woodward, 1995). Plot height, the average of five measurements taken on random plants as they stood naturally in the field, was measured in October 1998, June, July, and September 1999. In 1999, the heights taken in May/June, July, and September were individually correlated with average yearly height ($r = 0.81$, 0.95, and 0.95, respectively, $p < 0.0001$). Due to the high correlations, average yearly height for 1999 was used in correlations with other traits. The correlation (r) between autumn height in 1998 and average yearly height in 1999 was 0.71 ($p < 0.0001$).

Growth habit was visually scored on a 1 = most decumbent to 9 = most erect scale for each plot in May and August or September 1999. Maturity was scored on a 1 = early vegetative to 9 = ripe seed pod scale (Kalu and Fick, 1981) in May or June, July, and August or September 1999. Vigor was scored in May 1999 based on the density and amount of vegetative growth in each plot using a 1 = least to 5 = most scale. The amount and rate of regrowth was measured on a 1 = least to five = most scale four times during 1999. Spring regrowth was measured in April; midseason regrowth in June and July; and autumn regrowth in September.

Forage nutritive value was measured based on stem samples collected on 16 October 1998 and 27 May 1999 at Ames, and on 6 June 1999 at Nashua (Riday et al., 2002a). Traits measured included *in vitro* dry matter digestibility (IVDMD), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid determined lignin (ADL), crude protein, hemicellulose, cellulose, and leaf/stem ratio.

Each measured year-location combination was treated as a single environment for analysis of total yearly yield. Each measurement date-location combination was treated as a single environment for the analysis of 1999 heights, growth habit, midseason regrowth, maturity, and forage quality traits. For each trait, one experiment wide mean per entry was calculated using the PROC MIXED procedure of the SAS statistical software package (SAS Institute, 2000), with locations or environments considered to be random effects.

Heterosis Calculations

Two measures of yield heterosis were calculated for the progeny of each pair-wise combination of the fourteen parental genotypes: (i) specific combining ability percentage (SCA%) and (ii) mid-parent heterosis (MP-heterosis). SCA% was determined as:

$$SCA\%_{ij} = \frac{Observed\ Yield_{ij} - (\mu + GCA_i + GCA_j)}{(\mu + GCA_i + GCA_j)} \times 100 \quad [1]$$

where, *i* and *j* are parental genotypes (1 to 14), and *ij* are all ninety-one pair-wise progeny combinations, with the constraint that $i < j$.

Observed $Yield_{ij}$ = the observed progeny yield of parental combination *i* x *j*.

μ = mean yield performance of all progeny.

GCA = General Combining Ability of a given parental genotype.

Mid-parent heterosis (%) determined as:

$$MP\text{-}heterosis_{ij} = \frac{Observed\ Yield_{ij} - \left(\frac{Clonal\ Yield_i + Clonal\ Yield_j}{2}\right)}{\left(\frac{Clonal\ Yield_i + Clonal\ Yield_j}{2}\right)} \times 100 \quad [2]$$

where the terms are as defined above and

Clonal Yield = yield based on clonal performance of parental genotype.

The difference between [1] and [2] is that SCA% is based on the average performance of the progeny of specific parental genotypes, while mid-parent heterosis is based on actual parental genotype performance *per se*. For parental genotype *i*, the deviation between actual parental performance (i.e., that based on clonal measurements) and GCA (i.e., that based on progeny performance) represents the "average heterosis" across all genotypes in the experiment. Thus, specific combining ability percentage is a deviation from "average heterosis," while MP-heterosis includes both "average heterosis" and SCA%.

Genetic Distance

DNA was extracted from the fourteen parental genotypes, amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) polymorphic fragments between the parents were scored, and a genetic distance between each parental genotype was calculated as described previously (Riday et al., 2003).

