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Compositional Assessments of Key Maize Populations: B73 Hybrids of the Nested Association Mapping Founder Lines and Diverse Landrace Inbred Lines

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Supporting Information

ABSTRACT: The present study provides an assessment of the compositional diversity in maize B73 hybrids derived both from the Nested Association Mapping (NAM) founder lines and from a diverse collection of landrace accessions from North and South America. The NAM founders represent a key population of publicly available lines that are used extensively in the maize community to investigate the genetic basis of complex traits. Landraces are also of interest to the maize community as they offer the potential to discover new alleles that could be incorporated into modern maize lines. The compositional analysis of B73 hybrids from the 25 NAM founders and 24 inbred lines derived from landraces included measurements of proximates (protein, fat, ash, and starch), fibers, minerals, amino acids, fatty acids, tocopherols (α-, γ-, and δ-), β-carotene, phytic acid, and raffinose. Grain was harvested from a replicated trial in New York, USA. For each data set (NAM and landrace) canonical discriminant analysis allowed separation of distinct breeding groups (tropical, temperate, flint, mixed/intermediate) within each data set. Overall, results highlighted extensive variation in all composition components assessed for both sets of hybrids. The variation observed for some components within the landraces may therefore be of value for increasing their levels in modern maize lines. The study described here provided significant information on contributions of conventional breeding to crop compositional variation, as well as valuable information on key genetic resources for the maize community in the development of new improved lines.

KEYWORDS: *maize, landraces, Nested Association Mapping (NAM) founder lines, composition analysis*

INTRODUCTION

Maize (*Zea mays* L.) is a key agricultural commodity and a major source of animal food and feed as well as raw material for industrial processes worldwide.[1] Breeding programs committed to improving agronomic, compositional, and nutritional qualities extend globally and include renowned organizations such as the International Maize and Wheat Improvement Center (CIMMYT) headquartered in Mexico. In addition to its commercial significance, maize is also well-established in the scientific community as a model plant for the study of fundamental biological phenomena such as genome evolution and hybrid vigor.[2]

Maize was domesticated from teosinte (*Zea mays* ssp. *parviglumis*) approximately 9000 years in southern Mexico. This domestication and subsequent spread of maize across the Americas resulted in open-pollinated, heterogeneous populations adapted to specific environments called landraces. Although defined by distinct genetic and morphological characteristics, there is often more diversity within a landrace than between landraces.[3] Modern inbred lines developed from landraces through breeding and selection are nearly homozygous; that is, they breed true as additional seed is made by crossing the line with itself (i.e., self-pollinating). Maize hybrids are developed by crossing two distinct inbred lines. Hybridization was initiated in the late 1800s with commercialization of hybrids in the early 1900s.[4]

Today, *Zea* germplasm is defined in terms of teosinte, landraces, and inbred lines. The availability of these distinct germplasm pools has intriguing implications for breeding and the development of new maize hybrids. Although maize inbred lines contain an extraordinary amount of genetic diversity when assessed across all lines, subsets of genes are invariant or have reduced variation due to directional selection and bottlenecks associated with domestication and/or plant breeding.[5] Variation for these genes could be reintroduced into modern maize from landraces and teosinte to support further crop improvement.[6]

Diverse collections of genetically distinct maize inbred lines are now preserved in carefully maintained germplasm banks.[7] The USDA-ARS North Central Regional Plant Introduction Station (NCRPIS), for example, has conserved lines representing almost a century of breeding. The landrace accessions for this study were selected from such a collection representing the geographical diversity of maize adaptation in North and South America and were self-pollinated to generate landrace inbred lines,[8,9] In addition to naturally occurring collections, novel populations are being generated to understand the genetic

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complexity of maize. One extensively used population is the Nested Association Mapping (NAM) population. The NAM population[10] was generated by crossing 25 NAM founder lines to B73, an inbred line that has contributed extensively to the pedigree of many important commercial lines[11] and which serves as the source of the high-quality reference genome for maize.[12] The 25 NAM founder lines were chosen solely based on genetic diversity from a collection of 301 maize inbred lines adapted to various climatic regions including tropical, subtropical and temperate regions.[13]

Maize landraces are an extremely heterogeneous germplasm group, in terms of genetic and phenotypic diversity both within a landrace and between landraces.[3] Because any given landrace plant is unique, being heterozygous at a large percentage of loci across the genome, it is very difficult to assess the merit of specific landraces or landraces collections. In addition, landraces often display undesirable traits such as photoperiod sensitivity and unadaptedness to target environments. Thus, genetically stable genetic resources are needed to systematically explore diversity within landraces, just as the NAM population was used to initiate exploration of diversity within maize inbreds. The Seeds of Discovery (SeeD) Project (http://seedsofdiscovery.org) is a large-scale project to explore landrace diversity by sampling a single plant per landrace. Unfortunately, the single plant used for the SeeD project does not allow for follow-up genetic studies. However, a small set of inbred lines was derived by self-pollinating directly out of a set of landraces;[8] thus, these landrace inbred lines are true breeding and can be used for innumerable follow-up studies.

The purpose of the current study was to provide a compositional survey of maize hybrids derived from landraces adapted to diverse geographies and the genetically diverse NAM founder lines. An earlier comparative assessment of teosinte, landrace, and inbred lines revealed key differences in kernel proximates (protein, fat, ash, and starch),[14] but to our knowledge compositional surveys have not extended to diverse hybrids or to a diverse range of kernel components. The NAM population has been utilized to reveal key genetic loci associated with kernel compositional features such as starch, protein, and oil[15] and carotenoids.[16] There has been, however, no systematic compositional evaluation of the NAM founders. Such information could prove beneficial as there are genetic loci known to affect kernel composition such as crtRB1 that influences β-carotene concentration[16] or DGAT 1-2 (acyl-CoA:diacylglycerol acyltransferase) that affects oil content and fatty acid profiles.[17] Similarly, there have been no systematic studies on compositional variation in landrace populations of maize. Understanding the compositional diversity of these lines adapted to local environments could prove significant in improving the nutrient profile of new maize hybrids. Information on compositional diversity of these genetically diverse population is also of interest to researchers involved in compositional evaluation of new crops including genetically modified (GM) crops.[18]

MATERIALS AND METHODS

Biological Materials. The germplasm used in this study was selected to represent a broad diversity within maize landraces and inbred lines. Hybrids were used to decrease the range of maturity and thus decrease environmental effects on the composition data. For example, the inbreds flower over a period of 40−50 days in Missouri, but the B73 hybrids all flower within 22 days (S.F.-G., unpublished data).

The landrace inbred lines were derived from open-pollinated landrace accessions as described in Chia et al.[8] and Hufford et al.[9] The following landrace inbred lines were used in the study: MR01 (Araguito), MR02 (Assiniboine), MR03 (Bolita), MR04 (Canilla), MR05 (Cateto), MR06 (Chapalote), MR07 (Comiteco), MR08 (Costeno), MR09 (Cravo Riogranense), MR10 (Crystalino Norteno), MR11 (Cuban Flint), MR12 (Havasupai), MR13 (Hickory King), MR14 (Longfellow Flint), MR15 (Palomero de Jalisco), MR16 (Pepetilla), MR18 (Reventador), MR19 (Santo Domingo), MR20 (Shoe Peg), MR21 (Tabloncillo), MR22 (Tuxpeno), MR23 (Zapalote Chico), MR25 (Poropo), and MR26 (Pollo). The geographical origins of landrace inbreds are described in Hufford et al.[9] and are indicated in Supplementary Figure 7. On the basis of geographical origin and historical data[19] these inbreds have been classified as tropical/subtropical (Pepetilla, Cateto, Tuxpeno, Araguito, Tabloncillo, Comiteco, Canilla, Poropo, Zapalote Chico, Reventador, Pollo, Cravo Riogranense, Crystalino Norteno, Costeno, Bolita, and Cuban Flint), temperate (Shoe Peg and Hickory King), northern flint (Santo Domingo, Longfellow Flint, Assiniboine, and Havasupai), and intermediate (Palomero de Jalisco and Chapalote). The intermediate group refers to a geographical origin that is less clear, with membership in multiple groups (e.g., Palomero de Jalisco is a flint from a subtropical region).

