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Recovery of exotic alleles in semiexotic maize inbreds derived from crosses between Latin American accessions and a temperate line

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Abstract Genetic diversity of elite maize germplasm in the United States is narrow relative to the species worldwide. Tropical maize represents the most diverse source of germplasm. To incorporate germplasm from tropical maize landraces into the temperate gene pool, 23 Latin American maize accessions were crossed to temperate inbred line Mo44. During inbred line development, selection was practiced in temperate environments, potentially resulting in the loss of substantial proportions of tropical alleles. Genotyping 161 semiexotic inbreds at 51 simple sequence repeat (SSR) loci permitted the classification of their alleles as either Mo44 or tropical and allowed estimation of the proportion of detectable tropical alleles retained in these lines. On average, the percentage of detectable tropical alleles ranged among lines from 15% to 56%, with a mean of 31%. These are conservative, lower-bound estimates of the proportion of tropical germplasm within lines, because it is not known how frequently Mo44 and the tropical maize accession parental populations shared SSR alleles. These results suggest that substantial proportions of exotic germplasm were recovered in the semiexotic lines, despite their selection in temperate environments. The percent of tropical germplasm in semiexotic lines was not correlated to grain yield or moisture of lines testcrossed to a Corn Belt Dent tester, indicating that the incorporation of a substantial percentage of tropical germplasm in an inbred line does not necessarily negatively impact its combining ability. Thus, tropical maize accessions represent a good source of exotic germplasm to broaden the genetic base of temperate maize without hindering agronomic performance.

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Introduction

Despite the abundance of genetic variability within maize, the germplasm base of commercial maize in breeding programs within the United States is relatively narrow. Only one of the approximately 300 recognized races of maize, Corn Belt Dent, is represented in commercial US maize (Goodman 1985). Baker (1984) and Troyer (1999) reported that more than 50% of the germplasm used in contemporary hybrids descended from a single open-pollinated cultivar, Reid Yellow Dent. Lu and Bernardo (2001) reported small but significant reductions of genetic diversity caused by recycling public inbreds in breeding programs. Moreover, Smith et al. (1999) found that pedigree diversity of Pioneer brand inbreds and hybrids tended to decrease over time and were lower in the 1990s than any previous decade. Consequences of the narrow germplasm base of US maize include reduced potential for long-term gains in productivity and increased susceptibility to new pathotypes of disease-causing pests.

Tropical germplasm, the most genetically diverse source of exotic maize, is a possible source of favorable alleles for yield for incorporation into US Corn Belt maize (Uhr and Goodman 1995). Holland and Goodman (1995) reported that some populations developed from crosses between Latin American maize accessions and a temperate inbred had greater yields than the temperate parent in testcrosses evaluated in temperate environments.

However, tropical germplasm is poorly adapted to the temperate environments that represent the target production environments for many breeding programs. Photoperiod sensitivity and other agronomic deficiencies—such as high grain moisture, poor root and stalk strength, and high ear placement—are major problems facing breeders when introducing tropical material to a temperate environment (Goodman 1985). Maladaptation to the temperate envi-

ronment masks potentially beneficial alleles found in the tropical germplasm (Brown 1953; 1988; Crossa and Gardner 1987). Therefore, crosses between tropical germplasm and photoperiod-insensitive, temperate material are often performed in order to overcome the photoperiodicity of tropical germplasm (Brown 1975). For example, Holland and Goodman (1995) crossed 40 superior, tropical Latin American maize accessions to the temperate-adapted inbred line Mo44 and selected progenies for reduced photoperiod-sensitivity while intermating within semiexotic populations. Some semiexotic inbred lines developed by inbreeding from these selected populations had significantly superior combining ability for grain yield with a US Corn Belt Dent tester than their temperate parent, Mo44 (Tarter et al. 2003).

However, breeders face a dilemma when attempting to incorporate unadapted, exotic germplasm by crossing it to adapted, elite germplasm. Alleles from the elite parent that contribute to adaptation are needed to permit the identification of progeny that may possess favorable alleles from the exotic parent. Yet, selection for adaptation to the target environment may result in the substantial loss of exotic alleles, either due to their contribution to maladaptation or to their linkage to such alleles. It is not known to what extent superior semiexotic inbred lines derived from crosses between tropical maize accessions and temperate inbred lines actually possess tropical germplasm. Can high proportions of exotic germplasm be maintained in semiexotic lines with good combining ability? Rubino and Davis (1991) found that natural selection did not have a large impact on isozyme-allele frequencies in a random-mated semiexotic maize population. Gouesnard et al. (1996) also found that a semiexotic population maintained allele frequencies at expected proportions (based on pedigree contribution), following two generations of random mating and one generation of S_1 selection. However, these studies were based on five or fewer isozyme loci and addressed situations of relatively mild selection pressure and inbreeding.