Computation

The twenty trait measurements were compared with each other using Pearson's correlation coefficient for FFC, SFC, and SSC individually and for all cross groups combined. The cross types (FFC, SFC, and SSC) correlations were compared with each other for each pairwise correlation between the twenty traits as described in Steel and Torrie (1980). Additionally for FFC, SFC, and SSC slopes were calculated between pairwise regressions of the twenty traits. Slopes among cross types were compared using contrast statements in Proc Mixed (SAS Institute, 2000). For correlations involving genetic distance, r-value signs were reversed so that as genetic distance between parental genotypes increased, the correlated trait's value in the progeny increased as well. Correlations were considered to be significant at P=0.05 unless specified otherwise.

RESULTS AND DISCUSSION

Only phenotypic correlations are reported in this paper. Phenotypic correlations are affected by both environmental and genotypic effects, unlike genetic correlations, which measure relationships due to genetic effects such as pleiotropy and linkage (Falconer and Mackay, 1996). Although genotypic correlations are more desirable for determining the effects of selection on two traits, they are difficult to measure in unstructured populations such as those described in this experiment.

Table 1. Phenotypic correlations of total alfalfa biomass yield, specific combining ability percentage for yield (SCA%), mid-parent heterosis (MP-heterosis), parental genetic distance, agronomic traits, and nutritive value parameters based on data collected on intra and inter-subspecific progeny of nine *Medicago sativa* subsp. *sativa* and five *M. sativa* subsp. *falcata* genotypes at two Iowa locations over two years.

	Yield	SCA%	MP-heterosis	Genetic Distance
	--------- Correlation Coefficient (r) ---------			
Winter Injury	-0.28*	-0.34*	ns	ns
Plant Height	0.37**	0.35**	ns	-0.50***
Growth Habit	ns	ns	ns	-0.55***
Maturity	ns	ns	ns	-0.37**
Vigor	0.74***	0.62***	ns	ns
Spring Regrowth	0.27*	0.30*	ns	ns
Midseason Regrowth	ns	ns	ns	-0.54***
Autumn Regrowth	ns	ns	ns	-0.46***
IVDMD	ns	ns	ns	0.28*
NDF	0.29*	ns	ns	ns
ADF	ns	ns	ns	ns
ADL	ns	ns	ns	-0.34*
Hemicellulose	0.29*	ns	ns	ns
Cellulose	ns	ns	ns	ns
Crude Protein	ns	ns	ns	ns
Leaf/Stem Ratio	ns	ns	-0.33*	ns
Genetic Distance	ns	ns	ns	--

***, **, *** Phenotypic correlation significantly different from zero at the 0.01, 0.001, and 0.0001 level of probability. ns = not significant.**

Correlations Across All Cross Types

Yield, yield heterosis, genetic distance

Yield was negatively correlated with winter injury and positively correlated with plant height, vigor, and spring regrowth (Table 1). Yield showed no significant correlation with growth habit, maturity, midseason regrowth, or autumn regrowth. Although high yield might be expected to be correlated with more erect growth, the lack of association with growth habit could have resulted from our space planted experimental design; the relationship between the traits may be quite different under the sward conditions typical of production fields. Increased yield was correlated with increased NDF and hemicellulose (Table 1).

The two heterosis measures, MP-heterosis and SCA%, were positively correlated with each other ($r = 0.59$, $p < 0.0001$). Mid-parent yield, calculated from parental clonal yield data, was negatively correlated with MP-heterosis ($r = -0.69$, $p < 0.0001$). The negative correlation suggests that as parental yields increase, the potential to obtain heterosis in progeny decreases. In a previous study, we showed that high SCA% was indicative of the sativa-falcata heterotic pattern (Riday et al., 2003), with the largest SCA% values observed during the spring harvest of 1999 (Riday and Brummer, 2002a). Thus, the relationship of increasing SCA% with improved spring regrowth suggest that robust spring recovery is a feature associated with sativa-falcata hybrids (Table 1).

Table 2. Phenotypic correlations of agronomic traits and nutritive value based on data collected on intra and inter-subspecific progeny of nine *Medicago sativa* subsp. *sativa* and five *M. sativa* subsp. *falcata* genotypes at two Iowa locations over two years.

