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Diversity and heterotic patterns in North American proprietary dent maize germplasm

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Abstract

Publicly available maize (*Zea mays* L.) inbred lines with expired Plant Variety Protection certificates (ex-PVP) constitute the elite germplasm used in hybrids grown by North American farmers during the past half century. A set of 329 ex-PVP inbreds and eight public reference inbreds was evaluated for genetic diversity using molecular marker and pedigree-based relationships. The majority of inbreds originated from Dekalb Genetics Corporation, Holden's Foundation Seed, Pioneer Hi-Bred International, and Syngenta via Novartis and Northrup King. Flowering ranged from 968–1806 growing degree days to anthesis, supporting that these inbreds represent adaptation across the range of North American production environments. Admixture analysis supported eight subgroups (B14, B37, B73, Oh43, Iodent, Iodent-Lancaster, Lancaster, and Flint), reflecting heterotic patterns and company of origin. Five diallel-cross experiments identified primary yield heterotic patterns within early-, intermediate-, and late-maturity groups. Parental pedigree information was compiled for 2392 Plant Variety Protection and/or utility patented inbreds through 2017, allowing for multigenerational analysis of progenitors and the eight heterotic subgroups across current proprietary breeding programs. Key progenitors were identified for recently merged companies, Bayer Crop Science and Corteva Agriscience. The results of this study strengthen the value of the ex-PVP inbreds as a source of elite and relevant germplasm for breeders and researchers to exploit. This study provides information that will be logistically useful to researchers who use ex-PVP inbreds and adds to our understanding of heterotic grouping within North American proprietary germplasm.

1 | INTRODUCTION

Maize (*Zea mays* L., subsp. *mays*) is an important field crop in the United States with 33.1 million ha harvested for grain in 2018. Farmers predominantly grow hybrid cultivars derived from crossing two unrelated inbred parents. Maize breeders focus their breeding efforts on the identification of new inbreds and then cross combinations of elite inbred parents to generate superior hybrids. Today, nearly all commercial maize breeding occurs in the private sector because of limited public budgets, intellectual property protection, and globalization of agriculture. With increasing private sector consolidation, the number of active breeding programs for maize is decreasing (Howard, 2015; Mikel, 2008; Shelton & Tracy, 2017).

Abbreviations: CIMMYT, International Maize and Wheat Improvement Center; ex-PVP, inbred line with expired Plant Variety Protection, most also with expired patent protection; IBS, identity by state; LD, linkage disequilibrium; MAF, minor allele frequency; PVP, Plant Variety Protection; SNP, single nucleotide polymorphism; WiDiv-942, Wisconsin Diversity Panel with 942 individuals.

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The Plant Variety Protection (PVP) Act of 1970 granted breeders exclusive right to the commercialization of distinct and novel sexually or tuber-propagated plant varieties, with a protection period of 20 yr. Upon expiration of the PVP certificate, and barring additional utility patent protection, PVP maize inbreds become publicly available for distribution through the North Central Regional Plant Introduction Station (NC7) in Ames, IA. As of May 2019, there are ~460 publicly available maize inbreds with expired PVP certificates (ex-PVPs). By 2028, 750 additional ex-PVP inbreds will be available for breeders and researchers to use. This growing set of germplasm represents a group of elite inbred parents used in the generation of commercial hybrid seed across numerous parent seed companies in the United States since the 1980s. Ex-PVP germplasm is an increasingly important source material for public and private researchers to use, especially in the context of reduced public breeding effort and consequently fewer releases of new elite inbreds (Beckett, Morales, Koehler, & Rocheford, 2017, 2019; Mikel, 2008, 2011).

This study focused on inbreds submitted for PVP registration prior to 1997 and is representative of proprietary germplasm in the pre-transgenic era, which ended in 1996 with the sale of commercial maize hybrids containing the Mon810 *Bacillus thuringiensis* transgenic event (ISAAA, 2019; Levine, 2004). Since transgene use began, PVP registrations indicate that nearly all PVP-registered inbreds are nontransgenic. With a vast majority of commercial maize hybrids in the United States today containing multiple transgenic traits, major companies develop inbreds primarily using forward breeding of nontransgenic germplasm. Promising inbreds then have traits introgressed using rapid transgenic backcross conversion pipelines leveraged with marker selection and multiple growing generations per year. This system works well with commercial maize hybrids with the predominance of dominantly expressed transgenic traits requiring only one of a hybrid's two parental lines to have the trait. In addition, since events—not genes—are deregulated, it is prohibitively costly to produce a new transgenic event in each new inbred.

The PVP collection has numerous breeding entities that originated inbreds into the PVP system; however, acquisitions and mergers have consolidated the seed industry leaving relatively few independent breeding programs in the Corn Belt. Pioneer Hi-Bred International and later Dupont Pioneer merged with Dow AgroSciences to form Corteva Agriscience in 2019. Dekalb Genetics Corporation and Holden's Foundation Seed were acquired by Monsanto Technology in 1997, and in 2018, Bayer Crop Science acquired Monsanto Technologies. Novartis Agribusiness and Zeneca Agrochemicals merged to form Syngenta in 2000 and acquired Garst Seed Company and Golden Harvest Seeds in 2004. This study focuses on germplasm developed by the legacy seed companies for Pioneer Hi-Bred International (Corteva Agriscience), Monsanto Technology (Bayer Crop Science), and Syngenta.

Analysis of ex-PVP inbreds provides retrospective information on the establishment of heterotic groups, breeding strategies, and allelic shifts in response to selection with both practical and theoretical implications. Initial heterotic pools in commercial programs were established by empirical assembly of public inbreds into groups (Mikel, 2008; Tracy & Chandler, 2008). Multiple generations of breeding within companies structured and strengthened the use of those pools. Tester inbreds used in the evaluation and selection of new inbreds are often related to founding individuals and maintain strong general combining ability with the breeding population. Inbreds B14, B37, and B73 were broadly categorized under the Stiff Stalk heterotic group, and PH207, Oh43, and Mo17 were categorized as the non-Stiff Stalk heterotic group (Mikel, 2011).

Previous studies of ex-PVP inbreds support clusters within the context of the core founders. Nelson et al. (2008) found that 92 ex-PVP inbreds separated into six primary genetic clusters founded by inbreds B73, B37, A632 (derived from B14), Oh43, PH207, and Mo17. Recent studies with larger sets of ex-PVP inbreds and single nucleotide polymorphisms (SNPs) continue to support the primary heterotic pools forming subpopulations represented best by the B14, B37, B73, Oh43, Iodent, Iodent Mixed, Lancaster, and Mixed populations (Beckett et al., 2017).

Inbreds present in the ex-PVP pool are already contributing elite, high-yielding germplasm to breeding programs (Mikel, 2008, 2011). To optimize the use of ex-PVP germplasm, a greater understanding of origin and relationships is critical. This can be challenging, as some PVP certificates have limited interpretability because of the use of company-specific naming schema, references to experimental or noncommercial parental germplasm with unknown pedigrees, and phenotypic comparison with other unknown inbreds or hybrids. Molecular marker information and phenotypic analysis in common environments can help to disambiguate relationships and provide relative performance information that is useful in characterizing new germplasm and associating phenotypes with genotypes.

The goal of this study was to identify and evaluate the genetic diversity present within the available pool of ex-PVP inbreds using genomic and phenotypic measurements. To evaluate the heterotic structure of the ex-PVP population, hybrids generated using divergent ex-PVP parental lines were used to calculate combining ability estimates. This study reveals how heterotic patterns were formed within private company pools, highlights the contribution of key founder lines, and reveals the migration of germplasm among major breeding pools. This information will facilitate future use of ex-PVP inbreds as a source of elite germplasm adapted to North America.

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2 | MATERIALS AND METHODS

2.1 | Germplasm

Seed of 329 ex-PVP inbreds was acquired from the North Central Plant Introduction Station (NC7) in Ames, IA (Supplemental Table S1). This set of materials represents commercialized elite proprietary germplasm protected prior to 1997. In addition, eight public inbreds (B14, B37, B73, B84, H99, Mo17, Oh07, and Oh43) were included for reference based on previous studies of key public founder inbreds (Beckett et al., 2017; Melchinger & Gumber, 1998; Mikel & Dudley, 2006; Nelson et al., 2008; Troyer, 1999).

