ORIGINAL PAPER

Jochen C. Reif · Sonia Hamrit
Martin Heckenberger · Wolfgang Schipprack
Hans Peter Maurer · Martin Bohn
Albrecht E. Melchinger

Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years

Abstract It has been claimed that the system that delivers the products of plant breeding reduces the diversity of cultivated varieties leading to an increased genetic vulnerability. The main goal of our study was to monitor the temporal trends in genetic diversity over the past five decades among maize cultivars with the largest acreage in Central Europe. Our objectives were to (1) investigate how much of the genetic diversity present in important adapted open-pollinated varieties (OPVs) has been captured in the elite flint germplasm pool, (2) examine changes in the genetic diversity among the most important commercial hybrids as well as in their dent and flint parents, (3) analyze temporal changes in allele frequencies between the dent and flint parental inbreds, and (4) investigate linkage disequilibrium (LD) trends between pairs of loci within the set of parental dent and flint lines. We examined 30 individuals of five prominent OPVs from Central Europe, 85 maize hybrids of economic importance, and their dent and flint parental components with 55 SSRs. LD was significant at probability level $P = 0.01$ for 20.2% of the SSR marker pairs in the 82 dent lines and for 17.2% in the 66 flint lines. The dent and flint heterotic groups were clearly separated already at the beginning of hybrid breeding in Central Europe. Furthermore, the genetic variation within and among varieties decreased significantly during the five decades. The five OPVs contain numerous unique alleles that were absent in the elite flint pool. Consequently, OPVs could present useful sources for broadening the genetic base of elite maize breeding germplasm.

Keywords European maize · Genetic diversity · Linkage disequilibrium · SSRs · Temporal trends

Introduction

Hybrid maize breeding programs were started in Central Europe in the 1950s. As a promising heterotic pattern, high yielding US dent lines were crossed with adapted European flint lines (Schnell 1992). During the initial phase, dent inbreds from the US were primarily selected for earliness. The steady influx of dent germplasm from North America to Europe has continued over the past 50 years. In contrast, the parental flint inbreds were developed by selfing from a few European open-pollinated varieties (OPVs) such as Lacaune, Lizagaraute, Gelber Badischer Landmais, and Rheintaler (Messmer et al. 1992). Therefore, it can be conjectured that (1) a bottleneck occurred in the flint pool during the transition from OPVs to hybrids and (2) OPVs, which did not serve as germplasm source for the original flint inbreds, contain untapped allelic variation useful for future breeding progress. Detailed information about a reduction in genetic diversity could help to emphasize the importance of identifying germplasm sources for broadening the elite breeding pools.

In the second phase of hybrid breeding, new lines were primarily developed by second cycle breeding, i.e., from crosses among elite inbreds within heterotic groups. Moreover, outstanding elite lines were shared as parents of different commercial hybrids. In combination with intensive selection, this is expected to result in a

Communicated by A. Charcosset

J. C. Reif · S. Hamrit · M. Heckenberger · W. Schipprack
H. P. Maurer · A. E. Melchinger (✉)
Institute of Plant Breeding, Seed Science, and Population Genetics,
University of Hohenheim, 70593 Stuttgart, Germany
E-mail: melchinger@uni-hohenheim.de
Fax: +49-711-459-2343

M. Bohn
Crop Science Department,
University of Illinois,
S-110 Turner Hall MC 046 1102 S Goodwin Ave
Urbana Champaign, Illinois 61801, USA

reduced genetic diversity in the breeding pools but even more seriously in the cultivars grown by farmers. However, the risk of genetic erosion does not only depend on plant breeding practices but also on the system that delivers the final products of plant breeding to the market. In the European Community, this includes the regulations to register new varieties and the marketing of registered varieties. Statutory testing of new varieties is required to register them on the national lists (UPOV 2002). Afterwards, their acceptance by farmers depends on the amount and quality of the marketing effort of breeding companies but also on further series of voluntarily recommended lists based on regional trials. Consequently, only a few of the registered varieties are grown on a large scale.

Monitoring the genetic diversity available to farmers is important, because plant breeding practices, the registration procedures, and the marketing of new varieties could have caused a potential genetic erosion and, consequently, a potential increased genetic vulnerability of cultivated varieties. Snap-shots of the diversity present in maize breeding programs were reported (e.g., Messmer et al. 1992). In addition, the temporal trend of genetic diversity was investigated for a single US breeding program (Duvick et al. 2004a) as well as for important public US lines (Lu and Bernardo 2001). However, no information is available on the temporal trends in genetic diversity of important Central European maize varieties cultivated by farmers.