	Winter Injury	Height	Growth Habit	Maturity	Vigor	Spring Regrowth	Midseason Regrowth	Autumn Regrowth
				Correlation Coefficient (r)				
Growth Habit	ns	0.82***						
Maturity	ns	0.48***	0.52***					
Vigor	-0.60***	ns	ns	ns				
Spring Regrowth	-0.48***	0.56***	0.50***	0.39***	0.39***			
Midseason Regrowth	ns	0.69***	0.76***	0.77***	ns	0.53***		
Autumn Regrowth	ns	0.62***	0.72***	0.75***	ns	0.62***	0.90***	
IVDMD	ns	-0.61***	-0.58***	ns	ns	ns	-0.30*	-0.30*
NDF	ns	0.28*	0.27*	ns	ns	ns	ns	ns
ADF	ns	ns	ns	ns	ns	ns	ns	ns
ADL	ns	0.63***	0.63***	0.30*	ns	0.30*	0.39***	0.40***
Hemicellulose	ns	0.38**	ns	ns	ns	ns	ns	ns
Cellulose	ns	ns	ns	-0.36**	ns	ns	-0.41***	-0.31*
Crude Protein	ns	-0.27*	-0.28*	ns	ns	-0.30*	ns	ns
Leaf/Stem Ratio	ns	ns	ns	ns	ns	ns	0.39***	0.42***

***, **, *** Phenotypic correlation significantly different from zero at the 0.01, 0.001, and 0.0001 level of probability. ns = not significant.**

Genetic distance was neither correlated with yield nor with either measure of yield heterosis (Table 1; Riday et al., 2003). Selecting parents based on their genetic distance will not guarantee high yielding progeny. Greater genetic distances between the parents resulted in shorter, more decumbent, earlier maturing progeny that generally had poorer regrowth (Table 1). However, these results may reflect the genetic materials used in this experiment rather than a common phenomenon, since taller, faster regrowing parental genotypes were generally more closely related to each other than to decumbent, slower growing genotypes (Riday et al., 2003).

Agronomic Field Traits

Not surprisingly, greater winter injury was associated with lower spring regrowth and vigor (Table 2). We found a weak positive correlation between 1999 winter injury and autumn 1998 height ($r = 0.29$, $p < 0.01$) and found no correlation between October 1998 biomass yield and either autumn 1998 height or 1999 winter injury. These correlations (or lack thereof) support the lack of genetic correlation seen between winter injury and autumn height in a single F_1 population (Brummer et al., 2000). This interpretation offers hope that breeders can simultaneously improve yield and winter survival using the correct selection methodology.

Height and growth habit were highly correlated with each other, and both were positively correlated with all three regrowth measures and with maturity (Table 2). Maturity and regrowth were positively correlated, indicating that faster regrowing plants tended to mature faster (Table 2). Increasing vigor was correlated with increased spring regrowth (Table 2). Spring regrowth was not as strongly correlated with autumn regrowth and midseason regrowth as the latter two were with each other (Table 2), suggesting different genetic control for these traits.

Height and growth habit were correlated with many agronomic and nutritive value traits (Table 2). The correlations were negative for IVDMD and crude protein, and positive for NDF, and ADL (Table 2). Of all the nutritive value traits, IVDMD and ADL were most often correlated with agronomic traits. Given that stems need cell wall constituents, especially lignin, to stand erect and that increased fiber generally leads to decreased digestibility, these results are not unexpected. Compared with falcata, the sativa genotypes in this study had a greater capacity to produce lignin (ADL), had more midseason and autumn regrowth, and matured faster (Riday and Brummer, 2002b; Riday et al., 2002a). Falcata genotypes produced more cellulose than sativa (Riday and Brummer, 2002b; Riday et al., 2002a). These observations may account for the positive associations between ADL and maturity, midseason regrowth, and autumn regrowth and the negative associations between cellulose and maturity, midseason regrowth, and autumn regrowth (Table 2)

Stem Nutritive Value

In general, stem cell wall constituents (NDF, ADF, ADL, cellulose, and hemicellulose) were highly positively correlated with each other, but negatively associated with crude protein

Table 3. Phenotypic correlations of nutritive value based on data collected on intra and inter-subspecific progeny of nine *Medicago sativa* subsp. *sativa* and five *M. sativa* subsp. *falcata* genotypes at two Iowa locations over two years.