The 329 ex-PVP and eight public inbreds analyzed in this study are a subset of the Wisconsin Diversity Panel (WiDiv-942) containing 942 diverse maize inbreds (Mazaheri et al., 2019). The genotype matrix used in this study consisted of 899,784 biallelic SNPs; the details of the SNPs and methods are described in Mazaheri et al. (2019).

2.2 | Calculation of genetic distance and dendrogram

To assess relatedness, pairwise genetic distance was calculated as 1 − identity by state (IBS) using Tassel 5.0 (Bradbury et al., 2007), where IBS is the probability that two SNPs at a given locus are identical by state in two inbreds. The resulting distance matrix was used to generate a dendrogram using the neighbor-joining method (Saitou & Nei, 1987) in Tassel 5.0 (Bradbury et al., 2007), and the dendrogram exported as Newick tree format was visualized and annotated using FigTree (Rambaut, 2012).

2.3 | Admixture

To identify the presence of heterotic subgroups in the panel, an unsupervised, model-based estimation of ancestral populations among the 337 inbreds (329 ex-PVP plus eight public inbreds) was performed using Admixture (Alexander, Novembre, & Lange, 2009). The genotypic matrix consisting of 899,784 SNPs for the set of inbreds was first pruned for linkage disequilibrium (LD) using PLINK (Purcell et al., 2007). At a step interval of 10 SNPs, pairs of variants within the 50-SNP window with $R^2 > 0.1$ are noted, and variants were pruned from the window until no such pairs remained, leaving an LD-pruned subset of 109,605 SNPs. The pruned marker set was used in the admixture analysis without seeding founding individuals. Tenfold cross validation of the errors was minimized at K groups equal to eight, and therefore this was considered an appropriate model for this set of individuals and marker structure.

To enhance visualization of the heterotic subgroups, a multidimensional scaling analysis was completed on the genetic distance matrix using Tassel 5.0 (Bradbury et al., 2007). The first three components were visualized in a three-dimensional plot. Individuals were labeled using one of the eight admixture subgroups that represented the majority heterotic membership and scaled by the inbred's parental contribution in the generation of a new inbred that received a PVP certificate (R Core Team, 2012).

2.4 | Measures of allelic diversity among ex-PVP inbreds

Allele frequency was calculated for the subgroup of ex-PVP inbreds in their corresponding admixture heterotic subgroup across all 899,784 SNPs. To assess allelic variability among B14, B37, B73, Oh43, Iodent, Iodent-Lancaster, Lancaster, and Flint heterotic subgroups, each group was compared with the diverse WiDiv-942 (Mazaheri et al., 2019). Major allele frequency was calculated across the 899,784 SNPs for the inbreds in each of the eight heterotic subgroups. To visualize diversity across the largest chromosome, the resulting data were averaged in discrete windows of 200 SNPs for chromosome 1 and visualized using ggplot2 (R Core Team, 2012; Wickham, 2011). The proportion of SNPs monomorphic within a heterotic subgroup and polymorphic in the WiDiv-942 was calculated for the ex-PVP panel, Iodent, and B73 subgroups, which included 337, 62, and 66 inbreds, respectively.

On a chromosomal basis, diversity statistics, including pairwise divergence (p) (Nei & Li, 1979), Watterson estimator (Watterson, 1975), and Tajima's D (Tajima, 1993) were calculated with Tassel 5.0 (Bradbury et al., 2007) for the WiDiv-942 (Mazaheri et al., 2019), ex-PVP panel, Iodent, and B73 subgroups, with 942, 337, 62, and 66 inbreds, respectively. Pairwise divergence (p) measures the average number of different variants at polymorphic SNPs. Watterson estimator measures the number of polymorphic SNPs and mutation rate. Tajima's D is the comparison between the average pairwise divergence (π) and segregating sites within the population. These statistics use allele frequencies within populations and provide evidence regarding selective sweeps of rare alleles and population bottleneck events.

To view regions of conserved diversity specific to heterotic subgroups, the major allele at 899,784 SNPs for each of the eight heterotic subgroups was imputed using a sliding window of 200 SNP loci, with a step size of 40. Haplotypes unique or shared among each heterotic subgroup across the window were identified across the genome. The resulting windows were plotted to visualize regions of IBS among the eight heterotic subgroups in R (R Core Team, 2012).

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2.5 | Linkage disequilibrium

To measure the LD decay among ex-PVP inbreds, R^2 was calculated using 899,784 SNPs for the ex-PVP panel, Iodent, and B73 subgroups, which contain 337, 62, and 66 inbreds, respectively. For comparison, R^2 was calculated for 37 exotic and unadapted inbreds from the WiDiv-942 developed by the International Maize and Wheat Improvement Center (CIMMYT). The R^2 was calculated in Tassel 5.0 (Bradbury et al., 2007) using 1000-SNP windows. Pairwise window combinations were binned into distances of 0.1–1.0 kb, 1.0–10 kb, 10–100 kb, 100 kb–1.0 Mb, and 1.0–10 Mb in R (R Core Team, 2012). Violin boxplots for each bin of R^2 were plotted for chromosome one in R (R Core Team, 2012) using the ggplot2 package (Wickham, 2011).

2.6 | Inference of ex-PVP influence on current PVP inbreds

Pedigree information provided in the PVP Certificates and Utility Patents for 2,392 inbreds was compiled (Supplemental Table S1). This collection of inbreds represents all of the material with an expired or active PVP certificate from 1980 to 2017. As of May 2019, ~460 of the 2,392 inbreds have expired PVP certificates, and the remainder of the active certificates will expire during the next 20 yr. Admixture membership calculated for the ex-PVP inbreds was used to approximate the genetic contribution of each heterotic subgroup to actively protected PVP inbreds based on pedigree data provided in PVP certificates. Any genetic contribution that did not relate back to the current ex-PVP pool via pedigree was classified as "unknown." Utilization of the eight heterotic subgroups was approximated for Pioneer Hi-Bred International, Monsanto Technology, and Syngenta from the late 1980s to 2017, by decade.

To represent highly utilized ex-PVP germplasm, the 200 most referenced PVP inbreds used in the generation of new PVP material were plotted. To visualize the contributions of key backgrounds, the complete pedigree relationships of inbreds with an active or expired PVP certificate were visualized using Helium (Shaw, Graham, Kennedy, Milne, & Marshall, 2014). Key male and female backgrounds for Pioneer Hi-Bred International and Monsanto Technology were also generated.

2.7 | Yield analysis of hybrids from a subset of ex-PVP inbreds

To evaluate the yield interaction of inbreds in different heterotic groups, a series of diallel experiments were developed. A set of 48 ex-PVP inbreds was chosen based on their diversity and relevance in modern PVP germplasm (Table 1, Supplemental Tables S2a–S2e).

TABLE 1 List of parental inbreds for each of the five diallel experiments. The half-diallel crossing scheme produced hybrids without reciprocals or selfs (Griffing Method 4) for evaluation in hybrid yield trial

Early	Intermediate I	Intermediate II	Intermediate III	Late
3IBZ2	29MIBZ2	78010	3IIH6	HBA1
CR1HT	2FACC	3IIH6	LH145	LH195
PHAA0	AQA3	LH185	LH185	LH250
PHEM7	FBLA	MBST	LH198	PHG35
PHEM9	LH225	PHJR5	LH82	PHG39
PHGG7	LH82	PHK76	NKH8431	PHK93
PHJ40	NKH8431	PHN73	PHB47	PHR32
PHK05	PHJ89	PHT11	PHG72	PHW51
PHM81	PKE6	PHW20	PHJ89	PHW65
PHRE1	PHP02	PHG72	PHK56	PHMK0
	PHT69	PHB47	PHK76	PHR03
	PHW03		PHN46	
	LH145		PHP02	

The inbreds were grouped by maturity and crossed within groups in a half-diallel fashion without reciprocals or selfs, forming five experiments: one early-, three intermediate-, and one late-maturity diallel experiments. Hybrids were grown in two-row yield trial plots in a randomized complete block design. Rows were 6 m long, with 0.76-m row spacing at a planting density of 85,900 plants ha^{-1}. Four of the five diallel experiments were grown at the Arlington Agricultural Research Station (Arlington, WI) and the West Madison Agricultural Research Station (Verona, WI) in 2016 and 2017. The fifth diallel experiment (Intermediate-Maturity III) was grown in the same two locations in 2015, 2016, and 2017. The following agronomic traits were measured: stand count, anthesis and silking time in growing degree days (McMaster & Wilhelm, 1997), plant and ear height, grain moisture, test weight, and yield. General and specific combining ability were calculated in R (R Core Team, 2012) to provide estimates for additive and nonadditive effects for yield (Griffing, 1956). Specific combining ability effects between parental inbreds from all five diallel experiments were visualized using Circos (Krzywinski et al., 2009).