The main goal of our study was to monitor the temporal trends in genetic diversity over the past five decades among maize cultivars with the largest acreage in Central Europe. Our objectives were to (1) investigate how much of the genetic diversity present in important Central European OPVs has been captured in the elite flint germplasm pool, (2) examine changes in the genetic diversity among the most important commercial hybrids as well as in their dent and flint parents, (3) analyze temporal changes in allele frequencies between the dent and flint parental inbreds, and (4) investigate linkage disequilibrium (LD) trends between pairs of loci within the set of parental dent and flint lines.

Materials and methods

Plant materials

A set of 85 maize hybrids (Table 1) representing the most important cultivars grown in Germany over the last five decades was chosen based on acreage and seed sale figures. Estimates about the relative importance of these cultivars were obtained from (1) the Bundessortenamt, (2) the Deutsches Maiskomitee, and (3) maize experts from sales, breeding, production, and research. For 67 of the 85 hybrids, all parental inbred lines or parental populations were available. For 11 single-cross hybrids only one parent and for seven single-cross hybrids none of the parents was available. In a companion study (Reif et al. 2005), 30 individuals of each of five prominent flint populations from Central Europe (Gelber Badischer Landmais, Maleksberger, Mahndorfer, Strenzfelder, and Rheintaler) were examined. These populations had played an important role in the pre-hybrid breeding era in Germany and were grown in 1950 on more than 93% of the acreage (Oettler et al. 1976).

According to the year of release, the five OPVs and 85 hybrids, as well as their parents, were grouped into pre-hybrid breeding era (PH) (< 1951), Period A (1951–1975), Period B (1976–1985), Period C (1986–1995), and Period D (1996–2001). The decreasing trend in the length of the time periods reflects the decreasing number of years during which the cultivars played a significant role on the market.

SSR analyses

DNA was extracted from the leaf material of parental inbred lines employing a modified CTAB procedure (Saghai-Maroof et al. 1984). For the 11 single-cross hybrids, where only one parent was available, DNA was also extracted from the hybrids using the above mentioned procedure. For the seven single-cross hybrids, where none of the parents was available, DNA was isolated from leaf material and maternal pericarp

Table 1 Description of the hybrids grouped into four time periods: single cross (*2W*), three-way cross (*3W*), double cross (*4W*), and top-cross hybrids (*TO*) and open-pollinated varieties (OPVs) from the pre-hybrid breeding era (PH)

Period	Abbreviation	Varieties
< 1950	PH	*OPVs*: Gelber Badischer Landmais, Maleksberger, Mahndorfer, Rheintaler, Strenzfelder.
1951–1976	A	*TO*: Hymador, Perdux;4W:Alpha, Brilliant, Campo, Capella, Gamma, Infrafrüh, Inrakorn, Ipho, Limagold, Velox;3W:Anjou, Blizzard, Cargill Primeur, Forla, Limac, Zistron;2W;Forte, Kapio.
1976–1985	B	*4W*: Buras, Olymp, Protador;3W:Alize, Anko, Ass, Carlos, Golda, Eta, Fronica, Frontenac, Garant, Lixis, Mutin, Primat, Protagold, Tau;2W:Bastion, Dea, Felix, Mona.
1986–1995	C	*3W*: Alarik, Amadeus, Atlet, Aviso, Bonny, Diamant, Freya, Jericho, Kid, Saphir, Türkis;2W:Arsenal, Banguy, Boss, Fanion, Helga, Helix, Illias, Magister, Marshall, Noveta, Pirat.
1996–2001	D	*3W*: Attribut, Domenico, Pernel;2W:Acapulco, Benicia, Birko, Early Star, Effekt, Elita, Eurostar, Fjord, Gavott, Justina, Loft, Lukas, Oldham, PR39G12, Prinz, Probat, Romario, Sponsor, Symphony

tissue of the hybrids, using the GenElute Plant Genomic DNA miniprep Kit (Sigma, Cat Nr. G2N350). A total of 55 SSR markers uniformly distributed across the maize genome was used as described by Reif et al. (2005). Details about SSR amplification, detection, and allele calling procedure were described in our companion paper (Reif et al. 2005).