	IVDMD	NDF	ADF	ADL	Hemi-cellulose	Cellulose	Crude Protein
			Correlation Coefficient (r)				
NDF	-0.83***						
ADF	-0.75***	0.95***					
ADL	-0.98***	0.74***	0.66***				
Hemicellulose	-0.49***	0.47***	ns	0.47***			
Cellulose	-0.51***	0.85***	0.95***	0.39***	ns		
Crude Protein	0.44***	-0.46***	-0.34*	-0.36**	-0.49***	ns	
Leaf/Stem Ratio	ns	ns	ns	ns	ns	ns	ns

***, **, *** Phenotypic correlation significantly different from zero at the 0.01, 0.001, and 0.0001 level of probability. ns = not significant.**

Table 4. Phenotypic correlations differences among three cross types (sativa by sativa crosses (SSC), sativa by falcata crosses (SFC), and falcata by falcata crosses (FFC)) based on data collected on intra- and inter-subspecific progeny of nine *Medicago sativa* subsp. *sativa* and five *M. sativa* subsp. *falcata* genotypes at two Iowa locations over two years

Two Traits Correlated		Cross Type		
		SSC	SFC	FFC
		(n=36)	(n=45)	(n=10)
		Correlation Coefficient (r)		
Yield	Maturity	0.42$^{*\ a\S}$	-0.23^{b}	0.17^{ab}
Yield	Vigor	0.00^{b}	0.52$^{*\ a}$	0.81$^{**\ a}$
Yield	Spring Regrowth	-0.18^{b}	0.32$^{*\ a}$	0.64$^{*\ a}$
Yield	Midseas. Regrowth	-0.42$^{*\ b}$	-0.01^{a}	0.36^{a}
Yield	Autumn Regrowth	-0.37$^{*\ b}$	0.11^{a}	0.39^{a}
Mid-Par. Heterosis	Growth Habit	-0.18^{b}	0.07^{ab}	0.66$^{*\ a}$
Mid-Par. Heterosis	Midseas. Regrowth	-0.17^{b}	-0.02^{b}	0.66$^{*\ a}$
Mid-Par. Heterosis	Autumn Regrowth	-0.10^{b}	0.09^{b}	0.79$^{**\ a}$
Winter Injury	Growth Habit	-0.40$^{*\ b}$	0.31$^{*\ a}$	0.01^{ab}
Winter Injury	IVDMD	0.41$^{*\ a}$	-0.31$^{*\ b}$	0.20^{ab}
Winter Injury	NDF	-0.45$^{**\ b}$	0.23^{a}	-0.34^{ab}
Winter Injury	ADF	-0.44$^{**\ b}$	0.27^{a}	-0.31^{ab}
Winter Injury	ADL	-0.43$^{*\ b}$	0.32$^{*\ a}$	-0.23^{ab}
Winter Injury	Cellulose	-0.39$^{*\ b}$	0.23^{a}	-0.34^{ab}
Height	Autumn Regrowth	0.33$^{*\ a}$	-0.05^{b}	0.62$^{*\ a}$
Growth Habit	Spring Regrowth	0.54$^{***\ a}$	0.10^{b}	0.55^{ab}
Growth Habit	Hemicellulose	0.40$^{*\ a}$	-0.02^{b}	-0.70$^{*\ b}$
Growth Habit	Crude Protein	-0.60$^{***\ b}$	-0.08^{a}	0.13^{a}
Maturity	Midseas. Regrowth	-0.37$^{*\ b}$	0.40$^{**\ a}$	0.31^{ab}
Maturity	Autumn Regrowth	0.48$^{**\ a}$	-0.54$^{***\ b}$	0.37^{a}
Spring Regrowth	Hemicellulose	0.44$^{**\ a}$	-0.08^{b}	-0.59^{b}
Midseas. Regrowth	Hemicellulose	0.06^{a}	-0.43$^{**\ b}$	0.17^{ab}
Midseas. Regrowth	Crude Protein	-0.09^{b}	0.39$^{**\ a}$	0.69$^{*\ a}$
Autumn Regrowth	Hemicellulose	0.28^{a}	-0.43$^{***\ b}$	-0.64$^{*\ b}$
Autumn Regrowth	Crude Protein	-0.36$^{*\ b}$	0.39$^{**\ a}$	0.00^{ab}
Hemicellulose	IVDMD	-0.63$^{***\ b}$	-0.33$^{*\ ab}$	0.19^{a}
Hemicellulose	NDF	0.71$^{***\ a}$	0.45$^{***\ ab}$	-0.17^{b}
Hemicellulose	ADF	0.52$^{***\ a}$	0.11^{ab}	-0.39^{b}
Hemicellulose	ADL	0.62$^{***\ a}$	0.30$^{*\ ab}$	-0.31^{b}
Hemicellulose	Cellulose	0.40$^{*\ a}$	0.02^{ab}	-0.40^{b}