3 | RESULTS

3.1 | Identification of heterotic groups and subgroups

The three major heterotic groups (Stiff Stalk, Iodent, and non-Stiff Stalk) were visualized as the three largest

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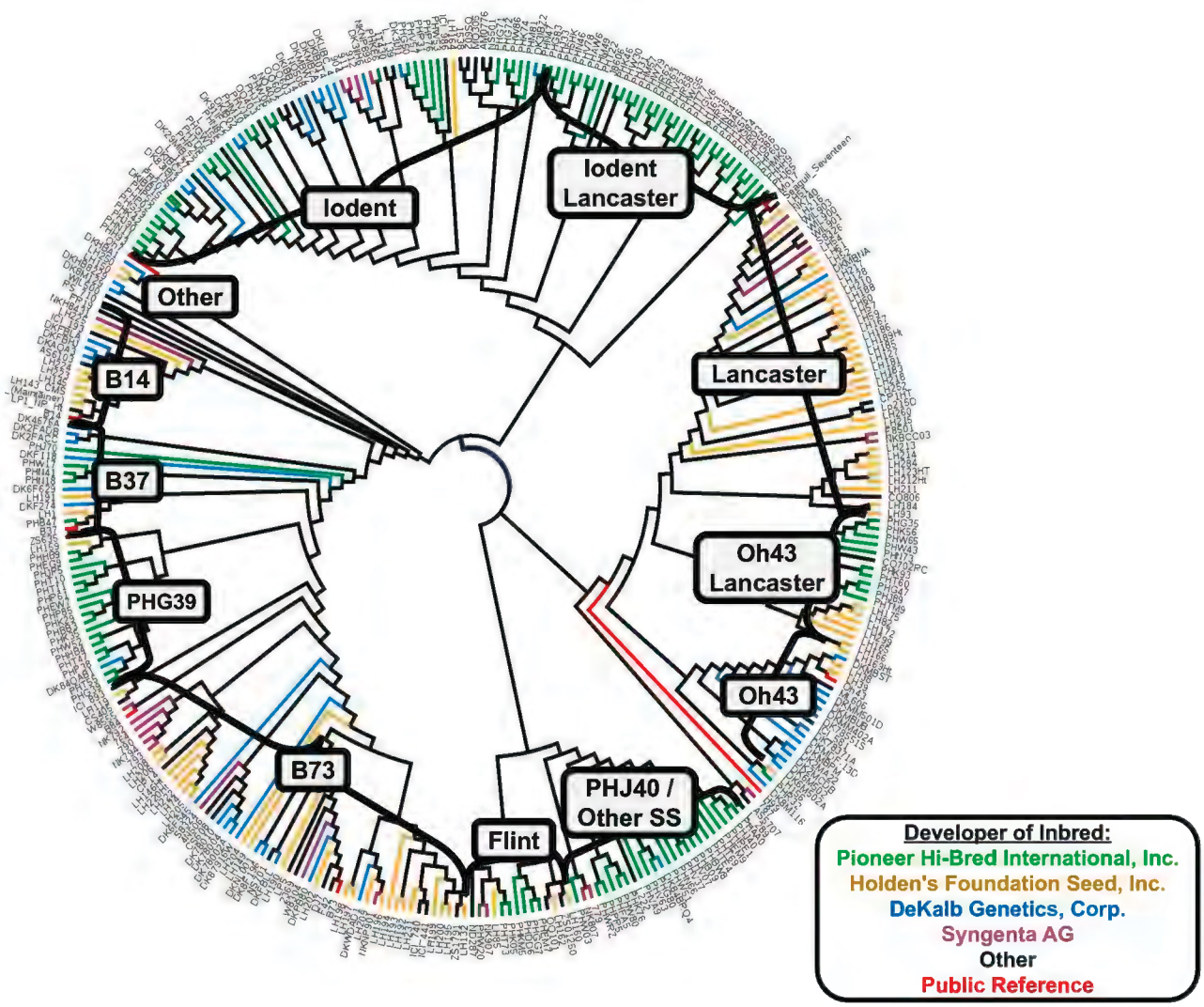


FIGURE 1 Dendrogram based on neighbor-joining clustering from genetic distance matrix consisting of 899,784 single nucleotide polymorphisms across 329 expired Plant Variety Protection inbreds and eight founder lines. Individuals are colored based on the developer. This circular representation highlights the relationship and heterotic subgroup structure present in this group of individuals. Major clusters of inbreds are labeled broadly based on their relevant founder

dendrogram branches (Figure 1) consistent with the broad heterotic groups used today. Membership of the eight heterotic subgroups was approximated for the inbreds in the ex-PVP panel from the admixture analysis (Supplemental Table S1). The eight *K* groups corresponded to the following heterotic subgroups: B14, B37, B73, Oh43, Iodent, Iodent-Lancaster, Lancaster, and Flint, with inbreds B14, B37, B73, Oh43, PH207, PHZ51, Mo17, and PHGG7, respectively, having the highest membership in each group. The founding inbreds B73, Oh43, PH207, and Mo17 clustered on the extremities of their respective heterotic arm (shown in Figure 2). Figure 2 reveals the separation of the three major heterotic groups, which is observed on the primary and secondary planes. The Stiff Stalk, Iodent, and non-Stiff Stalk heterotic groups defined the majority of the structure. The tertiary plane shows the separation of the non-Stiff Stalk heterotic group into the Lancaster and Oh43 heterotic subgroups. The individuals present in the less discrete Flint and Iodent-Lancaster heterotic subgroups fell within the internal structure of the graph and represented a smaller amount of the diversity present. Inbreds from multiple companies had a strong relationship to the heterotic subgroups resulting from the admixture analysis, which demonstrates that multiple companies used similar original source material.

The Stiff Stalk heterotic group was separated into subgroups associated with its primary founders from the Iowa Stiff Stalk Synthetic: B73, B37, and B14 (Figures 1 and 2). Interestingly, a subgroup of early-maturity inbreds founded by the ex-PVP inbred, PHJ40, considered to act within the Stiff Stalk pattern (Mikel, 2011), clustered near the Flint heterotic subgroup; this subgroup is labeled as PHJ40/Other Stiff Stalk. Inbreds founded by PH207 clustered in the Iodent subgroup. The Iodent-Lancaster subgroup contained inbreds developed

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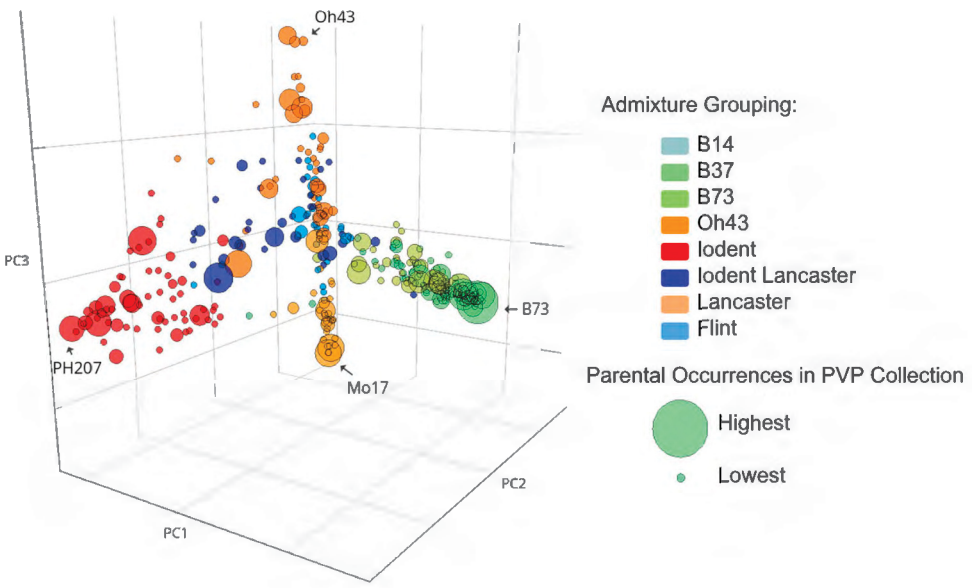