The first objective of this study was to estimate the percentages of alleles derived from tropical accession parents within the semiexotic inbred lines evaluated for testcross performance by Tarter et al. (2003). This was accomplished by surveying 51 simple sequence repeat (SSR) loci distributed throughout the genomes of 161 semiexotic inbred lines developed from crosses between Latin American maize accessions and Mo44 (Holland and Goodman 1995; Tarter et al. 2003) and comparing their allelic composition to their temperate parent, Mo44. The second objective was to determine the relationship between the estimated proportions of exotic germplasm retained in semiexotic inbred lines and mean grain yields and moistures of testcrosses of the lines to a Corn Belt tester evaluated in temperate environments.

Materials and methods

Inbred line development

Noninbred, semiexotic families were developed from photoperiod-insensitive crosses between Mo44 and 23 tropical accessions, as described by Holland and Goodman (1995). Briefly, tropical accessions were crossed to the photoperiod-insensitive, temperate line Mo44. Four generations of selection for photoperiod insensitivity were conducted within each segregating population. Selection for photoperiod insensitivity was accomplished by intermating the earliest maturing plants among families within populations grown in long-day photoperiod nurseries near Raleigh, North Carolina, USA. The resulting photoperiod-insensitive families had adaptive flowering times under long-day photoperiods and were only slightly inbred (F~0.17). Next, two generations of full-sib mating were followed by one generation of self-fertilization; then selected plants were self-fertilized to form inbred lines. The lines were derived from plants in a generation which had an inbreeding coefficient of F=0.74, and the lines have expected homogeneity approximately equivalent to $F_{4:5}$ lines. The plants within the derived lines have an expected inbreeding coefficient of F=0.87. At each generation of inbreeding, pedigree selection was used to choose superior ears from superior lines based on silk–tassel synchrony, early flowering, stalk strength, and leaf blight and ear rot diseases resistance, as described by Tarter et al. (2003). The final set of selected semiexotic lines consisted of 164 lines derived from 35 families, with from 1 to 36 line(s) developed from each family. The lines were testcrossed to the Corn Belt Dent hybrid LH132 × LH51, and the progeny were evaluated in seven North Carolina environments as described by Tarter et al. (2003).

SSR evaluation: agarose system

Seeds from 161 of the 164 semiexotic inbreds lines and Mo44 were available for genotypic analysis. Three lines were not surveyed because of limited seed availability. DNA was extracted from bulk tissue samples of at least ten plants per line, following the procedure described by Riede and Anderson (1996) or Saghai Maroof et al. (1994), with a second cold 95% ethanol extraction plus 3 M sodium acetate wash.

Each semiexotic inbred line and Mo44 were assayed at 51 SSR loci (Table 1). Loci were chosen to survey the genome, with markers spaced approximately every 35 cM apart (Davis et al. 1999). SSR primers were purchased from Research Genetics (Huntsville, Ala., USA), Gibco BRL Life Technologies (Rockville, Md., USA), or Genosys (Beverly, Mass., USA) and diluted to a final concentration of 5 μM. PCR reactions were performed as described by Senior et al. (1998) and separated by electrophoresis on 4% agarose gels prepared using MetaPhor Agarose (FMC Bioproducts, Rockland, Me., USA) or Agarose SFR (Amersco, Solon, Ohio, USA) and 1x TBE (Tris-Boric Acid-EDTA). PCR products from 30 semiexotic lines and two Mo44 samples plus three aliquots of evenly spaced size standard (50-bp ladder, Gibco Life Technologies) were run on each gel. DNAs were electrophoresed for 4–5 h at 95–100 V in 1× TBE buffer. RFLPscan analysis software version 3.2 (Scanalytics, Billerica, Mass., USA) was used for gel image analysis. Images were manually corrected for misalignments; compensations for curved electrophoretic fronts were performed; and the size of each PCR product was estimated by comparison to the three lanes of the 50-bp ladder.

SSR evaluation: acrylamide system

PCR products amplified by fluorescent primers obtained from PE Biosytems or MWG Biotech (High Point, N.C., USA) (Table 1) were produced using the system described by Senior et al. (1998), except the volumes of dNTPs, which increased to 150 μM and

Table 1 Chromosome and genomic bin positions, estimated proportions and their standard errors (SE) of semiexotic lines with tropical alleles, and genetically heterogeneous semiexotic lines at each of 51 simple sequence repeat (SSR) loci