Modern inbred lines used in the study are the parental lines of the NAM population:[10] B97, CML103, CML228, CML247, CML277, CML322, CML333, CML52, CML69, Hp301, IL14H, Ki11, Ki3, Ky21, M162W, M37W, Mo18W, MS71, NC350, NC358, Oh43, Oh7B, P39, Tx303, and Tzi8. The geographical origin of these inbreds is indicated in Supplementary Figure 7. The inbreds were classified by population structure using genetic marker data as described in Flint-Garcia et al.[13] as tropical/subtropical (NC350, CML103, CML333, Tzi8, Ki11, Ki3, CML69, NC358, CML228, CML247, CML52, CML322, and CML277), nonstiff stalk temperate (MS71, Oh43, B97, Ky21, M162W, and Oh7B), northern flint (P39, Il14H, and HP301), and mixed (Mo18W, M37W, and Tx303). Inbreds classified as mixed group have membership probabilities of <80% in any one of the groups, indicating admixture (shared breeding history) among the tropical and temperate groups. The common parent, B73, is a temperate stiff-stalk inbred and is therefore suitable as a tester for making hybrid seed. In addition, B73 is the reference genome line allowing for downstream genomic and bioinformatic analyses in these materials. Inbred seed material used in the study can be procured from the North Central Regional Plant Introduction Station (NCRPIS).

Production of Hybrid Seed. Hybrid seed of B73 crossed with the landrace inbred lines and the NAM founders was produced over four different seasons: 24 and two entries were produced, respectively, in Columbia, MO and Puerto Rico, USA, in 2008; 18 entries were produced in Puerto Rico in 2009; and six entries were produced in 2010 in Columbia, MO, USA. All hybrids were produced by controlled hand pollination, using B73 as the female for each hybrid.

Field Design. Materials for composition analyses were generated in 2012 near Aurora, NY, USA. The 25 B73 × NAM founder hybrids and 24 B73 × landrace hybrids were planted in a randomized complete block design in 3 m × 0.9 m rows in three replications. Three to five plants were self-pollinated for each row, and selfed ears were hand harvested and dried to 12−13% moisture. Dried ears from each row were bulk-shelled, and grain was stored at room temperature before shipping to Monsanto Co. in St. Louis, MO, USA. Grain samples were homogenized by grinding on dry ice to a fine powder and stored frozen at approximately −20 °C until compositional analysis.

Phenotypic Data. Plant, ear, and kernel traits were evaluated as follows. Anthesis dates were collected on a row basis, when half the plants in the row were shedding pollen, and subsequently converted to days to anthesis by subtracting the planting date from the anthesis date. Plant and ear heights were measured for three plants per plot after flowering from the ground to the collar of the flag leaf and the node of the top ear, respectively. Three self-pollinated ears were harvested per row and used to study the following ear and kernel traits: the number of kernel rows around the ear; the weight of the ear,

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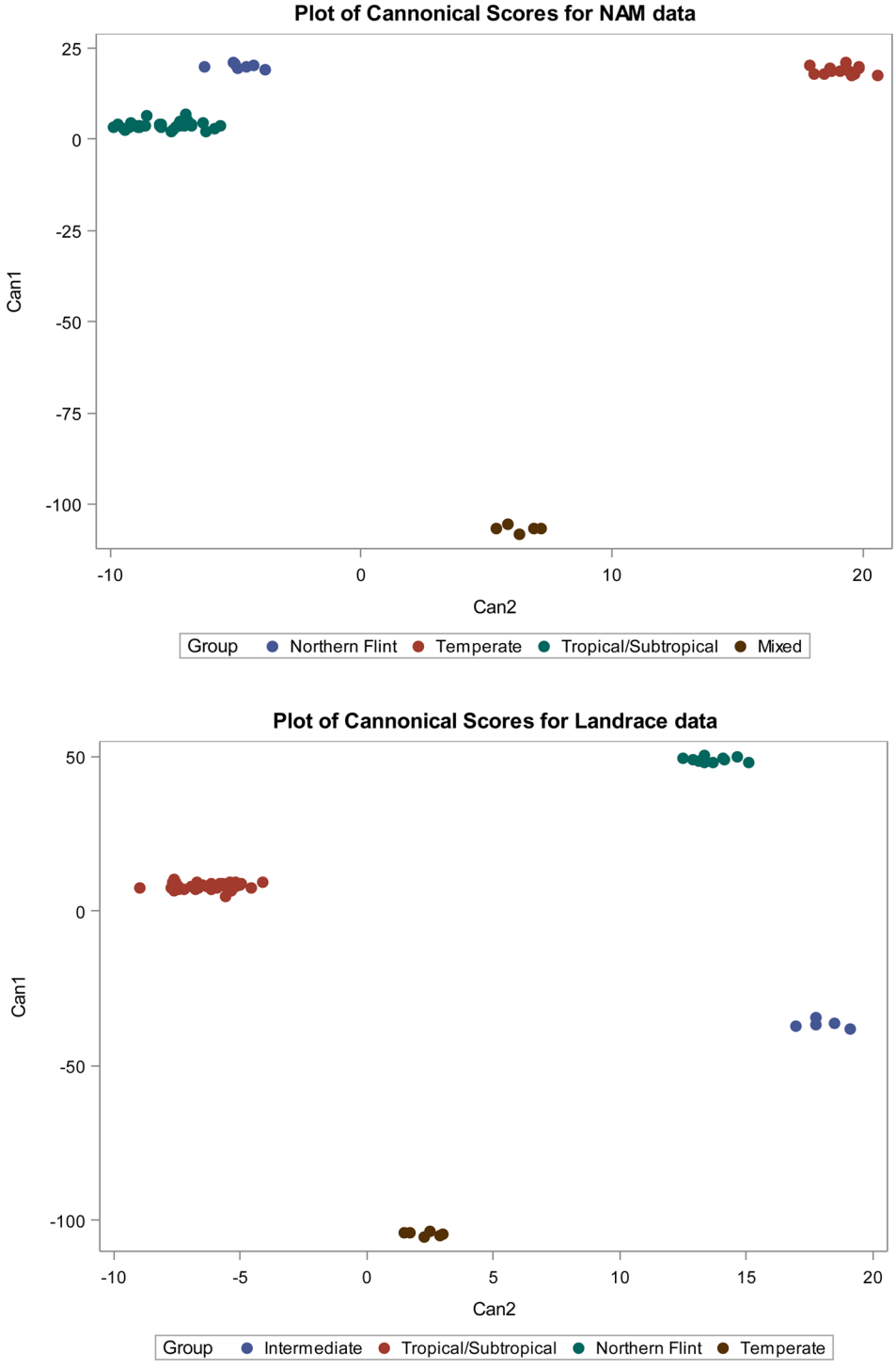

Figure 1. CDA of composiotional data from the NAM hybrids (top) and landrace hybrids (bottom).

the cob, and 50 kernels; the width of the ear and cob; and the length of the ear.

Composition Analysis. Assays for proximates (protein, fat, ash, and starch) except starch have been described before.[20] Starch was based on AOAC[21] protocols. Assays for fatty acids and amino acids,[22] minerals, raffinose, phytic acid, and β-carotene[20] have also been previously reported. Tocopherol analysis was based on a reversed-phase HPLC method using fluorescence detection with excitation at 290 nm and emission at 336 nm.[23] Tocopherols were extracted from ground lyophilized seed with 0.1% pyrogallol in ethanol. The reversed-phase HPLC system comprised a Keystone Aquasil C_{18} column at 40 °C and methanol as the mobile phase. The flow rate was 1 mL/min, and moisture was measured to allow for dry weight conversions. All compositional data are reported as percent dry weight (dwt) and are available in Supporting Information Files S1 and S2.