FIGURE 2 The first three dimensions of a multidimensional scaling plot from the genetic distance matrix containing 899,784 single nucleotide polymorphisms of the 337 expired Plant Variety Protection (ex-PVP) panel. Individuals are colored based on their majority admixture membership, highlighting population substructure present within each of the Stiff Stalk, Iodent, and Non-Stiff Stalk heterotic groups. PC, principal component

solely by Pioneer Hi-Bred International and was founded primarily by proprietary inbreds PH814, PH848, and PH595 introgressed with Iodent PH207 descendants. The Lancaster subgroup is largely based on inbreds with Mo17 and C103 heritage. The Oh43-Lancaster subgroup (Figure 1) features LH82, an inbred derived from crosses of parental lines with a diverse origin that is an important contributor to the pedigrees of multiple recent PVPs. LH38 is the primary ex-PVP progenitor of the Oh43 heterotic subgroup. Finally, the small representation of Flint germplasm within our study clustered within its own subgroup and includes some of the earliest flowering inbreds.

3.2 | Population genetics and allelic diversity of ex-PVP inbreds

Out of the 899,784 available SNPs, the WiDiv-942 had 430,695 SNPs with a minor allele frequency (MAF) > 0.05, whereas the ex-PVP panel had 384,211 SNPs with a MAF > 0.05 (Table 2). The proportion of monomorphic SNPs in the Iodent and B73 heterotic subgroups indicates strong selection or drift with fixation of up to 81.3% of SNPs, which are polymorphic in the WiDiv-942 (Table 2).

Average pairwise divergence (π), Watterson estimator, and Tajima's D provided evidence that alleles that are rare in WiDiv-942 are present at high frequencies in the Iodent and B73 heterotic subgroups (Table 3), indicating population expansion and/or purifying selection after a recent selective sweep or bottleneck event. Chromosome 4 contained the least diversity for the WiDiv-942, ex-PVP panel, and Iodent subgroups, whereas the B73 subgroup contained the least diversity on chromosome 2, as measured by MAF, π, and Tajima's D. In particular, chromosome 2 of the B73 heterotic subgroup had a Tajima's D value of −1.22, which provided evidence of a bottleneck on allelic diversity as a result of selection and population expansion (Table 3).

The visualization of allele frequency for the inbreds within each heterotic subgroup revealed different patterns of diversity at varying chromosomal locations across heterotic subgroups (Figure 3). Average major allele frequency for chromosome one was calculated to be 94.5, 93.8, 94.4, 92.4, 93.9, 90.5, 89.5, 89.2, and 91.1% for the inbreds present in the B14, B37, B73, Oh43, Iodent, Iodent-Lancaster, Lancaster, Flint, and Other heterotic subgroups, respectively. Haplotype > 20 Mb in length with <2% MAF were observed for the inbreds within the B14, B73, and Iodent heterotic subgroups, indicating a reduction in diversity as a result of selection towards the founder allele. The visualization of major allele frequency, across a sliding window of 200 SNPs indicated numerous regions of the genome of shared haplotypes between heterotic subgroups (Supplemental Figure S1).

3.3 | Linkage disequilibrium

Elevated levels of LD were observed among inbreds belonging to the B73 and Iodent heterotic subgroups relative to the ex-PVP group or CIMMYT-derived inbreds (Figure 4). The violin plots show the distribution and mean R^2 for

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TABLE 2 Diversity of the expired Plant Variety Protection (ex-PVP) inbred lines was compared with the larger Wisconsin Diversity Panel with 942 individuals (WiDiv-942), which included the ex-PVP inbreds. The table contains, per chromosome, the number of single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) > 5% for the WiDiv-942, the ex-PVP panel, and ex-PVP Iodent or B73 subsets, which contain 942, 337, 62, and 66 individuals, respectively. In addition, the proportion of monomorphic SNPs in the subset and polymorphic in the WiDiv-942 is reported for the ex-PVP panel, Iodent, and B73 subsets

	No. of SNP loci with MAF > 0.05				Proportion of SNPs monomorphic in each subset and polymorphic in WiDiv-942		
Chr.	WiDIV-942	ex-PVP-337	Iodent	B73	ex-PVP-337	Iodent	B73
1	68,813	61,013	44,512	35,371	0.315	0.523	0.624
2	51,654	47,733	34,185	26,096	0.410	0.682	0.813
3	48,564	43,785	33,846	26,605	0.290	0.501	0.584
4	39,466	34,711	22,498	20,386	0.298	0.538	0.606
5	52,953	46,908	36,510	28,405	0.269	0.463	0.540
6	34,728	31,019	23,229	19,008	0.304	0.511	0.641
7	34,986	30,940	21,151	18,482	0.323	0.563	0.646
8	37,413	33,062	24,927	21,750	0.304	0.513	0.586
9	32,499	29,286	21,844	18,678	0.291	0.494	0.566
10	29,619	25,754	17,497	15,656	0.301	0.546	0.583
All	430,695	384,211	280,199	230,437	0.301	0.522	0.606

TABLE 3 Diversity of groups compared using allelic diversity statistics: pairwise divergence (π), Watterson estimator, and Tajima's *D*. A comparison is provided for the Wisconsin Diversity Panel with 942 individuals (WiDiv-942) population relative to the expired Plant Variety Protection (ex-PVP) panel, and Iodent or B73 heterotic subgroups, which contain 942, 337, 62, and 66 individuals, respectively

	Pairwise divergence (π)				Watterson estimator				Tajima's *D*			
Chr.	WiDIV-942	ex-PVP-337	Iodent	B73	WiDIV-942	ex-PVP-337	Iodent	B73	WiDIV-942	ex-PVP-337	Iodent	B73
1	0.16	0.15	0.09	0.09	0.13	0.11	0.10	0.09	0.63	1.28	−0.31	−0.29
2	0.16	0.15	0.10	0.06	0.13	0.11	0.10	0.10	0.60	1.15	−0.04	−1.22
3	0.16	0.15	0.11	0.09	0.13	0.11	0.11	0.10	0.63	1.14	0.21	−0.07
4	0.16	0.14	0.08	0.07	0.13	0.11	0.10	0.09	0.51	0.98	−0.74	−0.81
5	0.16	0.15	0.11	0.08	0.13	0.11	0.10	0.10	0.65	1.17	0.18	−0.73
6	0.16	0.14	0.10	0.07	0.13	0.11	0.10	0.09	0.56	1.05	−0.16	−0.63
7	0.16	0.15	0.08	0.10	0.13	0.11	0.10	0.09	0.59	1.26	−0.47	0.26
8	0.16	0.15	0.10	0.08	0.13	0.11	0.10	0.10	0.60	1.09	−0.13	−0.74
9	0.17	0.15	0.10	0.09	0.13	0.11	0.11	0.10	0.72	1.21	−0.23	−0.25
10	0.16	0.15	0.08	0.08	0.13	0.11	0.10	0.09	0.59	1.08	−0.60	−0.67
All	0.16	0.15	0.1	0.08	0.13	0.11	0.10	0.09	0.61	1.15	−0.19	−0.53

each subset and bin by distance. Linkage disequilibrium was the highest within the B73 heterotic subgroup, which included 66 inbreds, with an average R^2 of 0.60 between windows of SNPs < 1 kb, compared with R^2 of 0.36 for the ex-PVP panel with 337 inbreds. The CIMMYT inbreds showed the least LD, less than half compared with the B73 and Iodent heterotic subgroups, consistent with their relatively broad diversity. Elevated LD (R^2 > 0.30) was found to persist for windows of SNPs 100 kb apart in the Iodent and B73 heterotic subgroups, indicating limited recombination among the elite ex-PVP inbreds within heterotic subgroups.