Locus	Bin[a]	Proportion of semiexotic lines with tropical alleles (%), (SE)	Proportion of heterogeneous semiexotic lines (%), (SE)
phi056	1.01	40 (4)	17 (1.29)
bnlg1429	1.02	59 (4)	8 (0.60)
bnlg1811	1.04	38 (4)	12 (0.90)
phi055	1.10	12 (3)	46 (3.58)
bnlg1017	2.02	53 (4)	9 (0.66)
phi083	2.04	26 (3)	50 (3.91)
bnlg108	2.04	29 (4)	3 (0.16)
nc003	2.06	21 (3)	4 (0.26)
bnlg1887[b]	2.06	25 (4)	0 (0.00)
phi090	2.08	34 (4)	12 (0.89)
bnlg1520	2.09	26 (3)	9 (0.70)
phi036	3.03	33 (4)	9 (0.65)
bnlg1647	3.04	21 (3)	4 (0.25)
phi053	3.05	11 (2)	4 (0.30)
phi047	3.09	7 (2)	1 (0.04)
nc135	4.01	17 (3)	1 (0.04)
nc004	4.03	19 (3)	7 (0.55)
umc1088[b]	4.04	48 (4)	5 (0.42)
bnlg1265	4.05	42 (4)	4 (0.30)
bnlg252	4.06	8 (2)	0 (0.00)
bnlg1444	4.08	50 (4)	5 (0.36)
nc007	5.01	21 (3)	0 (0.00)
umc1019	5.05	30 (4)	8 (0.57)
phi128	5.07	41 (4)	11 (0.80)
phi077	6.01	26 (3)	3 (0.15)
bnlg1371	6.02	11 (2)	4 (0.40)
umc1006	6.03	6 (2)	0 (0.00)
umc1014	6.04	68 (4)	13 (1.00)
nc013	6.05	51 (4)	0 (0.00)
phi070	6.07	9 (2)	1 (0.04)
bnlg1792	7.02	32 (4)	6 (0.45)
bnlg339[b]	7.03	30 (4)	4 (0.27)
bnlg469	7.05	11 (2)	0 (0.00)
phi116	7.06	40 (4)	4 (0.30)
phi119	8.02	44 (4)	2 (0.10)
bnlg669[b]	8.03	13 (3)	13 (0.99)
bnlg1651	8.05	29 (4)	6 (0.40)
bnlg1031	8.06	35 (4)	14 (1.10)
bnlg1828	8.07	11 (2)	0 (0.00)
bnlg1056	8.08	24 (3)	26 (2.02)
phi080	8.09	26 (3)	0 (0.00)
phi068	9.01	9 (2)	1 (0.04)
phi033	9.02	29 (4)	5 (0.35)
phi065	9.03	38 (4)	6 (0.40)
bnlg1884[b]	9.05	39 (4)	5 (0.35)
bnlg1525	9.07	46 (4)	7 (0.50)
bnlg128[b]	9.07	38 (4)	31 (2.49)
phi059	10.02	31 (4)	6 (0.40)
phi084	10.04	9 (2)	0 (0.00)
bnlg1185	10.05	96 (2)	16 (1.23)
umc1084	10.07	61 (4)	25 (1.92)
Mean		31 (3)	8

[a]Bin location based on recombination frequency. Bin positions are defined by core RFLP markers (Davis et al. 1999; Maize Database, October 10, 2001) and span approximately 20 cM
[b]SSR loci also surveyed using the acrylamide gel system

nonacetylated BSA increased to 15 µg/µl. DNA samples were electrophoresed at 3 kV on a 6% acrylamide gel for 2.5 h using an automated DNA sequencer (ABI Model 377, Applied Biosystems, Foster City, Calif., USA) and analyzed using GeneScan, version 3.1 and Genotyper, version 2.5 software (Applied Biosystems). DNA fragments were sized automatically by comparison to an in-lane standard (GeneScan 350 Tamra; Applied Biosystems) for a molecular-weight estimation using the local Southern algorithm (Elder and Southern 1987).

Statistical analysis

Two samples of Mo44 were loaded in every gel (in the first and last sample lanes), permitting estimation of the error variance associated with band-size estimation within gels. A preliminary analysis of Mo44 band sizes within gel and within marker—using the agarose system—suggested that error variance of SSR product-size estimates was heterogeneous among markers and that the variance increased as band size increased. Therefore, the data were partitioned into three marker classes according to product size within which error variance was homogenous. The small-size class contained loci with Mo44 products less than 120 bp. The medium-size class was composed of products between 120 bp and 180 bp, and the large class contained primers with products greater than 180 bp. An estimate of the variance of the Mo44 band size within a gel and within marker was pooled across gels and markers within each sizing class. For the acrylamide system, a single estimate of error variance of band-size estimate within gels was pooled across gels and markers.

Within a locus, the α=0.05 least significant difference (LSD) was used to compare sizes of products between semiexotic inbred lines and the mean Mo44 band size within a gel. Semiexotic lines with an SSR product that was significantly different in size from the Mo44 product were considered to have a tropical allele at that locus. If the PCR product size did not differ significantly, it was classified as having a Mo44 allele. In the case of some semiexotic lines that had more than one band at a SSR locus, one band was identified as a Mo44 allele, and the remaining bands were considered tropical alleles. Heterogeneous loci in semiexotic lines in which one allele matched Mo44 were considered to have Mo44 alleles for all calculations. PCR artifacts were observed in some of the semiexotic lines at ten loci. For marker loci *phi056* (bin 1.01), *phi083* (bin 2.04), *bnlg1371* (bin 6.02), *umc1014* (bin 6.04), *phi116* (bin 7.06), *bnlg1651* (bin 8.05), *bnlg1031* (bin 8.06), *bnlg1056* (bin 8.08), *phi065* (bin 9.03), and *phi059* (bin 10.02), additional bands outside of the region described by Senior et al. (1998) were observed and ignored for all calculations.