Three biological replicates were available for all samples except hybrids CML277, M37W, Oh43, and Oh7B, Bolita, Chapalote, Costeno, Cuban Flint, and Tabloncillo (two replicates each), and Canilla and Havasupai (one replicate each). Some assays were not repeated due to sample limitations. Thus, there is only one replicate of the Comiteco hybrid for protein, fat, starch, carbohydrates by calculation, β-carotene, raffinose, ADF, and NDF; one Araguito hybrid replicate for starch and phytic acid; one Tuxpeno hybrid replicate for raffinose; one CML103 hybrid replicate for NDF; and one

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Table 1. Mean Proximates and Fiber Values[a] for the NAM Founder Hybrids

group	founder × B73	protein	total fat	starch	carbohydrates[b]	ash	ADF[c]	NDF[d]
tropical	CML69	14.01	4.73	66.97	79.74	1.54	3.56	8.60
tropical	Tzi8	13.78	4.59	64.42	80.00	1.61	3.84	9.93
flint	P39	13.53	7.13	63.81	77.89	1.44	3.50	8.46
tropical	CML333	13.10	4.99	64.23	80.53	1.39	3.98	9.70
temperate	Oh43	12.99	4.54	65.62	81.13	1.33	3.64	9.10
tropical	CML52	12.95	4.27	66.31	81.21	1.54	3.83	10.19
tropical	CML228	12.94	4.96	69.03	80.66	1.48	3.35	8.22
tropical	CML277	12.86	3.87	66.37	81.75	1.50	3.64	9.93
flint	HP301	12.75	4.98	62.98	81.04	1.19	3.88	10.77
mixed	Tx303	12.57	4.60	67.77	81.46	1.35	3.63	9.41
temperate	Ky21	12.54	4.12	69.24	81.89	1.43	3.79	8.71
tropical	CML247	12.51	4.33	64.98	81.80	1.39	3.91	10.70
temperate	Oh7B	12.51	5.21	67.72	81.08	1.21	2.78	8.08
tropical	Ki3	12.32	4.42	64.89	81.76	1.49	3.49	10.06
flint	Il14H	12.25	5.72	66.71	80.59	1.47	4.11	10.39
temperate	B97	12.01	4.33	64.22	82.39	1.24	3.11	8.72
tropical	NC358	11.98	3.85	68.82	82.85	1.34	3.46	9.20
temperate	MS71	11.98	4.92	65.98	81.87	1.29	3.50	9.20
tropical	CML103	11.68	4.11	67.02	82.68	1.55	3.90	9.06
tropical	Ki11	11.48	4.16	67.28	83.06	1.32	3.48	8.76
mixed	Mo18W	11.10	4.97	77.89	82.58	1.37	3.80	9.18
tropical	CML322	11.09	4.83	67.50	82.69	1.37	3.96	10.07
temperate	M162W	11.08	4.40	75.49	83.32	1.21	3.32	9.18
tropical	NC350	10.56	4.73	67.69	83.31	1.42	3.23	9.26
mixed	M37W	10.25	3.97	67.19	84.33	1.44	3.98	10.09
	min	10.25	3.85	62.98	77.89	1.19	2.78	8.08
	mean	12.27	4.67	67.21	81.66	1.40	3.63	9.40
	max	14.01	7.13	77.89	84.33	1.61	4.11	10.77

[a]Values expressed as % dwt. [b]Carbohydrates by calculation. [c]Acid detergent fiber. [d]Neutral detergent fiber. For clarity, only means are presented, individual replicate values are presented in the Supporting Information (File S1).

Ky21 replicate for NDF. Samples with missing data due to sample limitations are noted in Supporting Information Files S1 and S2.

Statistical Analysis. Simple descriptive statistics were employed. Means, minimum replicate values, and maximum values were calculated in JMP 10 (SAS Institute, Cary, NC, USA). Analytes for which 50% of values were below the assay limit of quantitation (LOQ) were not included in the statistical summaries. These analytes included nine fatty acids (C11:0, C12:0, C14:0, C18:4n3, C20:2, C22:0, C22:5, C22:6, and C24:1), as well as sodium. No fatty acid data for one replicate of the M162W hybrid were assessed as one of the major fatty acids (oleic acid, C18:1) was assigned an LOQ during data acquisition. As in typical chemical analysis,[20a,24] analyte values below the LOQ were assigned a value of half of the assay LOQ. This included 54 values of C13:0, 20 values of C17:0, 25 values of C18:3n6, 22 values of C20:1n9, 4 values of C20:5, 24 values of C22:1n9, and 15 values of C24:0 with a value of 0.025% total fatty acid. The five values of β-carotene were assigned a value of 0.02 mg/100 g. All imputed values and associated samples are documented in Supporting Information Files S1 and S2.

We employed canonical discriminant analysis (CDA)[25] to determine if patterns existed in the data that could separate the hybrid groups, using the canonical discriminant CANDISC procedure in SAS. CDA is a dimension reduction technique related to principal component analysis and canonical correlation.[25] CDA analysis finds linear combinations of the quantitative variables that provide maximal separation between classes or groups. Given a classification variable and several quantitative variables, the CANDISC procedure in SAS derives canonical variables, linear combinations of the quantitative variables that summarize between-class variation in much the same way that principal components summarize total variation. The classification variable was hybrid groupings for NAM and landrace inbreds, and the quantitative variables were compositional components/phenotypic observations. Correlations between each of the analyte values and canonical scores 1 and 2 were derived using the multivariate CORR procedure in SAS.

RESULTS AND DISCUSSION

Maize is a highly diverse species, despite the narrow genetic base within U.S. commercial breeding programs.[26] Recently, genetic diversity within inbred lines has been explored to greater depth[7,10b] than ever before. However, systematic exploration of the landraces is lagging behind. In this study, we focus on the phenotypic evaluation of kernel composition traits in B73 hybrids derived from the NAM founders and a set of American landraces. The compositional analysis included quantitative measurement of proximates (protein, fat, ash, and starch), fatty acids, amino acids, minerals, raffinose, phytic acid, tocopherols, and β-carotene levels in mature grain of the two hybrid data sets.

CDA. CDA of the NAM hybrid data set established effective separation of the breeding groups assigned for the NAM hybrids (northern flint, temperate, tropical/subtropical, and mixed). CDA additionally provided effective separation of groups within the landrace hybrid set (northern flint, temperate, tropical/subtropical, and intermediate). Group assignments for each hybrid are provided in all tabulated data sets. The results clearly imply unique compositional characteristics that can distinguish between different sets of maize hybrids (Figure 1). The data also contrast the variation

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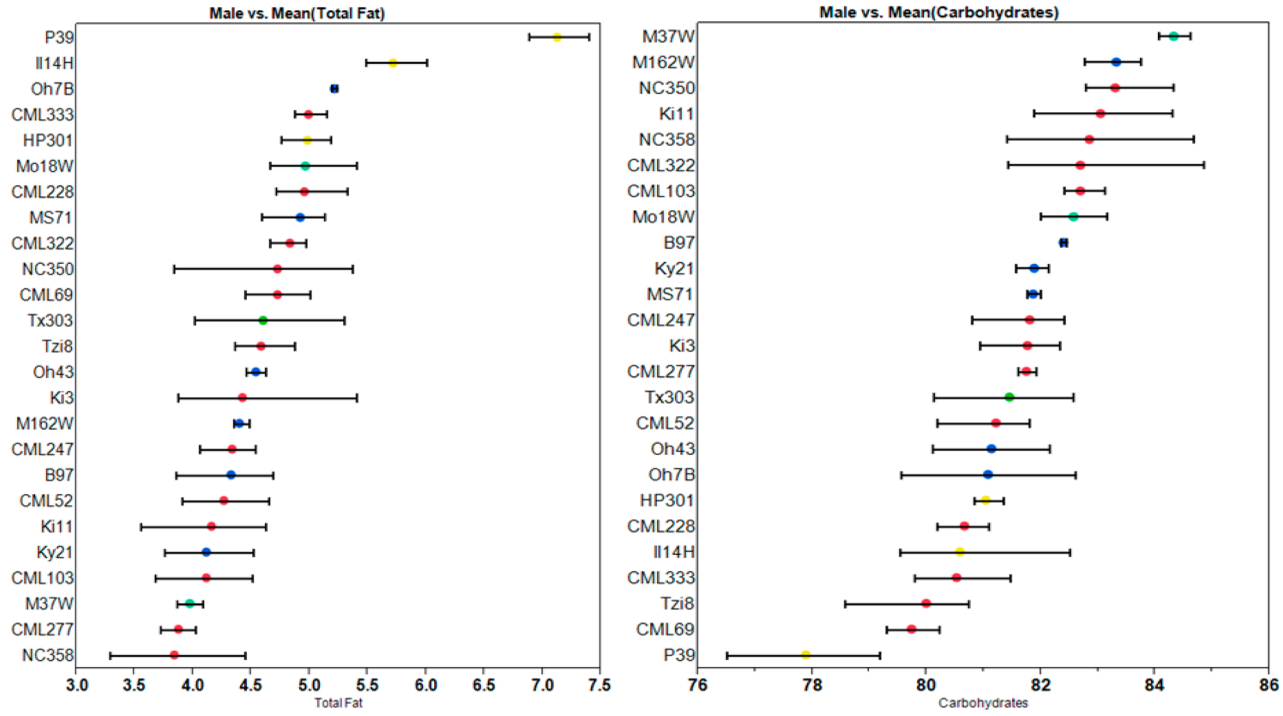