3.4 | Contribution of available ex-PVPs to current protected inbreds

Pedigrees provided in PVP certificates allow evaluation of the genetic contribution of ex-PVP inbreds to recently developed PVP inbreds currently under continuing protection. Estimation of admixture membership using pedigree information revealed that 317 of the 2,082 actively protected PVP inbreds contained 100% genetic contribution from ex-PVP inbreds available as of 2018. In addition, 1706 of the 2,082 actively protected PVP inbreds contained at least one-fourth genetic contribution directly from an inbred in the current

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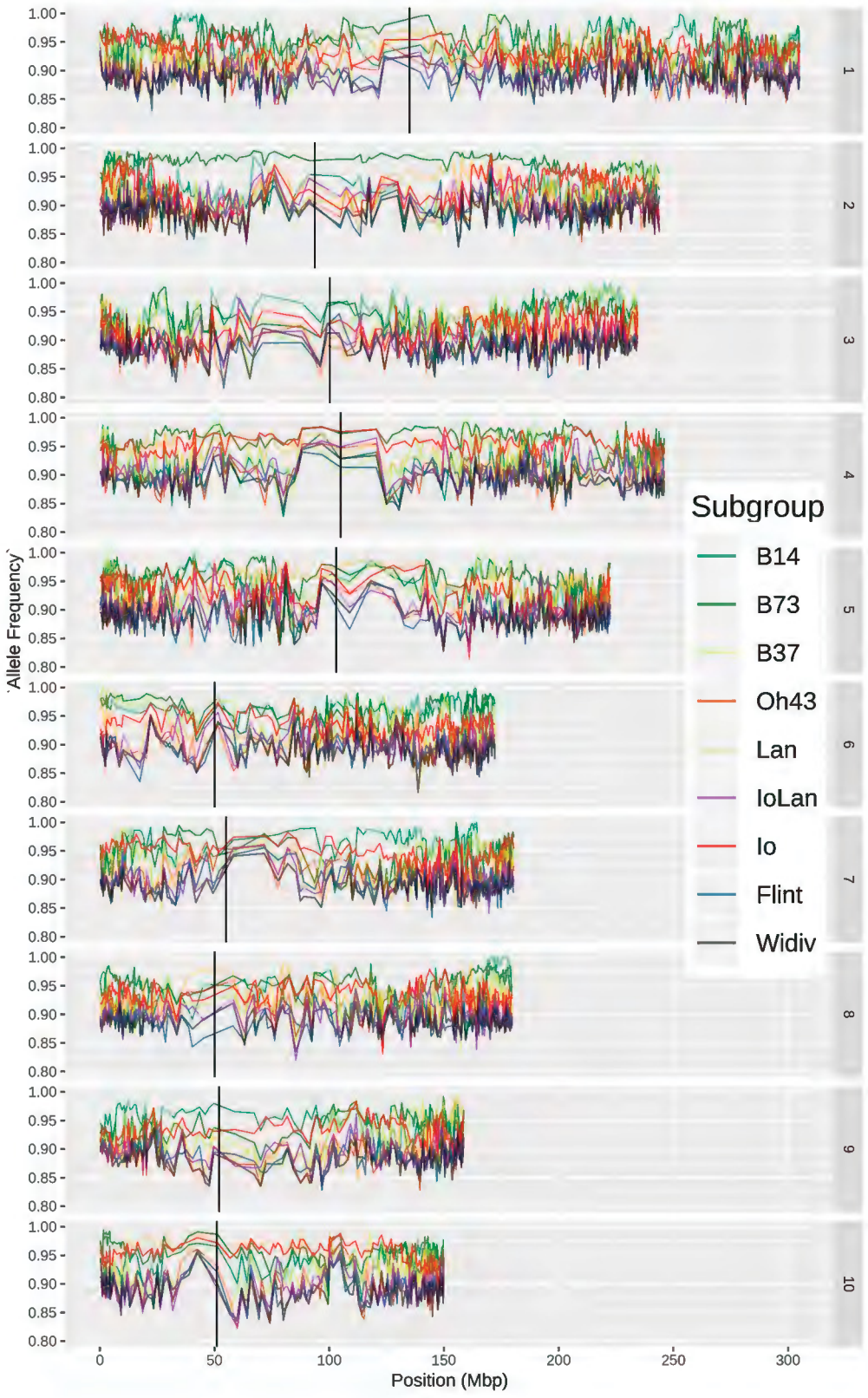


FIGURE 3 Allele frequency calculated using windows of 200 single nucleotide polymorphisms (SNPs) by chromosome across all 899,784 SSNPs derived from RNA sequencing for the B14, B37, B73, Oh43, Iodent (Io), Iodent-Lancaster (IoLan), Lancaster (Lan), and Flint heterotic subgroups. For comparison, the allele frequency for the Wisconsin Diversity Panel (WiDiv) with 942 inbreds was shown in black

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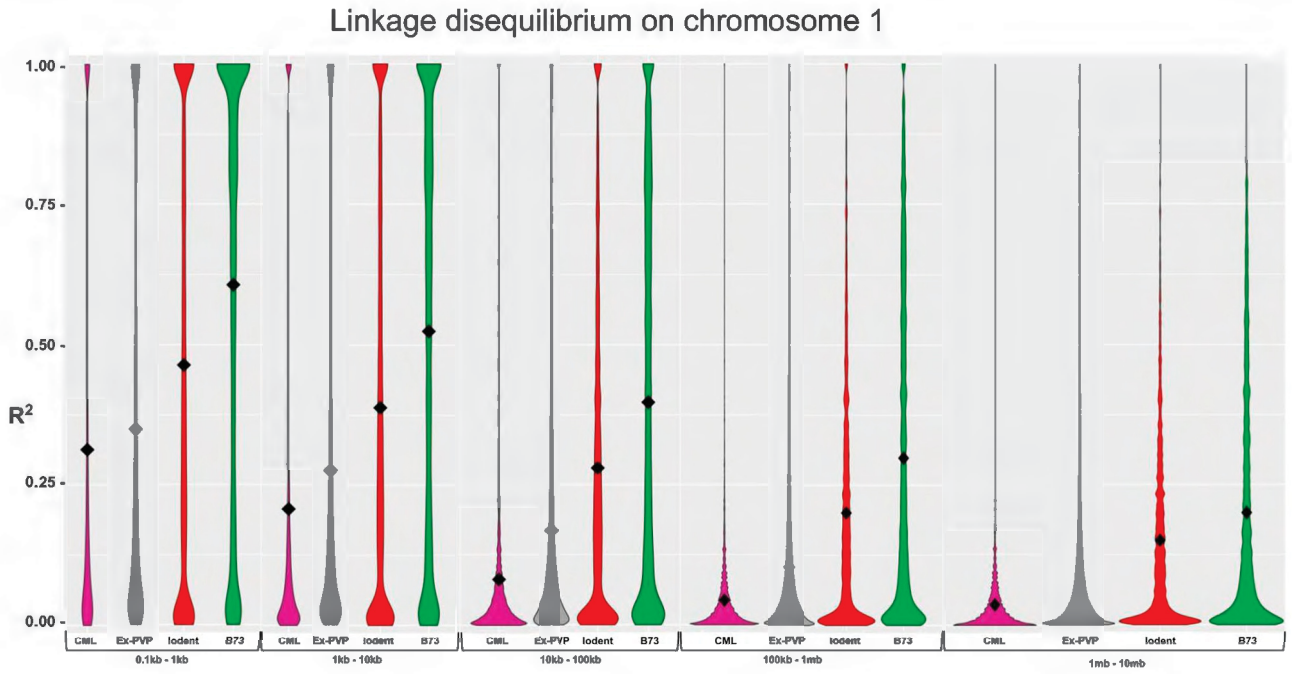


FIGURE 4 Linkage disequilibrium on chromosome one for the International Maize and Wheat Improvement Center (CIMMYT), expired Plant Variety Protection (ex-PVP), Iodent, and B73 subpopulations containing 37, 337, 66, and 60 individuals, respectively. Pairwise single nucleotide polymorphism R^2 values were binned on distance. The mean R^2 is displayed by the black point