Statistical analyses

Unknown marker genotypes of parental inbreds of single-cross hybrids were deduced by "subtracting" the genotype of one of the parental inbreds or the maternal pericarp tissue from the genotype of the hybrid (Stojsin et al. 1996; Wang et al. 2002). Thus, in total genotypes of 148 parental inbred lines were available. These lines were assigned to the dent and flint heterotic groups (1) according to pedigree information or (2) by applying the K-means clustering algorithm (Hartigan and Wong 1979) assuming two groups, if no pedigree information was available. This resulted unambiguously in 66 flint and 82 dent lines. Most hybrids consisted of dent × flint crosses except for five hybrids that had only dent parents. Allele frequencies of the hybrids were determined from their parental components. Associations among the hybrids were analyzed by applying principal coordinate analysis (PCoA) (Gower 1966) based on the modified Rogers' distance (Wright 1978), which was calculated from the allele frequencies of the hybrids. Associations among parental dent and flint inbreds were determined by PCoA based on modified Rogers' distance calculated from the allele frequencies of the groups of parental dent and flint lines from each of the four time periods. In addition, the number of alleles (n_A), the number of new alleles (n_N), and the number of lost alleles disappearing from one period to the next (n_L) were determined.

Rogers' distances (RD) (Roger 1972) were calculated among dent lines, among flint parental components, and among dent versus flint parental components. In addition, RD were determined between individual genotypes within OPVs and between individual genotypes of different OPVs. These RD estimates were averaged within each time period.

Consider two maize hybrids X and Y. The average RD between two randomly selected individuals from two different maize fields planted either with hybrid X or Y reflects the diversity between cultivated varieties. Thus, all pairwise comparison of hybrids of a certain time period represents a measure of the diversity present at that time between cultivated varieties at an individual level. The average expected RD between two randomly chosen individuals of hybrid X with its two parental components π' and π'' and hybrid Y with its two parental components π''' and π'''', was calculated according to Melchinger (unpublished data) as:

$$
E\big(\overline{RD}(X,Y)\big)
$$

$$
\begin{aligned}
= \frac{1}{m}\sum_{i=1}^{m}\Bigg[& \sqrt{\frac{1}{2}} + \left(\frac{1}{2}-\sqrt{\frac{1}{2}}\right) \\
& \times \left(H_0^{\mathrm{I\times III}} + H_0^{\mathrm{I\times IIII}} + H_0^{\mathrm{II\times III}} + H_0^{\mathrm{II\times IIII}}\right) \\
& + \left(\frac{1}{2}\sqrt{3} - \sqrt{\frac{1}{2}}\right)\left(H_0^{\mathrm{I\times II}} + H_0^{\mathrm{III\times IIII}}\right) \\
& + \left(\sqrt{2} - \frac{1}{2}\sqrt{3} - \frac{1}{2}\right) \\
& \times \left(H_0^{\mathrm{I\times II\times III}} + H_0^{\mathrm{I\times II\times IIII}} + H_0^{\mathrm{I\times III\times IIII}} + H_0^{\mathrm{II\times III\times IIII}}\right) \\
& + \left(\sqrt{\frac{1}{2}} - 1\right)\left(H_0^{\mathrm{I\times III}}H_0^{\mathrm{II\times IIII}} + H_0^{\mathrm{I\times IIII}}H_0^{\mathrm{II\times III}}\right) \\
& + \left(\sqrt{\frac{1}{2}} + 1 - \sqrt{3}\right)H_0^{\mathrm{I\times II}}H_0^{\mathrm{III\times IIII}} \\
& + \left(2\sqrt{3} + 1 - 3\sqrt{2}\right)H_0^{\mathrm{I\times II\times III\times IIII}}\Bigg]
\end{aligned}
$$