Figure 2. Means and ranges for total fat (left) and carbohydrates (right) of the NAM founder hybrids. Colored circles represent different subgroups: red, tropical; blue, temperate; green, mixed; yellow, flint.

associated with conventional breeding to the relative lack of impact of GM on composition.[18] Overall, however, correlations between the canonical scores (Can1 and Can2; Supplementary Table 1) and individual analytes that provided the separation were generally low (Supporting Information), and no one component was uniquely different in one breeding group versus another. In general, all components were characterized by extensive variation in grain levels, although values were typical for maize hybrids (ILSI, 2014).[27] Variation for individual components and their association with canonical scores is discussed in more detail below.

NAM Founder Hybrid Proximates and Fibers. Results for proximates and fibers are presented in Table 1, where the NAM founder hybrids are tabulated in order of highest to lowest mean protein values. The CML69 × B73 hybrid had the highest mean protein value (14.01% dwt) and M37W × B73 the lowest (10.25% dwt); the corresponding amino acid data are presented in Supplementary Table 2. Overall, all proximates values were variable, and no clear distinctions between the different NAM hybrid subsets were observed. We found that the hybrids with flint ancestry (derived from the sweetcorn lines P39 and Il14H and the popcorn HP301) were characterized by a relatively high-fat, low-carbohydrate profile (see Figure 2). For example, P39 × B73 and Il14H × B73 had the highest mean fat values (7.13 and 5.72% dwt), respectively; P39 × B73 had the lowest mean carbohydrate values (77.89% dwt). These observations are consistent with the abnormal starch content of sweetcorn lines due to the *sugary1* locus.[28] The extensive variation in protein values observed is presented in Supplementary Figure 1. Interestingly, the correlation between the inbred values for the same NAM parents reported by Cook et al.[15] for protein, oil, and starch were $r = -0.02$, 0.58, and 0.38, respectively. It appears that the B73 parent of the hybrids used in this study is contributing strongly to differences in protein content, but not for starch or oil. Correlations between protein, starch, and carbohydrates with canonical score 1 were $r = 0.358$, −0.363, and −0.333, respectively (Supporting Information). These were among the highest correlations observed, and these components are clearly some of the larger contributing factors to the separation of the different breeding groups.

NAM Founder Hybrid Fatty Acids. Results for fatty acids are presented in Table 2. The NAM founder hybrids are tabulated in order of highest to lowest mean linoleic acid (C18:2) values, the major fatty acid in maize. B97 × B73 had the highest mean value (58.92% total FA) and M162W × B73 the lowest mean value (41.68% total FA). The sweetcorn hybrids (derived from P39 and Il14H) and the popcorn HP301 were characterized by a relatively low oleic acid (C18:1) and high linolenic acid (C18:3) profile (see Figure 3), consistent with prior observations that high oil materials have high linolenic acid (C18:3) levels.[15] Correlations of the major individual fatty acids with the canonical scores were generally low; palmitic acid (C16:0) had a value of $r = -0.384$ with canonical score 2, representing the highest correlation for these components.

NAM Founder Hybrid Vitamins and Metabolites. Results for vitamin and metabolites are presented in Table 3. The NAM founder hybrids are tabulated in order of highest to lowest mean α-tocopherol values. Overall, vitamin and metabolite values were highly diverse; values for γ-tocopherol and β-carotene are presented in Figure 4 and for raffinose presented in Supplementary Figure 2. Despite this variation, some of these metabolites represented some of the highest correlations with the canonical scores; for example, raffinose and canonical score 1 ($r = -0.381$), phytic acid and canonical score 2 ($r = -0.418$), and γ-tocopherol and canonical score 2 ($r = 0.353$). Again, none of these components, by themselves, uniquely distinguished any breeding group. The β-carotene values (Table 3) in the hybrids were well correlated with the

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Table 2. Mean Fatty Acid Values[a] for the NAM Founder Hybrids

group	founder × B73	C10:0	C13:0	C16:0	C16:1	C17:0	C18:0	C18:1n9	C18:1n7	C18:2n6	C18:3n3	C18:3n6	C20:0	C20:1n9	C20:5	C22:1n9	C24:0
temperate	B97	0.21	0.05	13.19	0.13	0.04	1.82	19.65	0.80	58.92	1.54	0.13	0.32	0.11	0.18	0.23	0.21
flint	HP301	0.11	0.22	11.84	0.13	0.06	1.97	23.28	0.77	54.58	1.32	0.14	0.30	0.10	0.16	0.18	0.16
temperate	Oh43	0.12	0.46	11.84	0.10	0.04	1.37	18.55	0.68	53.87	1.53	0.19	0.17	0.11	0.07	0.23	0.16
tropical	Ki11	0.14	0.16	14.43	0.08	0.05	1.71	22.77	0.71	53.87	1.56	0.06	0.31	0.12	0.21	0.14	0.38
temperate	MS71	0.13	0.10	11.28	0.11	0.05	1.65	26.03	0.77	53.75	1.51	0.13	0.30	0.16	0.19	0.14	0.27
tropical	CML277	0.20	0.14	14.37	0.11	0.05	1.84	21.38	0.74	53.59	1.49	0.21	0.34	0.11	0.20	0.11	0.37
tropical	CML52	0.13	0.12	13.72	0.10	0.07	1.63	25.36	0.74	53.06	1.43	0.15	0.33	0.17	0.16	0.16	0.20
tropical	NC358	0.18	0.08	14.79	0.12	0.07	1.49	23.98	0.84	52.37	1.74	0.08	0.29	0.15	0.14	0.14	0.07
tropical	CML322	0.16	0.11	13.14	0.11	0.08	1.79	25.08	0.77	51.85	1.46	0.11	0.33	0.13	0.24	0.22	0.19
tropical	CML333	0.14	0.07	13.62	0.15	0.07	1.73	26.29	0.87	51.76	1.22	0.16	0.34	0.16	0.22	0.14	0.23
tropical	CML103	0.20	0.04	13.90	0.10	0.08	1.79	27.74	0.77	51.64	1.53	0.17	0.29	0.20	0.29	0.22	0.10
tropical	CML228	0.11	0.31	12.68	0.08	0.06	2.85	23.70	0.58	51.51	1.26	0.11	0.34	0.12	0.18	0.13	0.27
tropical	CML69	0.15	0.07	13.11	0.09	0.06	2.28	26.25	0.67	51.45	1.48	0.06	0.43	0.15	0.23	0.17	0.18
tropical	Tzi8	0.17	0.04	13.44	0.10	0.08	1.81	26.90	0.75	51.02	1.31	0.14	0.35	0.10	0.14	0.12	0.12
temperate	Ky21	0.14	0.19	13.03	0.10	0.04	1.74	26.00	0.77	50.61	1.66	0.21	0.29	0.16	0.19	0.18	0.12
tropical	CML247	0.17	0.08	13.32	0.13	0.07	1.71	26.95	0.82	50.18	1.30	0.11	0.31	0.16	0.28	0.18	0.20
flint	P39	0.14	0.04	11.70	0.11	0.07	2.29	30.77	0.68	50.11	1.12	0.09	0.40	0.16	0.17	0.06	0.15
tropical	NC350	0.16	0.15	13.21	0.13	0.08	1.96	27.09	0.75	49.66	1.56	0.16	0.37	0.15	0.20	0.18	0.21
mixed	Tx303	0.16	0.10	13.81	0.12	0.08	2.17	29.51	0.82	49.26	1.30	0.16	0.37	0.13	0.27	0.16	0.22
mixed	M37W	0.23	0.07	14.96	0.09	0.07	1.54	25.70	0.83	49.03	1.86	0.28	0.34	0.15	0.54	0.11	0.26
tropical	Ki3	0.16	0.07	13.87	0.12	0.07	1.98	29.35	0.73	47.97	1.30	0.14	0.44	0.14	0.15	0.13	0.26
temperate	Oh7B	0.14	0.08	12.75	0.12	0.04	2.07	30.83	0.72	47.89	1.29	0.13	0.36	0.13	0.20	0.12	0.16
mixed	Mo18W	0.11	0.32	12.40	0.11	0.07	1.92	29.12	0.67	46.64	1.20	0.08	0.30	0.12	0.19	0.10	0.28
flint	Il14H	0.18	0.05	12.33	0.11	0.07	2.74	34.44	0.74	42.60	1.25	0.19	0.36	0.21	0.17	0.20	0.05
temperate	M162W	0.12	0.29	12.54	0.13	0.06	1.95	32.37	0.76	41.68	1.44	0.10	0.37	0.11	0.18	0.17	0.20
	min	0.11	0.04	11.28	0.08	0.04	1.37	18.55	0.58	41.68	1.12	0.06	0.17	0.10	0.07	0.06	0.05
	mean	0.15	0.14	13.17	0.11	0.06	1.91	26.36	0.75	50.75	1.43	0.14	0.33	0.14	0.21	0.16	0.20
	max	0.23	0.46	14.96	0.15	0.08	2.85	34.44	0.87	58.92	1.86	0.28	0.44	0.21	0.54	0.23	0.38