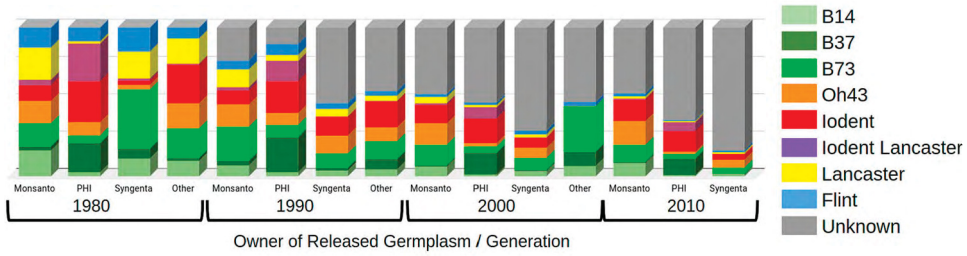


FIGURE 5 The utilization of admixture indicated heterotic subgroups present in the current set of 329 expired Plant Variety Protected inbreds by major developers from 1980 to 2010. Gray represents unknown contributions in the pedigree of a line that does not trace back to the Plant Variety Protection collection. PHI, Pioneer Hi-Bred International

ex-PVP set of materials. In total, 87% of the actively protected PVP inbreds were documented to be at least partially derived from the available ex-PVP inbreds. These are likely conservative estimates because of ambiguity of pedigrees in PVP certificates, which are classified as "unknown" germplasm in this analysis. Unknown germplasm often includes inbreds derived from competitor hybrids or unreferenced, proprietary germplasm. Pedigree analysis estimated that at least 50 and 30% of the germplasm developed since 2010 by Monsanto Technology and Pioneer Hi-Bred International, respectively, was derived directly from the current ex-PVP pool (Figure 5). Because of ambiguity in the pedigrees provided in the PVP certificate, it is likely that a majority of the remaining 50–70% of the germplasm is also derived from within the primary historical breeding pools, although introgression of new alleles has been emphasized by breeders within these companies.

3.5 | Hybrid performance of inbred lines varying in origin and genetic relatedness

To assess the performance of inbreds from crosses within and between company germplasm pools, and as another measure of genetic relatedness, we conducted an evaluation of five diallel sets of ex-PVP hybrids grown in a multiyear, hybrid yield trial (Table 4).

General and specific combining ability estimates for grain yield as indicators of additive and nonadditive (dominance and epistatic) variance were significant for all five sets of hybrids ($p < 6.68 \times 10^{-15}$) (Supplemental Tables S2a–S2e). For the early-maturity diallel (Supplemental Table S2a), inbreds PHM81 and PHK05 had the highest general combining ability for grain yield. The hybrids PHEM9 × PHGG7 and PHAA0 × PHEM7 had the highest specific combining ability

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TABLE 4 Summary of the five diallel experiments showing number of inbred parents, number of environments, and average anthesis and silking growing degree days (GDD), grain moisture at harvest, and adjusted grain yield

Experiment	No. of parental inbreds	No. of environments	Avg. pollen date	Avg. silk date	Avg. grain moisture hybrid	Avg. yield
			—GDD—		%	Mg ha^{-1}
Early I	10	4	1079	1075	20.11	9.3
Intermediate I	13	4	1193	1197	19.26	12.4
Intermediate II	11	4	1216	1213	22.50	11.7
Intermediate III	13	6	1238	1235	21.50	12.7
Late I	11	4	1391	1394	32.00	9.5

for grain yield. Both of these hybrids featured a Pioneer Hi-Bred International B37/PHJ40 × Flint heterotic pattern, which performed best in the 92- to 100-d maturity group within the tested germplasm and environments.

Three diallels represented intermediate maturity (Supplemental Tables S2b–S2d) (100- to 105-d maturity), with some parents overlapping across the diallel groups. In the Intermediate I diallel, inbreds PHJ89 and PHP02, and 2FACC had the highest general combining abilities for grain yield. The hybrids LH225 × PHKE6 and LH225 × LH82 had the highest specific combining ability for grain yield. In the Intermediate II diallel, 78010 and LH185 had the highest general combining ability for grain yield. The hybrids 78010 by PHB47 and PHK76 by PHW20 had the highest specific combining abilities for grain yield. In the Intermediate III diallel, PHB47 and LH198 had the highest general combining ability for grain yield. The hybrids PHB47 × NKH8431 and PHB47 × PHJ89 had the highest specific combining ability for grain yield. It is notable that hybrids (PHB47 × NKH8431, PHB47 × LH145, and 78010 × PHB47) had high specific combining ability and came from crosses between inbreds originating from different companies' Stiff Stalk subgroups. These results highlight the complexity of predicting heterosis in maize and suggest opportunities for noncanonical hybrids, such as those between Stiff Stalk subgroups, and in crosses among rarely documented commercial hybrids featuring an Iodent × Oh43 or Lancaster heterotic pattern (PHP02 × PHJ89). The high performance of inbreds from smaller germplasm groups is also of interest and it is exemplified by inbreds, such as PHJ89 from the Oh43 group and LH82 representing a mixed origin.

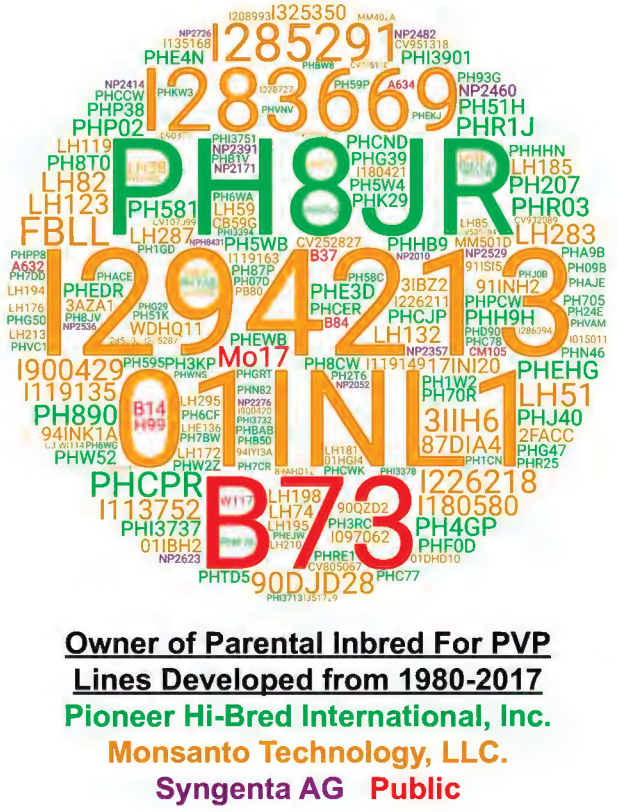
In the late-maturity diallel (Supplemental Table S2e) (105- to 112-d maturity), PHW51 and PHR32 had the highest general combining ability for grain yield. The hybrids PHR03 × PHG39 and PHG39 × PHMK0 had the highest specific combining ability for grain yield, demonstrating two potential high performing hybrids. These represent the Pioneer Hi-Bred International B37 × Iodent-Lancaster heterotic pattern. However, the average yield of the late-maturity diallel was 2.6 Mg ha^{-1} less compared with the intermediate maturity diallels in similar environments. In addition to the higher grain moisture at harvest, the average maturity of the late-maturity diallel hybrids was not optimized for performance in the northern environments in which the study was conducted.

The specific combining abilities of hybrids for grain yield from the ex-PVP diallel experiments helped illustrate the relevance of specific heterotic patterns. Hybrids with a positive specific combining ability often contain two parental inbreds that belong to opposite heterotic subgroups. Conversely, hybrids with a negative specific combining ability were often composed of parental inbreds within the same heterotic subgroup (Supplemental Figure S2). The maturity of closely related inbreds is often similar, specifically in the Iodent and Flint heterotic subgroups. Although similar in background, the separation between the Iodent and Iodent-Lancaster heterotic subgroups is evident when comparing the maturity of inbreds.

4 | DISCUSSION

The canonical concept of heterosis in maize centers on Stiff Stalk vs. non-Stiff Stalk heterotic patterns used broadly across industry and academia. Our analysis indicates that this is overly simplistic. Analysis of inbred relationships in the context of company developer reveals that major industry breeding programs likely leveraged different subgroup heterosis in their breeding efforts through this period. Monsanto Technology, via Dekalb Genetics Corporation and Holden's Foundation Seed, substantially used the B73 subgroup often crossed with inbreds in the Mo17/C103 subgroup, leveraging the canonical B73 × Mo17 heterosis. Pioneer Hi-Bred International developed private clusters of inbreds representing the Iodent-Lancaster subgroup and Stiff Stalk predominated by the PHG39 and B37 subgroups, consistent with the notion that heterosis was maximized between these groups in their breeding program. Pioneer Hi-Bred International also developed the Iodent subgroup, important for shorter-season hybrids. Iodent germplasm was subsequently accessed by other

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Owner of Parental Inbred For PVP Lines Developed from 1980-2017

Pioneer Hi-Bred International, Inc.