***, **, *** Phenotypic correlation significantly different from zero at the 0.05, 0.01, and 0.001 level of probability. ns = not significant.**
§ Significant correlation and slope differences between cross types at *P* = 0.05

and IVDMD (Table 3). Leaf/stem ratio showed no significant correlation with any stem nutritive value traits (Table 3). Because stem nutritive value traits rarely exhibit genotype by environment interaction, these correlations are similar to those reported in other studies (Julier et al., 1996; Jung et al., 1997; Fonseca et al., 1999).

Correlation Comparisons between Inter and Intra-Subspecific Crosses

In this study, we produced three types of crosses: the two intra-subspecies hybrids (SSC and FFC) and the inter-subspecies hybrid (SFC). This allowed us to determine if correlations between particular traits differed among cross types. Due to the morphological divergence between these falcata and sativa genotypes that we have documented (Riday and Brummer, 2002b, Riday et al., 2002a), we expected that the relationships between traits might differ, particularly in the inter-subspecies crosses, due to the presence of complementary allelic interactions and effects between subspecies (Bingham et al., 1994).

Comparisons among SSC, SFC, and FFC revealed that in 30 pairwise trait comparisons correlations and slopes differed among one or more of the cross types (Table 4). Due to small sample size of FFC (n = 10) correlations were usually not significant, although comparatively correlations values were equivalent to significant SSC and SFC values. Particular traits tended to be in correlations that differed between cross types more often.

Hemicellulose, in pariticular, showed differences, especially, between SSC and FFC. Correlations of hemicellulose with ADF, ADL, autumn regrowth, cellulose, growth habit, IVDMD, midseason regrowth, NDF, and spring regrowth, showed differences between SSC and FFC. Sativa x falcata cross correlations were either intermediate to the subspecies correlations or they were equivalent to one of the parental types. Compared with falcata, sativa have a higher ratio of hemicellulose to cellulose (Riday et al., 2002a). Genes regulating these components may also be involved in the regulation of morphological features of plant growth form. Different alleles in the two subspecies may interact in different ways, leading to the radically different correlations in SSC and FFC.

Winter injury showed differences between cross types in correlations with ADF, ADL, cellulose, growth habit, IVDMD, and NDF (Table 4). Correlations were different between SSC and SFC. In every case FFC correlations was intermediate to the parental correlations. Almost all cross type differences in winter injury correlations are with forage quality traits.

Maturity correlated with autumn regrowth, midseason regrowth, and biomass yield show differences between cross types (Table 4). The correlations of maturity and autumn and midseason regrowth, show significant correlations for SSC and SFC, however, they are in the opposite direction. The relationship between maturity and yield depends on the rapidity of regrowth, growth rate of the plant, and the timing of harvest. Biomass accumulation declines as plants begin to flower (Sheaffer et al., 1988). Thus, for plants with similar growth patterns, those with later maturity will produce larger yields than early maturing plants if harvest is delayed. Our three-harvest system, under which plants were clipped after the early maturing crosses reached their maximum biomass accumulation, allowed later maturing SFC to continue increasing yield. The highest yielding SFC, therefore, combine two parental characteristics: later maturity of falcata with faster regrowth of sativa. Interestingly, however, the within species crosses with delayed maturity did not have greater yield, suggesting that only the hybrids *per se* have this genetic ability.