[a]Values expressed as % total fatty acid. For clarity only means are presented, individual replicate values are presented in the Supporting Information (File S1).

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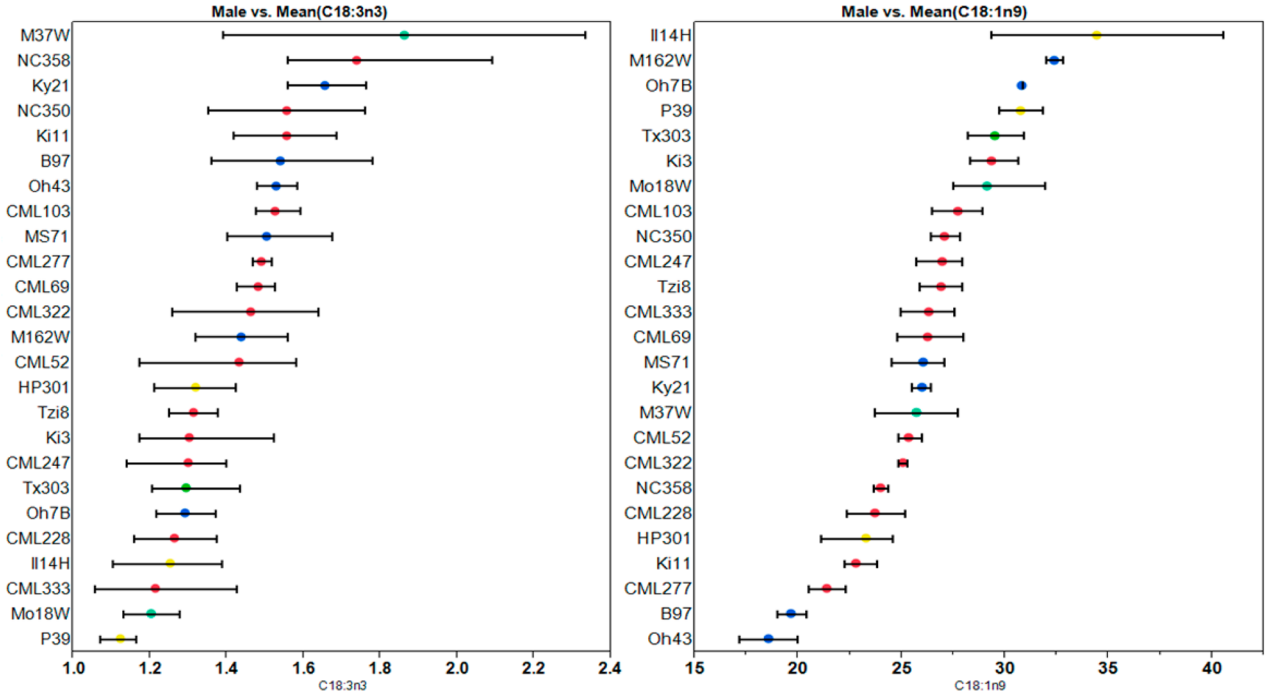

Figure 3. Means and ranges for oleic acid (left) and linolenic acid (right) of the NAM founder hybrids.

Table 3. Mean Vitamin and Metabolite Values[a] for the NAM Founder Hybrids

group	founder × B73	α-tocopherol	γ-tocopherol	δ-tocopherol	β-carotene	raffinose	phytic acid
tropical	NC350	15.33	23.33	1.33	0.13	0.12	1.02
tropical	CML103	13.00	32.67	1.00	0.04	0.13	1.03
tropical	CML333	12.67	26.33	1.33	0.03	0.15	1.11
flint	P39	12.67	39.33	1.33	0.09	0.26	1.10
temperate	MS71	12.00	26.33	1.67	0.10	0.18	1.03
temperate	Oh43	12.00	37.50	1.50	0.16	0.18	0.92
tropical	Tzi8	11.33	25.33	2.67	0.04	0.06	1.13
flint	Il14H	11.00	34.67	3.00	0.02	0.15	1.04
tropical	Ki11	10.67	24.00	1.00	0.23	0.16	0.92
tropical	Ki3	10.67	35.67	2.00	0.18	0.17	1.06
temperate	B97	8.33	35.33	1.67	0.07	0.13	0.91
tropical	CML69	8.33	15.00	1.00	0.14	0.16	1.11
mixed	Mo18W	8.33	34.67	2.67	0.03	0.18	0.87
tropical	NC358	8.33	20.00	0.67	0.09	0.16	1.08
tropical	CML228	8.00	35.33	1.67	0.16	0.18	1.08
tropical	CML247	8.00	19.67	1.00	0.03	0.15	1.07
mixed	M37W	8.00	33.50	1.50	0.07	0.25	1.07
tropical	CML52	7.67	12.00	1.00	0.26	0.12	1.01
temperate	Ky21	7.67	38.33	1.33	0.03	0.13	1.03
temperate	M162W	7.33	22.33	1.67	0.08	0.11	0.85
temperate	Oh7B	7.00	36.50	3.00	0.10	0.11	0.94
mixed	Tx303	6.00	30.67	1.67	0.20	0.21	0.85
tropical	CML322	5.67	27.00	2.33	0.05	0.15	1.00
flint	HP301	5.00	33.33	1.33	0.15	0.21	0.96
tropical	CML277	3.50	25.00	1.50	0.04	0.11	0.95
	min	3.50	12.00	0.67	0.02	0.06	0.85
	mean	9.14	28.95	1.63	0.10	0.16	1.01
	max	15.33	39.33	3.00	0.26	0.26	1.13

[a]Tocopherol values are expressed as mg/kg dwt, β-carotene values are expressed as mg/100 g dwt, raffinose and phytic acid are expressed as % dwt. For clarity only means are presented; individual replicate values are presented in the Supporting Information (File S1).

kernel color (Supplementary Table 7) of the non-B73 parent, with the white parents (Il14H, CML333, Mo18W, CML247, Ky21, CML103, Tzi8, CML277, CML322, M37W, and M162W) having the lowest β-carotene values, the orange