Monsanto Technology, LLC.

Syngenta AG **Public**

FIGURE 6 Set of 200 most frequently used parental inbreds in the development of new Plant Variety Protection (PVP) Certificates from 1980 to 2016, colored by developer. Pioneer Hi-Bred International (green), Monsanto Technology (orange), Syngenta (purple), and public (red). The names of inbreds are scaled based on appearance frequency

breeding programs through the selfing of hybrids, such as Pioneer brand hybrid 3737, which included crosses between the noncanonical subgroups Iodent by Oh43/Lancaster (Mikel, 2011). 3IIH6 is an inbred derived from Pioneer brand hybrid 3737, initiating an important Iodent–Oh43 non-Stiff Stalk lineage within Monsanto Technology. Broader access to the Iodent group is indicated by the number of companies with inbreds within the Iodent subgroup (Figure 1). Major commercial breeding programs during this era had relatively distinct germplasm pools, which likely strengthened heterosis between those pools.

Germplasm utilization across companies continues to evolve. The 200 most frequently used parental lines for the generation of elite inbred parents from 1980–2017 are shown in Figure 6, and the top 15 most frequently used are listed in Table 5. Use of the non-Stiff Stalk Lancaster heterotic subgroup has dropped sharply, followed by the increased utilization of the Iodent and Oh43 heterotic subgroups (Figure 5). Direct utilization of competitor germplasm via breeding from hybrids, which was allowed under PVP, has largely ceased after the 1990s because of the increased practice of patenting both the inbred and hybrids with utility patents in addition to PVP certificate.

The top five parental lines are I294213 (Monsanto Technology) and B73 (public) from the Stiff Stalk heterotic subgroup, and PH8JR (Pioneer Hi-Bred International), Inbred I283669 (Monsanto Technology), and 01INL1 (Monsanto Technology) from the Iodent/Oh43 heterotic subgroups. I294213 was developed by Monsanto Technology from a biparental cross between two proprietary inbreds, 01DHD10 and 90DJD28.

TABLE 5 Fifteen most frequently used maize inbreds based on Plant Variety Protection certificates through 2017. Genotype, number of parental occurrences, pedigree, year of filing, and the responsible entity in 2019 are shown

Genotype	Parental occurrences in PVP collection	Pedigree	Year developed	Responsible entity in 2019
I294213	57	01DHD10/90DJD28	2004	Bayer
PH8JR	47	PH24E/PH1B8	2004	Corteva
I283669	44	01INL1/M37AZ-25//01INL1	2006	Bayer
B73	44	BSSSC5	1973	Public
I285291	39	01INL1/17INI20	2005	Bayer
90DJD28	39	P3180/FBLL	1997	Bayer
01INL1	39	3IIH6/MM501D//3IIH6	1999	Bayer
I226218	38	01INL1/LH283	2005	Bayer
PHCPR	34	PH1W2/PH57T	2005	Corteva
I180580	30	87DIA4/WQCD10	2002	Bayer
I900429	26	01INL1/91ISI5	2004	Bayer
I113752	24	01HGI4/LH283	2004	Bayer
FBLL	23	5B2C-A/PB80	1991	Bayer
I119135	22	3AZA1/87DIA4	2004	Bayer
PHR1J	22	PH4GP/PH6WA	2008	Corteva

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I294213 continues the B73 lineage from both of its parents and has been extensively bred as a direct parent of 57 PVP inbreds developed during 2006–2015, indicating that it is a core component of Monsanto Technology temperate Stiff Stalk germplasm (Supplemental Figure S3).

The Pioneer Hi-Bred International Iodent inbred PH8JR was derived from the cross PH24E and PH1B8, both of which are descendants of the Iodent progenitor PH207. Inbred PH8JR was a parent in the development of 47 new Pioneer Hi-Bred International inbreds from 2007–2015, constituting a large component of contemporary Pioneer Hi-Bred International's germplasm (Supplemental Figure S4).

Monsanto Technology Iodent inbred 01INL1 was developed from a three-way cross of 3IIH6 and MM501D, with its recurrent parent 3IIH6 bringing a genetic contribution of the Iodent PH207 lineage from Pioneer Hi-Bred International via Pioneer Brand hybrid 3737. Inbred 01INL1 has been extensively bred into Monsanto Technology non-Stiff Stalk germplasm as a direct parent of 40 new PVP inbreds from 2004–2011. One progeny of 01INL1 is I283669, which was developed from the three-way cross of 01INL1 and M37AZ-25, with the recurrent parent 01INL1 contributing three-fourths to I283669. Inbred I283669 further extends the Iodent/Oh43 01INL1 lineage as a direct parent of 44 new PVP inbreds from 2006–2015. This mixed Iodent/Oh43 lineage through 3IIH6, 01INL1, and I283669 constitutes a large portion of Monsanto Technology's non-Stiff Stalk germplasm (Supplemental Figure S5).

The fourth most referenced parental inbred was B73, a descendent from the Iowa Stiff Stalk Synthetic. B73 had been used since the 1970s in breeding Stiff Stalk inbreds in the initiation of its heterotic components across all hybrid maize breeding programs. B73-derived inbreds were used to a great extent by Syngenta and Monsanto Technology acquired companies. Pioneer Hi-Bred International's use of B73 germplasm initially lagged, but its genetic contribution to their Stiff Stalk germplasm has increased largely from breeding with the PHW52 (PHG39 × B73) lineage. This research confirms the dominant contribution of B73 to Stiff Stalk germplasm across contemporary proprietary programs.

Stiff Stalk founders B14 and B37 maintain legacy importance in PVP pedigrees. B14-derived inbreds have affected contemporary short-season germplasm primarily through its descendants A632, A634, and CM105, enabling hybrid placement further north in the Corn Belt. The yield potential and combining ability of B14 were increased through breeding with the fifth cycle BSSS B73, facilitating advantageous recombination between the B14 and B73 subgroups (Troyer, 1999). Several noteworthy B14-derived inbreds include Monsanto Technology inbreds FBLA, AQA3, and LH145, and also Syngenta inbred NKH8431.

The B37 heterotic subgroup was largely derived from breeding with the first-cycle Stiff Stalk B37. This germplasm was used across commercial programs, generally in the development of longer season hybrids, because of the late maturity and health characteristics of this group. The Pioneer Hi-Bred International breeding program used B37 germplasm most extensively and was able to gradually breed B37 germplasm into shorter-season areas of the northern Corn Belt. The most important progenitor of this heterotic subgroup is PHG39. Pedigree information indicates that the PHG39/B37 subgroup has a small amount of exotic germplasm from Maiz Amargo (Mikel, 2011; Mikel & Dudley, 2006).

PHJ40 is a progenitor of Pioneer Hi-Bred International's short-season Stiff Stalk germplasm and was developed from the biparental cross of two inbreds, PHB09 and PHB36. Although the background of PHB36 is not known publicly, admixture membership places PHJ40 primarily as B37 with minor Oh43 and Lancaster contributions, confirming the known pedigree contributions (PVP 9600171 Exhibit A). Compared with its first-cycle Stiff Stalk relatives, PHJ40 represents a relatively unique subgroup because of its diversity, as illustrated by the clustering analysis of genomic marker data. This behavior was also exhibited in the early-maturity diallel, providing evidence of high performance via general and specific combining abilities with both Flint and Iodent heterotic subgroups. Recently, PHJ40's lineage has been carried forward in combination with B73, such as in the case of Pioneer Hi-Bred International inbred PHBW8 (Supplemental Figure S6).