Mean grain yield and moisture of the testcross of each line to LH132 × LH51 averaged across seven North Carolina environments (Tarter et al. 2003) were regressed on the percentage of tropical

germplasm within each line using Proc REG in SAS, version 8.0 (SAS Institute 2000). Proc CORR in SAS was used to estimate the correlation coefficient between percent tropical germplasm at each SSR locus averaged across semiexotic lines and percent of the semiexotic lines heterogeneous at the locus.

Results

Precision of SSR band-size estimates

To declare that a semiexotic line possessed an allele different from Mo44 at an SSR locus, we estimated the error variance associated with fragment-size estimation within gels, based on replicate samples of Mo44 included within 75% of the gels. The error variance differed among SSRs of different-sized classes. Therefore, we calculated LSDs separately for three different marker-size classes. In addition, 77 (25%) gels were missing one of the duplicate Mo44 bands due to PCR failure; therefore, we computed appropriate LSDs for these gels. Finally, 18 (6%) gels were missing both Mo44 lanes, and for these, the semiexotic line SSR products were compared to the mean Mo44 product-size estimate for the same locus using data from the other gels. In these cases, differences between semiexotic lines and Mo44 mean product sizes were compared with an appropriate LSD that incorporated the error variance among, as well as within, gels.

The LSDs for band-size comparisons in agarose gels ranged from 5 bp to 7 bp for the small- and medium-size classes and from 7 bp to 10 bp for the large-size class, depending on the number of Mo44 replicate samples contained in each gel.

Genetic heterogeneity within semiexotic lines

The inbreeding process did not eliminate within-line heterogeneity (loci segregating within the line because the plant that gave rise to the line was heterozygous at those loci). The number of heterogeneous semiexotic lines estimated ranged among loci from 0 to 80 (50%), with a mean of 13 (8%) heterogeneous loci (Table 1). For 30 of the 51 tested loci (59%), fewer than ten lines were heterogeneous at the marker locus (Table 1). Nine loci (18%) did not exhibit any residual heterogeneity. At the other extreme, five loci were heterogeneous in 30–80 of the semiexotic lines (19–50%). Regions of low heterogeneity included all of chromosome 3, with between 1% and 9% heterogeneous lines across all loci on that chromosome. Chromosomes 4 and 7 also displayed low heterogeneity, with 0–7% of the semiexotic lines being heterogeneous across loci on each chromosome. Finally, the largest percentage of heterogeneous semiexotic lines was at locus *phi083* (bin 2.04), at which 80 of the 161 (50%) semiexotic lines were heterogeneous.

Estimates of the percent heterogeneity averaged across SSR loci within each semiexotic line varied from 0% to 24% with a mean of 8% (data not shown). One hundred and twenty-eight (80%) of the semiexotic lines were heterogeneous at <10% of tested loci. Thirty-one (19%) of the lines were heterogeneous at between 10% and 20% of the loci.

Proportion of tropical germplasm retained in semiexotic lines

Estimates of the lower bound of percent of tropical germplasm averaged across loci ranged from 15% to 56% among the semiexotic lines, with a mean of 31% (Fig. 1). Most (147 or 91%) of the semiexotic lines had at least 20% tropical germplasm (Table 2; Fig. 1). Seven lines had 10–20% tropical germplasm, and seven other lines had at least 40%. To eliminate the differential weighting of accessions caused by variation in the number of inbred lines that were derived from them, we computed an unweighted average across accessions and obtained the same result—31% of the alleles were derived from the tropical parents. The accession with the lowest proportion of exotic germplasm was Cubano Tusón ECU 542, represented by one line with 24% tropical alleles. The accession with the highest proportion of exotic germplasm was Tepecintle GUA 597, represented by two lines with a