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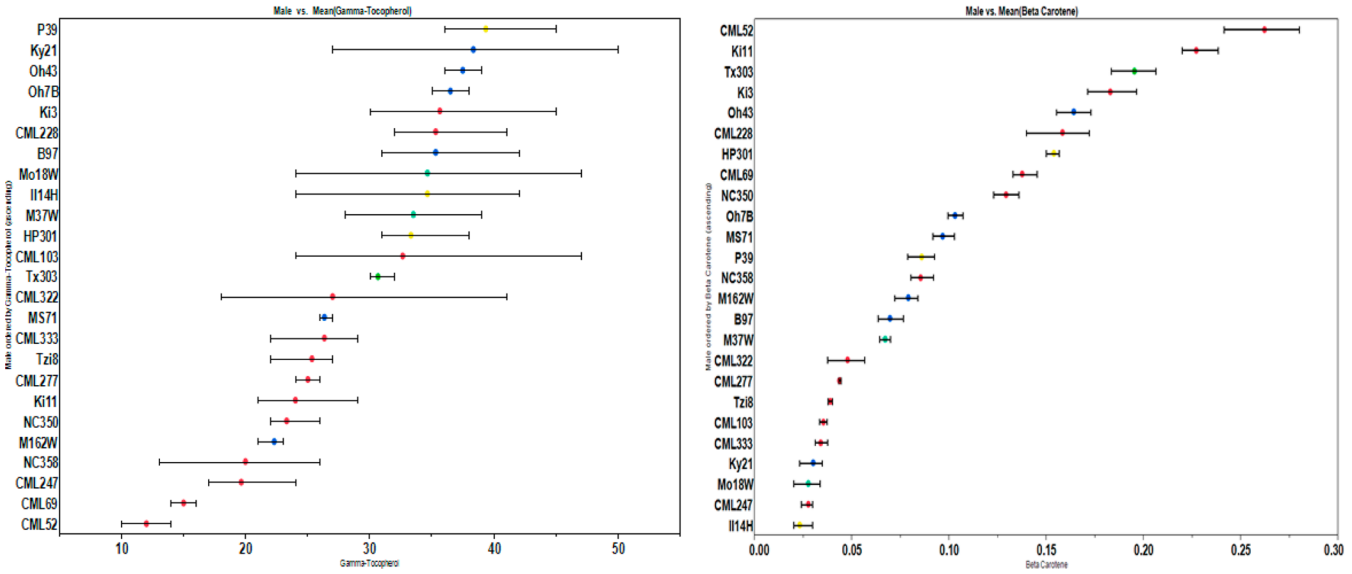

Figure 4. Means and ranges for γ-tocopherol (left) and β-carotene (right) of the NAM founder hybrids.

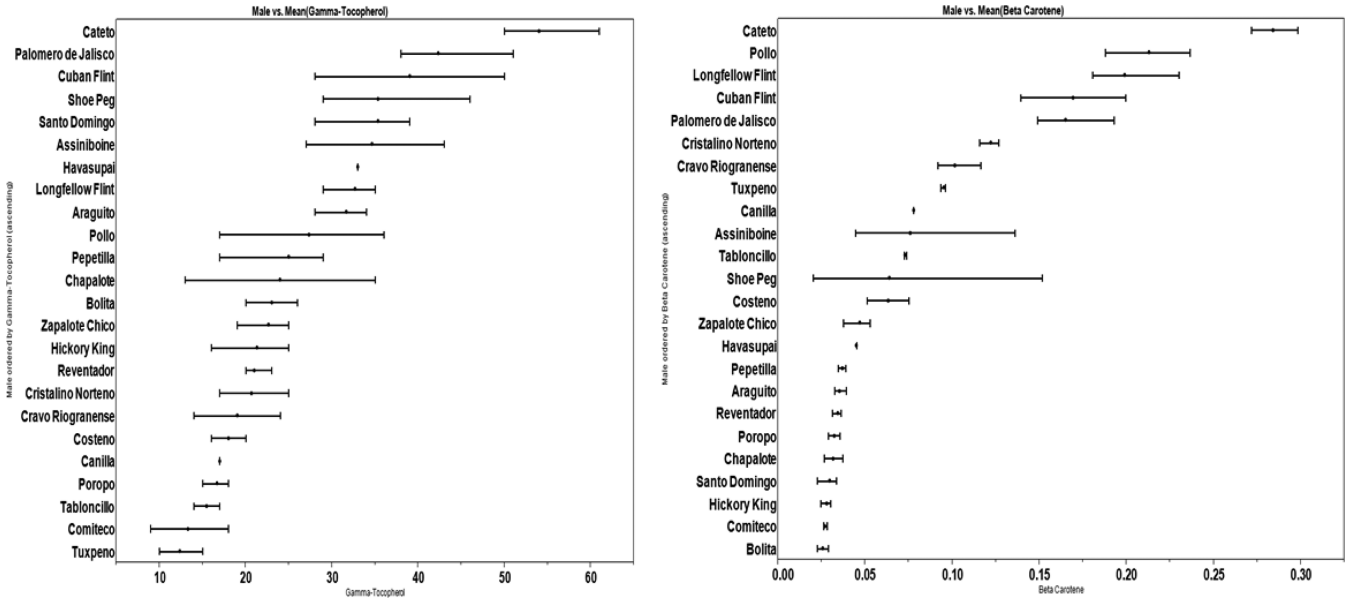

Figure 5. Means and ranges for γ-tocopherol (left) and β-carotene (right) of the landrace hybrids.

parents (P39, NC358, NC350, CML69, HP301, CML228, Ki3, Tx303, Ki11, and CML52) having the highest β-carotene values, and the yellow parents having intermediate values. However, the relationship between kernel color and carotenoid content is not perfect, which is consistent with previous results.[16a] The red kernel color observed for the CML52 hybrid (Figure 6; Supplementary Table 7) originates in the pericarp, the outer layer of the seed, which is derived from maternal tissue. The pericarp does not contain carotenoids, but rather contains other phenolic compounds such as anthocyanins, flavonoids, and phlobaphenes.[29] It is interesting that both the B73 and CML52 inbreds have clear pericarps, but the F1 ear produces red pericarp. This was observed during the development of the NAM population (S.F.-G., unpublished data) and is likely due to independent mutations in the *P1* locus in the two inbred lines that were complemented in the hybrid.

NAM Founder Hybrid Minerals. Mineral values among the NAM founder samples varied within the different subgroups (Supplementary Table 3). Two minerals showed some correlation with the canonical scores (in contrast with the landrace hybrid results shown below). These were manganese and canonical score 1 (r = 0.372) and phosphorus and canonical score 2 (r = −0.535). The phosphorus results are consistent with those of phytic acid (a phosphorus storage compound in maize grain).

Landrace Hybrid Composition Traits. Results for proximates and fibers, minerals, fatty acids, and vitamins and metabolites are presented in Table 4, Supplementary Table 5, Table 5, and Table 6, respectively. Overall, proximates and mineral values were typical for maize hybrids.[27] It is interesting that the hybrid derived from the popcorn Palomero de Jalisco had the highest mean protein value (14.69% dwt) just as was observed for the NAM popcorn parent HP301. Shoe Peg × B73, a landrace that is commonly eaten fresh in the United States, had the lowest protein value (10.12% dwt) (Supplementary Figure 3); the corresponding amino acid data are

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Figure 6. Photographs of the B73 × NAM founder ears: (top row) B97, CML103, CML228, CML247, CML277, CML322, CML333, CML52, CML69, Hp301, IL14H, Ki11, Ki3l; (bottom row) Ky21, M162W, M37W, Mo18W, MS71, NC350, NC358, Oh43, Oh7B, P39, Tx303, Tzi8.