Non-Stiff Stalk subgroups of importance in PVP inbreds include Lancaster, Iodent, Iodent-Lancaster, Oh43, and Flint. Initially, Mo17/C103 based Lancaster germplasm was extensively used in the development of non-Stiff Stalk germplasm across all of the proprietary corn programs with the exception of Pioneer Hi-Bred International. A few decades later, Mo17 was bred into Pioneer Hi-Bred International non-Stiff Stalk germplasm in the PH581 and PH8PG lineages (PVP200200190 Exhibit A, PVP200100250 Exhibit B). The contribution of Mo17-based Lancaster germplasm to the broader non-Stiff Stalk pool has decreased during the past few decades, and it has been replaced by and introgressed with Iodent germplasm.

The Iodent subgroup originated from the landrace Reid Yellow Dent in 1902, undergoing numerous cycles of recurrent selection for early maturity by Professor Lyman C. Burnett at Iowa State University (Troyer, 1999). The major Iodent progenitor is Pioneer Hi-Bred International inbred PH207, whose lineage has also permeated the non-Stiff Stalk germplasm of Monsanto Technology and Syngenta. In the 1980s, Iodent germplasm was established outside of Pioneer Hi-Bred International through prolific breeding of Pioneer brand hybrids containing PH207 lineage (Mikel, 2011). Notable early cycle descendants of PH207 are PHP02, PHG29, and 3IIH6. Key features of Iodent inbreds include excellent general combining ability as well as tolerance

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to elevated planting densities and biotic stress (Troyer, 1999).

The Iodent-Lancaster heterotic subgroup represents Pioneer Hi-Bred International's effort to adapt high-yielding, full-season Lancaster germplasm to become better adapted to the central Corn Belt by breeding with shorter season Iodent germplasm. Notable founders of Pioneer Hi-Bred International Iodent-Lancasters are inbreds PH814, PH848, and PH595 (Smith & Smith, 1989) originating from Oh07, proprietary synthetic female composite, Lancaster, Midland Yellow Dent, and Osterland Yellow Dent. Inbreds derived from these populations were combined with PH207-based Iodent germplasm to formulate a 108- to 114-d maturity group. As PH814, PH848, and PH595 are not currently publicly available, admixture results from the ex-PVP panel placed half-siblings PHZ51 (PH848 × PH814) and PHG84 (PH848 × PH595) as putative founders of this Iodent-Lancaster heterotic subgroup. The Iodent-Lancaster heterotic subgroup is frequently crossed against complementary PHG39-based Stiff Stalk germplasm for use in intermediate- to late-maturity zones. This is supported by the performance of the PHG39 by PHR03 hybrid in our late-maturity diallel.

G.H. Stringfield developed the early-flowering, short-stature inbred Oh43 in 1943 with lineages from synthetic-derived breeding populations containing Lancaster and Minn13 germplasm (Troyer, 1999). Ex-PVP progenitors of this heterotic subgroup are LH38, 3IIH6, and MM501D. Oh43 haplotypes remain prevalent in contemporary Monsanto Technology non-Stiff Stalk lineages through previously mentioned 01INL1. The diallel analysis showed a viable Iodent × Oh43 heterotic pattern supported by the known parentage of Pioneer brand hybrid 3737 (PHG29 × PHG47) (Mikel, 2011) and exemplified by the strong performance of the hybrid PHP02 (progeny of PHG29) by PHJ89 (progeny of PHG47).

Flint germplasm is available in the ex-PVP panel primarily through Pioneer Hi-Bred International. The Flint heterotic subgroup is complemented in hybrids by the Iodent heterotic subgroup (Larièpe et al., 2017). The true "Flint" seed characteristic has been purged to maintain the dominant, market-driven dent phenotype. The 150 inbreds in the PVP system with the highest Admixture membership to the Flint heterotic subgroup have a negative correlation ($r^2 = -0.30$) for flowering time (growing degree days), illustrating the impact of the Flint heterotic subgroup in earlier maturity zones in North America.

Large-scale visualization of pedigrees for elite backgrounds highlight the utilization of B37 (PHG39), B73 (B73), Iodent (PH207), Oh43 (LH38), and Iodent-Lancaster (PHZ51, PHG84) heterotic subgroups, as well as the addition of external germplasm largely composed from competitor hybrids and exotic introgressions (Supplemental Figure S3–S6). Across time, the heterotic subgroups that best structure the diversity present among the ex-PVP panel studied remain mostly intact and have made considerable contributions to the development of new elite material.

4.1 | Allelic diversity within ex-PVPs

Sufficient genetic diversity is necessary to make breeding progress and maintain a reservoir of alleles to address future biotic and abiotic stresses. Selection reduces the frequency of unfavorable alleles, but the positive or negative effect of an allele may be context dependent, and useful alleles may be lost because of linkage or genetic drift. Our results support that allelic diversity has been reduced in PVP material relative to a representative set of available public and proprietary inbreds. Reduction of diversity is most pronounced in groups of related individuals tracing back to primary heterotic group founders such as B73, Mo17, and PH207. Linkage disequilibrium is also high in pools of inbreds with close relationships and limited generations of recombination, which increases the probability of allelic shifts in large haplotypes because of selection through breeding. We observed regions of limited diversity varying by heterotic subgroup and location in the genome. These haplotypes can be of considerable length (>20 Mb), with allele frequencies near fixation within specific heterotic subgroups. Fixed haplotypes in opposing heterotic groups likely strengthen combining ability relationships but should be considered targets for next-generation breeding through enhanced recombination or gene editing to resolve repulsion-phase linkages and release new variation for breeding. These fixed haplotype regions may also be targets for introgression of novel alleles to increase diversity and possibly enhance heterosis.

As developers continue to breed commercial maize in new environments, diversity is critical to future selection efforts. Our analysis of available ex-PVPs through the 1990s shows that major breeding programs have somewhat unique, albeit overlapping, germplasm. Consolidation in the industry, coupled with licensing of parental inbreds carrying transgenic traits has reduced the uniqueness of breeding programs. Opportunities to develop unique germplasm pools are reduced by the current practice of licensing transgenic inbreds to other companies. Without a strategic change in the tester strategy and/or through the implementation of a genomic selection strategy that favors enriched diversity, the progress of incorporating new diversity in a breeding population will be limited to the inbreds that combine well with the established heterotic scheme.

Haplotypes that show evidence of selection across generations merit further research to explore their implications to heterosis theory, complementary gene action, unfavorable linkage drag, and genomic selection, which drives selection for superior commercial hybrids in modern agronomic

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settings. In addition, selection studies that investigate phenotypes are possible using inbreds with expired PVP certificates (Pinnisch, Mowers, Trumpy, Walejko, & Bush, 2012; Bari and Carena, 2015; Gage, White, Edwards, Kaeppler, & de Leon, 2018; Mastrodomenico, Hendrix, & Below, 2018).

4.2 | Implications of making hybrids and breeding populations between company crosses

The 329 ex-PVP inbreds present in the ex-PVP diversity panel represent a core set of germplasm used in breeding the generation of commercial hybrids today. Ex-PVP inbreds may also be a resource for identifying new hybrids that were not previously considered, such as by crossing inbreds originating from different companies. We identified high-yielding hybrids from crosses within a developer's germplasm, such as LH198 × LH185, PHP02 × PHJ89, PHG39 × PHR03, 29MIBZ2 × 2FACC, and 3IIH6 × FBLL. Interestingly, within tested germplasm and environments, some of the highest-performing hybrids were noncanonical, cross-company hybrids, including 2FACC × PHJ89 and PHP02 × FBLA (Supplemental Table S2a–S2e).

4.3 | Process to categorize new ex-PVPs as they become available

With ~750 new ex-PVP inbreds becoming publicly available by 2028, the task of test-crossing new materials against multiple testers to confidently sort germplasm would be daunting for public and small-share breeding programs. Rather, genotyping new germplasm using low-cost molecular markers and implementing genomic tools, such as those outlined in this paper, have the potential to expedite the process of sorting germplasm into established heterotic patterns (Bari & Carena, 2016; Beckett et al., 2017, 2019). Through the utilization of admixture and other clustering methods to sort new germplasm into workable families of diversity, researchers can efficiently source new germplasm to initiate or improve existing populations while attempting to maintain the desirable heterotic scheme. This study has demonstrated the potential value of the germplasm present in the maize PVP collection and offers methods of analysis with publicly available statistical software tools to enable small-share breeders to make informed decisions on sourcing germplasm.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to conception, data acquisition, analysis, and writing of the manuscript.

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