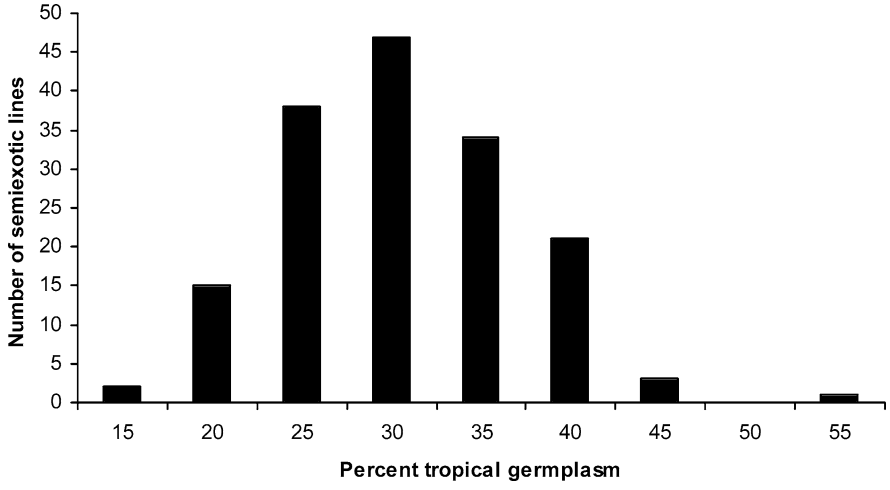

Fig. 1 Histogram of percentage of tropical germplasm identified in 161 semiexotic lines averaged across 51 simple sequence repeat (SSR) loci. Mean is 31%

mean of 52% tropical alleles. Semiexotic lines produced from the race Cubano Amarillo Duro retained large percentages of detectable tropical alleles among the semiexotic lines (Table 2). Fifty-three of the 57 (93%) lines derived from the six Ecuadorian accessions of Cubano Amarillo Duro had at least 20% tropical germplasm. Lines with germplasm from the race Tusón also retained a large percentage of detectable tropical alleles (Table 2). Twenty-five semiexotic lines derived from accessions of Tusón possessed at least 20% tropical germplasm while 16 lines possessed at least 30%.

The proportion of detectable tropical alleles averaged across semiexotic lines ranged among SSR loci from 6% to 96%, again with a mean of 31% (Table 1; Fig. 2). At 14 of the 51 SSR loci sampled (27%), fewer than 20% of the lines possessed detectable tropical alleles. At only 12 loci (24%) did more than 40% of the lines have detectable tropical alleles. *Umc1006* (bin 6.03) and *phi047* (bin 3.09) were the loci with the smallest percentage of detectable tropical alleles across semiexotic inbreds (6% and 7%, respectively). In contrast, *umc1014* (bin 6.04) and *bnlg1185* (bin 10.05) had the largest percentage of detectable tropical alleles across inbreds (68% and 96%, respectively, Table 1).

The correlation between mean proportion of detectable tropical alleles across semiexotic lines and mean percent heterogeneity was not significant (P=0.99). Thus, the proportion of detectable tropical alleles was not related to the amount of residual heterogeneity in the semiexotic lines.

Relationship between proportion of tropical germplasm retained and agronomic performance of testcrosses of semiexotic lines

There was no apparent relationship between percent tropical germplasm and either grain yield or moisture. Coefficients of regression of testcross grain yield and moisture on the percentage of tropical germplasm retained within lines were not significant (P=0.09 and P=0.60, respectively). The eight highest-yielding semiexotic lines had 18–35% tropical germplasm, while the eight lowest-yielding semiexotic lines had 20–32% tropical germplasm (Table 3). Among the eight highest-yielding semiexotic lines, the lines with the smallest and largest proportion of detectable tropical alleles had equal or very similar grain moisture and days to silk.

Table 2 Frequency distributions of the proportions of tropical germplasm estimated using the agarose system retained at 51 SSR loci in semiexotic lines derived from 23 Latin American maize accessions

Race	Accession	Percentage of tropical germplasm				
		10–20	20–30	30–40	40–60	Total
		Number of lines				
Costeno	ATL 314			2		2
Costeno	ATL 329			5		5
Tusón	BAI III	1	3	12	1	17
Tusón	CUB 57	2	6	3		11
Cubano Dentado	BOV 585		1	1		2
Cuban Flint	CUB 63	1		2		3
Chandelle	CUB 68			2		2
Chandelle	VEN 352		2	3		5
Cubano Cateto	ECU 339		2	2		4
Cubano Tuson	ECU 542		1			1
Cubano Amarillo Duro	ECU 326		3			3
Cubano Amarillo Duro	ECU 653	1	1			2
Cubano Amarillo Duro	ECU 770	1	5	6		12
Cubano Amarillo Duro	ECU 904		21	17	2	40
Tuxpeño	ECU 942		1	1		2
Cateto Nortista	GIN I			1		1
Tepecintle	GUA 597				2	2
Perla	LIM 13		10	8	2	20
Puya	MAG 322		1		1	2
Puya	SAN 349	1	9	3		13
Tuxpeño	VEN 598		2	1	1	4
Tuxpeño	VEN 767		3			3
Canilla	VEN 981		2	3		5
Total		7	73	72	9	161