Here, $H_0^{\mathrm{I\times II}} = \sum_j^{n_i} p'_j p''_j, H_0^{\mathrm{I\times II\times III}} = \sum_j^{n_i} p'_j p''_j p'''_j$, and $H_0^{\mathrm{I\times II\times III\times IIII}} = \sum_j^{n_i} p'_j p''_j p'''_j p''''_j$, where p'_j, p''_j, p'''_j, and p''''_j are the frequencies of allele j at locus i in parental component π', π'', π''', and π'''', respectively, whereas m refers to the number of loci and n_i to the number of alleles at locus i. $E\big(\overline{RD}(X,Y)\big)$ ranges from 0 (both hybrids are identical single cross hybrids) to 1 (both hybrids share no alleles in common). The average RD between individual genotypes of one hybrid reflects the diversity present in a certain field. The average RD between individual genotypes within hybrid X with parental components π' and π'' was also calculated with the above formula using $\pi' = \pi'''$ and $\pi'' = \pi''''$. The average of these values within time periods is therefore a measure of the diversity present at that time within cultivated varieties at an individual level. The maximum average RD between individual genotypes ranges from 0 for single-cross hybrids to 0.43 for double cross hybrids. The Wilcoxon rank sum test was applied to examine the significance between pairwise comparisons of average RDs (Hollander and Wolfe 1973).

Linkage disequilibrium between all pairs of loci was tested within the dent and flint groups with an exact test described by Weir (1996). The power for detection significant LD between pairs of markers depends on the sample size of the germplasm groups, which varied in our study. Therefore, a re-sampling strategy was used to obtain comparable estimates: a random sample of 15 individuals from each time period for the set of dent and flint lines was chosen for the above analyses; sampling was repeated 50 times, and the results were averaged. All analyses were performed with the software Plabsoft (Maurer et al. 2004), which is implemented as an extension to the statistical software R (Ihaka and Gentleman 1996).

Table 2 Number of open-pollinated varieties (OPVs), hybrids, or parental components (*N*), number of different parental components (N_D), number of alleles (n_A), number of new alleles (n_N) and number of lost alleles disappearing (n_L) from one period to the next in the varieties of Period PH, A, B, C, and D in marker assays with 55 SSR markers

Period	Hybrids				Dent lines					Flint parents[a]				
	N	n_A	n_N	n_L	*N*	N_D	n_A	n_N	n_L	*N*	$N <_D$	n_A	n_N	n_L
PH										5		323		
A	20	323			33	22	225			35	21	278	28	73
B	21	293	46	76	32	25	255	64	34	29	15	203	26	101
C	22	282	35	46	29	24	232	32	55	26	21	215	44	32
D	22	255	38	65	28	20	219	42	55	19	16	190	33	58
Total[b]	85	404			122	91	328			109	73	337		

[a]Period A includes two open-pollinating varieties[b]Include Period A,B,C,and D

Results

A total of 404, 323, 328, and 337 alleles were observed in the hybrids, OPVs, as well as in dent and flint parental components, respectively (Table 2). In total, 28% of the alleles present in the five OPVs were not recovered in the flint lines. Most of them (97%) had an allele frequency below 0.1 in the OPVs. Population Mahndorfer had the highest number of unique alleles (35) and Rheintaler the lowest number of unique alleles (20) not present in the flint lines.

The PCoA of the 85 hybrids revealed a shift in allele frequencies from Period A to D (Fig. 1). This shift was also observed in the PCoA of the parental dent and flint lines grouped into the four time periods with respect to the second principal coordinate (PC2) (Fig. 2). In addition, PC1 revealed that for each time period, the group of dent lines was clearly separated from the group of flint lines.

The number of alleles (n_A) in the hybrids decreased consistently from Period A to D (Table 2). Period B showed both the highest number of new alleles (n_N) and lost alleles (n_L). While n_A decreased for the flint parental components from Period A to D, with the highest loss of alleles from Period A to B, n_A increased for the dent lines from Period A to B but decreased thereafter monotonically to Period D. Across all four periods, the dent and flint lines had 65% of the alleles in common. The dent lines contained 22% and the flint lines 13% unique alleles not present in the opposite heterotic pool.