Growth habit had positive correlations with spring regrowth for SSC and FFC (although not significant), while SFC was close to zero (Table 4). No correlation was observed in SFC between autumn regrowth and average yearly height, while in both SSC and FFC the two traits were positively correlated (Table 4). However, autumn regrowth had a high positive correlation with autumn 1998 height for all cross types, which mirrored the overall correlation ($r = 0.78$, $p <$

0.0001, data not shown). These results suggest that in intra-subspecific crosses, plants with increased autumn regrowth were taller, both throughout the year and in the autumn. In contrast, inter-subspecies crosses had a positive relationship between autumn regrowth and autumn height, but regrowth had no relationship with average yearly height, suggesting the two traits act independently in SFC, unlike SSC or FFC. Autumn regrowth likely reflects the amount of autumn dormancy expressed by the plants; those with an early dormancy will produce less regrowth as photosynthate is diverted to acclimation related uses (McKenzie et al., 1988). The dormancy response may be related to plant growth throughout the year, and hence it may be genetically related with average yearly height. The fact that SFC behave differently than intra-subspecies crosses may indicate that they do not have a genetic relationship between these traits, which could be useful for future improvement efforts.

Phenotypic correlations and the cause of yield heterosis

In this report, we have evaluated the relationships among a broad suite of traits associated with alfalfa biomass yield and nutritive value in a set of genetically diverse germplasm. In addition, we independently considered these relationships in three major categories of alfalfa germplasm: within subspecies *falcata*, within subspecies *sativa*, and within crosses of the two subspecies (reflecting subspecies x *varia*). We had previously reported that the inter-subspecific crosses exhibited substantial heterosis for biomass accumulation (Riday and Brummer, 2002a). In this experiment, we began to explore the causes of biomass yield heterosis.

Correlations that radically differ between SSC and FFC provide evidence that independent suites of alleles control the traits of interest, a point strengthened when inter-subspecies crosses show no relationship between the specific traits. These results suggest that complementary gene interactions are occurring as a result of genetically divergent crosses, as described previously (Hallauer and Miranda, 1988; Bingham et al., 1994; Woodfield and Bingham, 1995). Due to the extreme morphological divergence of falcata and sativa (Riday and Brummer, 2002b; Riday et al., 2002a), we hypothesize that different suites of alleles may regulate dormancy (and associated traits such as winter injury and regrowth), maturity, and growth habit (including height, basal vs. apical growth, and stem thickness) in the two subspecies. When creating sativa-falcata hybrids, the collective effect of merging the differing sets of alleles controlling a variety of traits helps explain the emergent property of increased fitness–i.e., improved biomass accumulation–that we have observed in this material (Riday and Brummer, 2002a,b).

This study represents an attempt to dissect the basis of alfalfa biomass heterosis, but further work remains. Biomass accumulation reflects a measurable phenotype that is the end result of a host of underlying interactions among traits, some of which are not easily observable. The difficulty of identifying the underlying causes of heterosis lies in examining relationships among a few traits in isolation while retaining the holistic view of biomass production. The correlations we found in this study need to be verified in other experiments designed to test genetic, rather than phenotypic, correlations. In addition, the relationship of these correlations under different management systems (e.g., different harvest schedules), different environmental conditions, and different plant life histories (e.g., after several years in the field) needs to be evaluated. By assembling evaluations of the individual relationships among morphological and physiological constituents (and eventually among metabolites, proteins, and genes), we may be able to develop a conceptual model of heterosis that can lead us to a more rational means of routinely capturing its value and of increasing the forage yield of alfalfa.

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