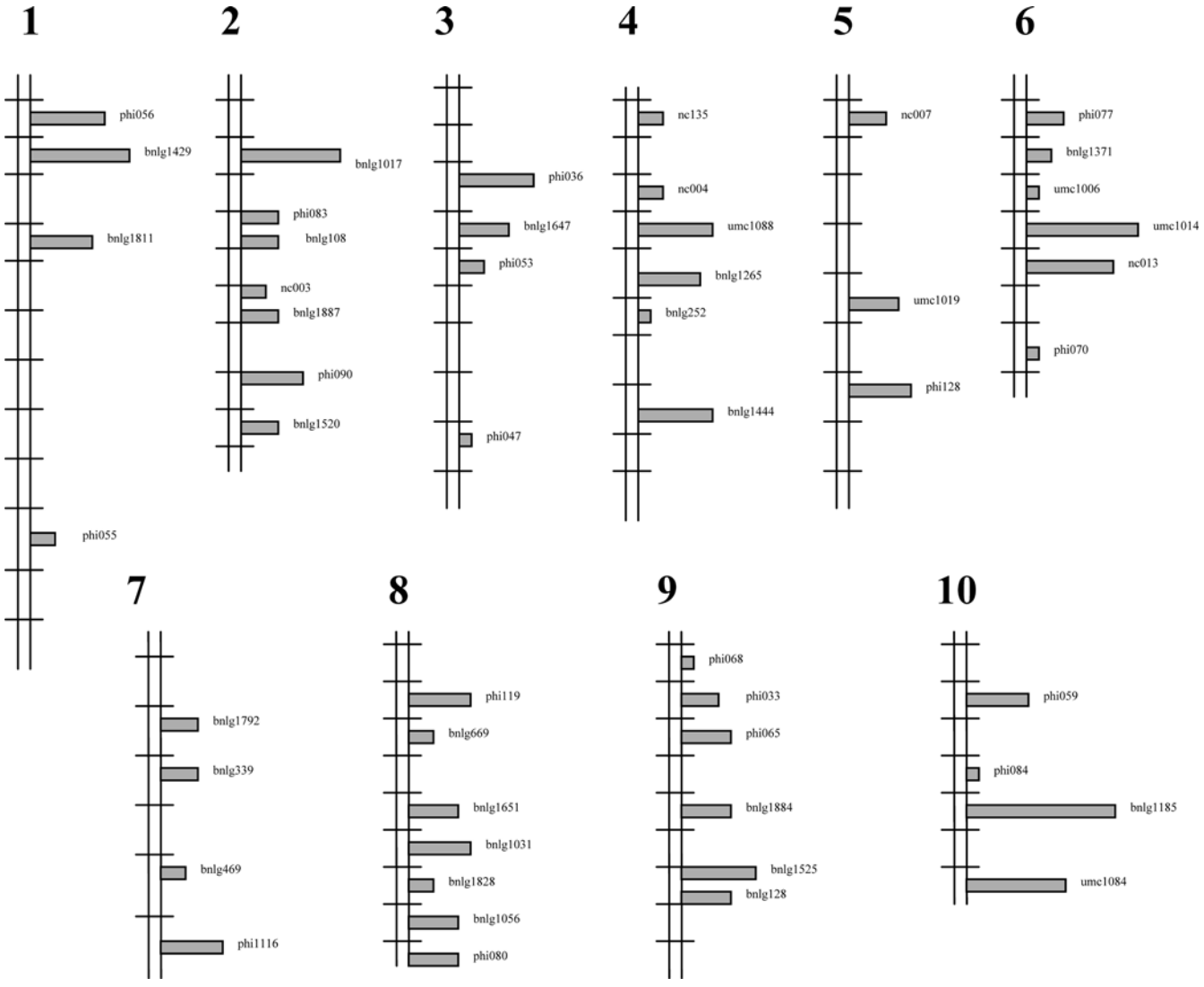

Fig. 2 Percent of tropical germplasm alleles at 51 SSR loci averaged over 161 semiexotic lines. *Crosshatch marks* represent bin sizes of approximately 20 cM (Davis et al. 1999)

Discussion

Precision of SSR fragment-size estimation

The precision of SSR fragment-size estimations reported for this study is substantially lower than that for other recent studies in maize. Romero-Severson et al. (2001) estimated a standard error for SSR fragment sizes of less than 1 bp. Similarly, Heckenberger et al. (2002) suggested that technical error rates of maize SSR fragment estimated are very low. In contrast to our study, both these studies used acrylamide gels—which have a higher level of resolution than agarose gels—and also used automated scoring systems that use in-lane size standards that correct for variation among lanes within a gel.

To determine if the SSR assay method impacted our results, we surveyed a subset of the six SSR loci listed in Table 1 using an acrylamide gel and automated scoring system. The LSDs for band-size comparisons in acrylamide gels ranged between 3 bp and 4 bp, still larger than those of previously reported error rates, but substantially lower than the error rates of the agarose gel system. Averaged across these six loci, we estimated 32% tropical germplasm in the semiexotic lines using the agarose system (almost identical to the result using all 51 loci), compared to 41% using the acrylamide system (data not shown). This suggests that our agarose gel-scoring method substantially underestimated the proportion of tropical germplasm in the semiexotic lines. Magnifying the effect of using agarose gels instead of acrylamide gels, the LSD values used to declare an allele exotic were likely to be overly stringent because the two replicate Mo44 lanes were farther apart on any gel than any other pair of lanes. Since the error rate may increase as the physical distance between lanes increases, we estimated larger error variances than were probably correct for most comparisons between band sizes. This would result in a higher type II error rate (i.e., alleles that were truly different than