Table 4. Mean Proximates and Fiber Values for the Landrace Hybrids[a]

group	landrace × B73	protein	total fat	starch	carbohydrates[b]	ash	ADF[c]	NDF[d]
intermediate	Palomero de Jalisco	14.69	4.11	56.68	79.75	1.46	3.69	8.85
tropical	Pepetilla	14.40	4.62	60.98	79.79	1.18	4.33	10.54
tropical	Cateto	13.82	5.20	64.50	79.56	1.41	3.79	7.81
tropical	Tuxpeno	13.76	4.60	66.62	80.04	1.60	3.68	8.92
intermediate	Chapalote	13.58	5.18	69.90	79.82	1.39	3.40	8.46
tropical	Araguito	13.57	4.85	67.69	80.17	1.42	3.21	8.89
flint	Santo Domingo	13.51	5.24	64.40	79.70	1.55	3.85	10.12
flint	Longfellow Flint	13.49	3.88	67.61	81.25	1.43	3.19	8.65
tropical	Tabloncillo	13.48	5.05	65.53	79.97	1.49	2.84	7.87
tropical	Comiteco	13.41	5.13	58.90	79.90	1.47	3.88	9.51
temperate	Hickory King	13.28	4.00	65.34	81.39	1.31	3.75	10.29
tropical	Canilla	13.26	3.92	65.02	81.52	1.31	3.76	9.13
tropical	Poropo	13.21	5.10	64.09	80.26	1.39	4.00	9.97
tropical	Zapalote Chico	13.19	4.06	63.29	81.07	1.71	3.63	9.45
tropical	Reventador	12.89	4.82	68.09	80.87	1.37	3.45	8.86
tropical	Pollo	12.61	4.95	70.04	81.01	1.40	3.27	7.56
tropical	Cravo Riogranense	12.43	3.93	70.22	82.29	1.41	3.74	9.99
tropical	Crystalino Norteno	12.11	4.76	65.51	81.86	1.28	3.82	8.31
flint	Assiniboine	12.07	4.35	64.59	82.05	1.57	4.34	11.58
tropical	Costeno	11.73	4.65	64.80	82.07	1.53	3.25	8.49
tropical	Bolita	11.40	4.00	65.88	83.25	1.34	3.30	8.95
tropical	Cuban Flint	11.31	4.92	68.36	82.46	1.34	3.57	8.64
flint	Havasupai	10.97	4.20	65.32	83.62	1.20	3.22	9.38
temparate	Shoe Peg	10.12	5.25	71.04	83.32	1.33	3.88	8.64
	min	10.12	3.88	56.68	79.56	1.18	2.84	7.56
	mean	12.85	4.62	65.60	81.12	1.41	3.62	9.12
	max	14.69	5.25	71.04	83.62	1.71	4.34	11.58

[a]Values expressed as % dwt. [b]Carbohydrates by calculation. [c]Acid detergent fiber. [d]Neutral detergent fiber. For clarity only means are presented, individual replicate values are presented in the Supporting Information (File S2).

presented in Supplementary Table 4. Similar variations in mineral levels were reported by Aliu et al.[30] in local populations of maize grown in Kosovo.

Fatty acids were highly variable for the landrace hybrids (Table 5; tabulated in order of highest to lowest mean linoleic acid (C18:2) values. Tuxpeno × B73 had the highest mean value (55.18% total FA) and Zapalote Chico × B73 the lowest (42.11% total FA). The oleic (C18:1) and linolenic (C18:3) fatty acid values are represented in Supplementary Figure 4. Likewise, there were high levels of variability for vitamins and metabolites in the landrace data set, including carotenoids, tocopherols, phytic acid, and raffinose. Values for γ-tocopherol and β-carotene are presented in Figure 5, and those for raffinose are presented in Supplementary Figure 5. Just as in the NAM founders, the carotenoid content of the landrace hybrids closely followed the kernel color. The striking exceptions were the Comiteco and Revenatdor hybrids, where the pink and red

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Table 5. Mean Fatty Acid Values[a] for the Landrace Hybrids

group	landrace × B73	C10:0	C13:0	C16:0	C16:1	C17:0	C18:0	C18:1n9	C18:1n7	C18:2n6	C18:3n3	C18:3n6	C20:0	C20:1n9	C20:5	C22:1n9	C24:0
tropical	Tuxpeno	0.18	0.23	13.86	0.12	0.07	1.72	21.68	0.77	55.18	1.50	0.16	0.28	0.20	0.24	0.06	0.31
flint	Longfellow Flint	0.13	0.25	13.53	0.12	0.06	1.60	20.16	0.78	54.50	1.68	0.16	0.22	0.12	0.16	0.19	0.14
tropical	Araguito	0.14	0.16	13.58	0.13	0.03	1.31	23.06	0.90	53.62	1.43	0.12	0.22	0.07	0.13	0.10	0.31
tropical	Canilla	0.13	0.26	14.20	0.15	0.08	1.56	23.39	0.86	51.95	1.56	0.03	0.26	0.15	0.26	0.09	0.12
tropical	Comiteco	0.18	0.07	13.84	0.10	0.07	1.76	26.09	0.76	51.35	1.30	0.17	0.31	0.16	0.18	0.17	0.22
tropical	Cuban Flint	0.14	0.04	13.85	0.10	0.09	2.20	26.12	0.66	51.32	1.36	0.17	0.48	0.12	0.16	0.03	0.16
temperate	Hickory King	0.24	0.10	14.51	0.12	0.04	1.69	27.57	0.86	50.24	1.65	0.18	0.29	0.14	0.33	0.27	0.27
intermediate	Chapalote	0.14	0.19	14.63	0.11	0.04	1.63	27.48	0.71	49.71	1.50	0.14	0.24	0.15	0.21	0.19	0.03
tropical	Pepetilla	0.09	0.25	14.03	0.10	0.05	1.97	24.18	0.70	49.59	1.42	0.11	0.32	0.08	0.11	0.16	0.10
tropical	Cateto	0.12	0.05	12.96	0.13	0.08	1.68	28.26	0.87	49.13	1.35	0.22	0.37	0.15	0.29	0.06	0.13
tropical	Tabloncillo	0.11	0.28	14.08	0.11	0.08	2.23	24.77	0.65	48.90	1.35	0.10	0.34	0.09	0.12	0.03	0.34
flint	Havasupai	0.19	0.03	14.00	0.13	0.08	2.10	30.42	0.77	48.89	1.65	0.20	0.40	0.20	0.38	0.23	0.21
tropical	Pollo	0.16	0.10	14.53	0.12	0.08	2.07	27.52	0.73	48.80	1.32	0.17	0.47	0.15	0.26	0.16	0.21
intermediate	Palomero de Jalisco	0.17	0.16	13.55	0.10	0.07	2.01	28.58	0.74	47.45	1.91	0.13	0.30	0.14	0.20	0.13	0.31
tropical	Crystalino Norteno	0.14	0.03	12.10	0.11	0.09	2.11	32.66	0.80	46.76	1.55	0.11	0.35	0.19	0.19	0.16	0.13
tropical	Bolita	0.13	0.13	13.55	0.12	0.08	2.39	29.20	0.69	46.03	1.37	0.15	0.36	0.13	0.14	0.17	0.23
tropical	Poropo	0.15	0.07	13.37	0.16	0.08	2.10	32.28	0.71	45.67	1.42	0.12	0.35	0.17	0.14	0.09	0.19
tropical	Costeno	0.18	0.03	13.63	0.13	0.07	1.95	31.45	0.83	45.52	1.38	0.22	0.34	0.12	0.54	0.12	0.11
flint	Santo Domingo	0.15	0.12	12.67	0.11	0.07	1.94	31.72	0.71	44.98	1.22	0.07	0.33	0.17	0.37	0.09	0.23
temparate	Shoe Peg	0.19	0.11	12.38	0.12	0.08	1.98	33.23	0.82	44.80	1.27	0.13	0.31	0.20	0.17	0.09	0.29
flint	Assiniboine	0.13	0.08	12.72	0.13	0.08	1.93	33.90	0.76	44.52	1.36	0.18	0.36	0.17	0.19	0.19	0.20
tropical	Cravo Riogranense	0.18	0.06	14.07	0.14	0.06	1.68	32.08	0.91	44.38	1.55	0.27	0.33	0.14	0.32	0.09	0.14
tropical	Reventador	0.09	0.32	13.95	0.14	0.04	2.14	31.32	0.79	42.84	1.36	0.14	0.29	0.12	0.23	0.09	0.27
tropical	Zapalote Chico	0.21	0.11	14.73	0.12	0.06	1.84	32.43	0.79	42.21	1.81	0.25	0.35	0.14	0.21	0.21	0.24
	min	0.09	0.03	12.10	0.10	0.03	1.31	20.16	0.65	42.21	1.22	0.03	0.22	0.07	0.11	0.03	0.03
	mean	0.15	0.13	13.68	0.12	0.07	1.90	28.31	0.77	48.26	1.47	0.15	0.33	0.14	0.23	0.13	0.20
	max	0.24	0.32	14.73	0.16	0.09	2.39	33.90	0.91	55.18	1.91	0.27	0.48	0.20	0.54	0.27	0.31

[a]Values expressed as % total fatty acid. For clarity only means are presented, individual replicate values are presented in the Supporting Information (File S2).