Table 3 Mean proportion of exotic germplasm across 51 loci and mean grain yields, grain moistures, and silking dates averaged across seven North Carolina environments (as a proportion of the mean values of the Mo44 testcross) of the eight highest-ranking and eight lowest-ranking semiexotic lines for grain yield in testcross to LH132 × LH51

Race	Accession	Family	Inbred line ID	Grain yield (Mg ha^{-1})	Grain moisture (g kg^{-1})	Days to silk (days)	Proportion tropical germplasm (%)
Highest-yielding semiexotic line testcrosses							
Tusón	CUB 57	c	2	122.6	101.8	104.3	24
Tusón	CUB 57	c	3	121.0	104.3	100.4	18
Tusón	BAI III	b	6	119.4	103.1	101.5	25
Tusón	CUB 57	c	1	119.4	105.5	102.4	22
Tusón	BAI III	b	3	117.7	105.5	101.1	29
Tusón	CUB 57	c	4	117.7	103.7	103.3	24
Tusón	CUB 57	c	5	116.1	104.3	102.0	35
Cubano Amarillo Duro	ECU 770	b	2	113.8	99.4	101.5	28
Lowest-yielding semiexotic line testcrosses							
Perla	LIM 13	d	10	93.5	108.0	99.2	29
Cuban Flint	CUB 63	d	3	92.3	99.4	96.9	37
Cuban Flint	CUB 63	d	4	92.3	98.2	99.1	20
Cubano Cateto	ECU 339	c	2	92.3	100.6	98.0	25
Perla	LIM 13	d	8	91.9	110.4	99.2	27
Perla	LIM 13	d	9	91.9	108.6	102.0	32
Costeño	ATL 314	d	1	90.8	100.6	99.7	31
Cubano Cateto	ECU 339	c	1	90.8	96.5	97.8	32
LSD[a]				0.5	6	1.0	20

[a]*LSD* Least significant difference is appropriate to compare means of two semiexotic line testcrosses at α=0.05

Mo44 were declared equivalent to Mo44 because they did not differ from Mo44 by more than the threshold LSD). Therefore, we suggest that, because of technical limitations, our estimate of an average of 31% tropical germplasm remaining in the semiexotic lines is conservative.

Residual heterogeneity estimates

Additional evidence that we underestimated the proportion of tropical germplasm in the semiexotic lines is that the amount of heterogeneity within loci was lower than expected in these lines. As the semiexotic lines are approximately equal in homogeneity to $F_{4:5}$ lines, the residual heterogeneity expected is approximately 12.5% at those loci which were polymorphic in the original tropical-by-temperate populations. However, if Mo44 and the tropical accession parent from which a semiexotic line was derived possessed similarly sized SSR alleles at a locus, then the locus would be monomorphic within that particular breeding population and would be noninformative about the true level of heterogeneity within lines. Including such locus/population combinations within the survey would tend to decrease the estimated proportion of heterogeneous loci within lines. Since we do not know the genotypes of the plants from the accession that were parents of the semiexotic lines, it was not possible to identify such loci and exclude them from the survey. However, we can retrospectively determine that the level of 8% heterogeneity within semiexotic lines was significantly (P<0.001) lower than the expected values of 12.5%, indicating that about 36% of the locus-breeding population combinations were noninformative. These noninformative locus-population combinations also resulted in an underestimation of the proportion of exotic germplasm maintained in the semiexotic lines. Correcting for this level of noninformative loci by dividing by the estimated proportion of tropical germplasm by the proportion of informative loci (0.31/0.64), we suggest that the true proportion of tropical germplasm retained in the semiexotic lines was approximately 48%.

Chromosomal linkage

As expected due to linkage, in most cases, SSR loci within the same bin had similar estimated percentages of detectable tropical alleles across semiexotic inbreds (Table 1; Fig. 2). At loci *phi083* and *bnlg108*—both located on chromosome 2 in bin 4—the percentages of inbreds with detectable tropical alleles were estimated at 26% and 29%, respectively. At loci *nc003* and *bnlg1887*—located in bin 6 of chromosome 2—the percentage of inbreds with detectable tropical alleles were estimated at 21% and 25%, respectively. Finally, the percentage of inbreds with detectable tropical alleles at *bnlg1525* and *bnlg128*—located on chromosome 9 in bin 7—were estimated at 46% and 38%, respectively.

Exceptions to the generally positive correlation of the amount of tropical germplasm retained at linked loci were observed on chromosomes 4 and 6. Two loci, *umc1088* and *bnlg1265*, with large percentages of detectable tropical alleles (48% and 42%) are located on chromosome 4 in bins 4 and 5, respectively. Flanking both of the loci are SSR loci *nc004* (bin 4.03) and *bnlg252* (bin 4.06), which had detectable tropical alleles in only 19% and 8% of the semiexotic lines, respectively. On chromosome 6, in bins 4 and 5, *umc1014* and *nc013* had detectable tropical alleles in 68% and 51% of the semiexotic lines, respectively. However, these loci were surrounded on either side by *umc1006* and *phi070* in bins 3 and 7, containing detectable tropical alleles in only 6% and 9% of the semiexotic lines.

The semiexotic lines were developed by intermating with limited inbreeding for four generations, followed by two generations of sib-mating before self-fertilization was initiated. Relative to typical maize inbred line development methods, this slower approach to homozygosity provided more chances for recombinations to occur between chromosome blocks containing temperate and tropical germplasm. This scheme likely increases opportunities to recover lines with recombinant chromosomal segments containing favorable combinations of alleles from both tropical and Mo44 parents. The high frequency of regions on chromosomes 4 and 6 that appear to represent recombinant linkage blocks could be a result of linkages between favorable Mo44 alleles and favorable alleles from most tropical accessions in these regions or favorable epistatic effects between Mo44 and detectable tropical alleles in neighboring chromosomal segments.

Coincidence of candidate gene loci and chromosomal regions

Chromosomal regions at which few lines possessed tropical alleles may represent linkage blocks containing genes that were targets of selection during line development. Photoperiod-response genes and flowering-time genes would be the most obvious targets of selection, since selection pressure for earlier flowering in a long-day environment was maintained through all phases of line development. Some loci at which smaller percentages of lines had tropical alleles were found on chromosomal regions that may possess photoperiod-response or flowering-time QTL or candidate genes (Moutiq et al. 2002). *Phi055*—at which only 12% of lines carried tropical alleles—is located on chromosome 1, bin 10. In the same bin are candidate genes *d8* (dwarf plant 8), which were reported to affect flowering time in both long- and short-day environments (Thornsberry et al. 2001), and *phyA1*, a phytochrome locus that helps regulate developmental responses to light (Gyula et al. 2003). Nearby is *id1* (indeterminate growth 1) in bin 1.08, which requires short days for flowering. *Phi047*—on chromosome 3—and *bnlg252*—on chromosome 4, at which 7–8% of lines had tropical alleles—are located in regions that contained epistatically interacting QTL for photoperiod response (Moutiq et al. 2002). Additionally, Imaizumi et al. (2003) recently reported that the flavin-binding, kelch repeat, F-box (FKF1) protein in *Arabidopsis* plays a key role in photoperiod-dependent regulation of flowering time. A 109-amino acid portion of the FKF1 protein is 46% identical to the nonphototropic hypocotyl 1 maize protein. An SSR within the locus coding for this protein, *nph1*, maps to the same chromosomal bin as *phi047*, 3.09. The *phi047* locus had the lowest percentage of tropical germplasm averaged across lines (7%), suggesting strong selection pressure against tropical alleles in this region, perhaps due to their effect on photoperiodicity at the *nph1* candidate gene.

In contrast, other regions that have been identified as containing candidate flowering-time genes did not have unusually low proportions of lines with tropical alleles. For example, bin 10.08 demonstrates synteny with *Sorghum* linkage group D, which contains the *Ma1* flowering-time gene (Lin et al. 1995; Moutiq et al. 2002; Quinby and Karper 1945), but the nearest locus tested in this study, *umc1084* in bin 10.07, had tropical alleles in 61% of lines. Similarly, Koester et al. (1993) identified a putative photoperiod-response QTL near *umc44* in bin 8.05, but in our study, the SSR sampled in this bin, *bnlg1651*, had tropical alleles in 29% of lines. The moderate-to-high recovery of tropical alleles in these regions suggests that these candidate genes are not generally segregating for large effects on flowering time in crosses between Mo44 and tropical accessions.

Use of tropical germplasm to enhance agronomic performance

Tarter et al. (2003) reported that some of the semiexotic lines used in this study (including all eight of the highest-yielding lines listed in Table 3) produced testcross hybrids with significantly greater yields than testcrosses of their temperate parent, Mo44. This was interpreted as evidence that the lines contained alleles from the tropical accessions that contributed to increased hybrid yield. The lack of a significant correlation between overall percent tropical germplasm and grain yield or moisture reported in this study indicates that it is possible to incorporate substantial proportions of tropical germplasm into the temperate maize genetic base without hindering agronomic performance. Together, the results of these two studies suggest that semiexotic lines with both substantial proportions of exotic germplasm and superior combining ability can be recovered from crosses with tropical landrace accessions. These results also support Uhr and Goodman (1995), who reported that tropical-derived inbreds produced testcross hybrids with Corn Belt Dent testers that were competitive with commercial hybrids and provided a wider genetic background than commercial temperate maize. Tropical maize accessions harbor alleles that can simultaneously improve productivity and enhance the genetic base of commercial US hybrids.

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