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Table 6. Mean Vitamin and Metabolite Values[a] for the Landrace Hybrids

group	landrace × B73	α-tocopherol	γ-tocopherol	δ-tocopherol	β-carotene	raffinose	phytic acid
tropical	Cuban Flint	13.00	39.00	1.00	0.17	0.11	1.01
tropical	Cateto	12.67	54.00	3.00	0.28	0.16	1.23
tropical	Cravo Riogranense	11.33	19.00	1.00	0.10	0.13	0.90
intermediate	Palomero de Jalisco	11.33	42.33	3.00	0.16	0.14	1.13
tropical	Crystalino Norteno	11.00	20.67	0.67	0.12	0.20	0.92
tropical	Zapalote Chico	10.67	22.67	0.67	0.05	0.16	1.25
tropical	Pollo	9.00	27.33	1.00	0.21	0.11	0.98
intermediate	Chapalote	8.00	24.00	1.00	0.03	0.12	1.00
flint	Assiniboine	7.67	34.67	2.33	0.08	0.24	1.11
flint	Longfellow Flint	7.67	32.67	2.33	0.20	0.14	0.99
tropical	Bolita	7.50	23.00	1.00	0.03	0.10	0.97
flint	Santo Domingo	7.33	35.33	1.33	0.03	0.15	1.21
tropical	Comiteco	7.00	13.33	0.33	0.03	0.09	1.02
flint	Havasupai	7.00	33.00	1.00	0.04	0.12	0.91
tropical	Tabloncillo	7.00	15.50	1.00	0.07	0.16	1.13
temperate	Hickory King	6.67	21.33	1.33	0.03	0.16	0.89
temparate	Shoe Peg	6.33	35.33	3.67	0.06	0.08	0.82
tropical	Araguito	6.00	31.67	3.00	0.04	0.20	0.96
tropical	Tuxpeno	6.00	12.33	2.33	0.10	0.10	1.06
tropical	Pepetilla	5.67	25.00	1.00	0.04	0.23	0.94
tropical	Reventador	5.67	21.00	1.67	0.03	0.14	1.07
tropical	Poropo	4.67	16.67	1.00	0.03	0.17	1.00
tropical	Costeno	4.50	18.00	1.00	0.06	0.10	0.94
tropical	Canilla	4.00	17.00	1.00	0.08	0.21	1.12
	min	4.00	12.33	0.33	0.03	0.08	0.82
	mean	7.82	26.45	1.53	0.09	0.15	1.02
	max	13.00	54.00	3.67	0.28	0.24	1.25

[a]Tocopherol values are expressed as mg/kg dwt, β-carotene values are expressed as mg/100 g dwt, and raffinose and phytic acid are expressed as % dwt. For clarity only means are presented; individual replicate values are presented in the Supporting Information (File S2).

Figure 7. Photographs of the B73 × landrace ears: (top row) MR01 (Araguito), MR02 (Assiniboine), MR03 (Bolita), MR04 (Canilla), MR05 (Cateto), MR06 (Chapalote), MR07 (Comiteco), MR08 (Costeno), MR09 (Cravo Riogranense), MR10 (Crystalino Norteno), MR11 (Cuban Flint), MR12 (Havasupai); (bottom row) MR13 (Hickory King), MR14 (Longfellow Flint), MR15 (Palomero de Jalisco), MR16 (Pepetilla), MR18 (Reventador), MR19 (Santo Domingo), MR20 (Shoe Peg), MR21 (Tabloncillo), MR22 (Tuxpeno), MR23 (Zapalote Chico), MR25 (Poropo), MR26 (Pollo).

pericarp colors masked white endosperm (Supplementary Table 8; Figure 7).

Correlations with Canonical Scores within Landraces. Correlations between proximates values and the canonical scores showed a pattern different from that for the NAM hybrid data set with, in general, much lower correlations (Supplementary Table 1; Figure 1). The minerals represented some of the highest correlations with canonical score 1; for example, iron, magnesium, manganese, and phosphorus were all >0.4 (Supporting Information). It is therefore probable that these components contribute more so than other components to separation of the breeding groups in the landrace set. This

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contrasts slightly with results from the NAM set where minerals (with the possible exceptions of manganese and phosphorus) were less affected. Correlations between fatty acid values and canonical scores were low, suggesting that these components are unlikely to contribute to any separation of the different breeding groups. Of the metabolites, phytic acid showed the highest correlation with canonical score 1 (r = 0.394), whereas γ-tocopherol showed the highest correlation with canonical score 2 (r = 0.384).

Comparison of NAM to Landraces for Composition Traits. Box plots comparing compositional variation of NAM and landrace hybrids for selected analytes discussed above are presented in Supplementary Figure 6 (a−e). Sevaral composition components such as iron, manganese, phosphorus, ash, γ-tocopherol, raffinose, and phytic acid had the highest correlations for both hybrid sets. For most composition traits, the landrace hybrids had either the same or wider ranges of phenotypic variation than the inbred lines. The exceptions were the proximates fat, starch, and carbohydrates (Tables 1 and 4), several of the fatty acids such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) (Tables 2 and 5), the vitamins and minerals α- and γ-tocopherol and raffinose (Tables 3 and 6), the amino acids methionine and proline (Supplementary Tables 2 and 4), and the minerals copper, manganese, and zinc (Supplementary Tables 3 and 5). Interestingly, for most of these exceptions the lower range for the landrace hybrids did not exceed the lower range of the inbreds, whereas the upper range of the landrace hybrids still exceeded the inbred range, indicating that the landraces may be more valuable for increasing these trait values rather than decreasing them. The wide diversity of phenotypes for all of the kernel composition traits in the landrace hybrids warrants further studies of the genetic architecture underlying composition traits, as well as the exploration of this diversity for maize improvement.

Plant, Ear, and Kernel Phenotypic Data. Results of the CDA on the phenotypic data for both the NAM and landrace hybrid sets are provided in Supplementary Table 6. Positive correlations with canonical scores 1 or 2 were observed for days to anthesis, ear weight, ear width, kernel weight, cob weight, and ear height in the NAM hybrid set. In the landrace hybrid set positive correlations with canonical scores 1 or 2 were observed for days to anthesis, plant height, ear height, ear length, and ear fill length. The majority of the ear/kernel phenotypes showed negative correlations in the landrace hybrid data set. There was wide variability for all plant, ear, and kernel traits in both the NAM and landrace hybrid sets (Supplementary Tables 7 and 8). As with the composition data, the landrace hybrids had wider ranges of plant, ear, and kernel phenotypes. The exceptions were ear and cob weight, which are likely a function of the unadaptedness of the landraces to the temperate environment in which they were evaluated, where the landrace hybrids were not able to produce full-sized ears. These phenotypes have been studied extensively at the genetic level in the NAM population,[31] but as for the composition traits discussed above, they have not been explored in landraces.

The development of modern plant breeding techniques has greatly facilitated wider use of a wealth of genetic diversity from many sources including landraces. Exploitation of genetic diversity in breeding programs has allowed food production to keep up with population growth.[32] The development of improved maize hybrids will require optimization of current resources to guide breeding strategies. The genetic diversity preserved in germplasm banks represents a key current resource. In this study we have provided the maize research community a first survey of kernel composition of hybrids from two important genetic resources: the NAM founder lines and landraces selected from wide geographic areas in the Americas. Multivariate analysis (CDA) showed that subsets within each of the NAM and landrace hybrid data sets could be distinguished by breeding group. Overall, results highlighted extensive variation in all compositional components assessed for both sets of hybrids reflecting the underlying genetic diversity of these lines. Data generated here will be of value to plant breeders and plant biotechnologists involved in the nutritional and agronomic improvement of corn and will be a resource for the wider research community utilizing the NAM founder lines to understand the genetic basis of compositional and other complex traits. The data also contrast the variation associated with conventional breeding to the relative lack of impact of GM on composition.[18b] The natural variability associated with composition observed in these conventional natural populations may therefore also be of interest to regulatory scientists and policy makers involved in safety evaluation of transgenic crops from a food and feed perspective.[33]

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and tables. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b00208.

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Notes

The authors declare no competing financial interest.

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