The average RD between individual genotypes was higher within OPVs (0.37) than within hybrids of Period A (0.18) (Table 3). The average RD between individual genotypes within hybrids decreased almost linearly from Period A to D. In addition, the average RD between individual genotypes of different varieties was higher for the OPVs (0.51) than for the hybrids of Period A (0.45). The average RD between individual genotypes of dif-
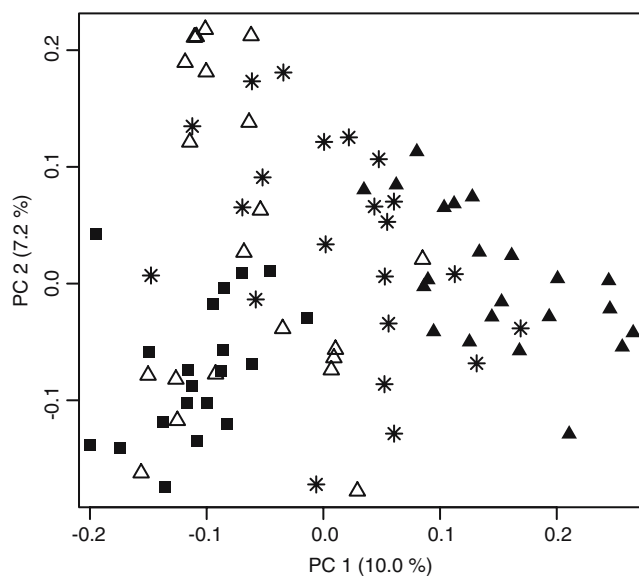

Fig. 1 Principal coordinate analysis based on modified Rogers' distances between the 85 hybrids of Period A (*filled square*), B (*open triangle*), C (*asterisk*), and D (*filled triangle*). PC1 and PC2 are the first and second principal coordinate, respectively
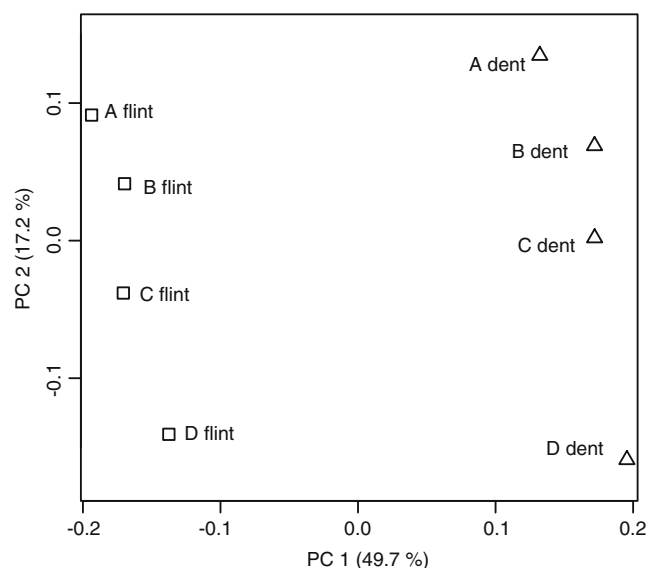

Fig. 2 Principal coordinate analysis based on modified Rogers' distances of the 82 dent and 66 flint lines grouped into four time periods. PC1 and PC2 are the first and second principal coordinate, respectively

Table 3 Average pairwise Rogers' distances based on 55 SSRs between individual genotypes within hybrids (W-HY), between individual genotypes of different hybrids (A-HY), among 82 dent lines (DE), among 68 flint parental components (FL), and between dent and flint parental components (DE × FL) grouped into the four time periods (A, B, C, and D)

Period	W-HY	A-HY	DE	FL	DE × FL
A	0.179^{c*}	0.447^a	0.567^b	0.467^a	0.644^a
B	0.128^c	0.488^c	0.619^c	0.525^c	0.698^c
C	0.068^b	0.487^c	0.612^c	0.511^b	0.682^b
D	0.014^a	0.463^b	0.530^a	0.504^{ab}	0.682^b

*Estimates followed by the same letter are not different at the 0.01 probability level

ferent hybrids increased from Period A to B but decreased from Period C to D.

For all time periods, the average RD among lines was greater for dent (0.62) than for flint inbreds (0.54). The RD between dent lines increased from Period A to B and decreased substantially from Period C to D (Table 3). The RD between flint parental components also increased from Period A to B but decreased slightly to Period D. The RD between dent and flint parental components from the same time periods increased from Period A to B, with a minor decrease to Period D.

Linkage disequilibrium was significant at a probability level $P = 0.01$ for 20.2% of the SSR marker pairs in the 82 dent lines, and for 17.2% in the 66 flint lines (Table 4). We observed almost as much inter-chromosomal as intra-chromosomal LD. The percentage of SSR loci pairs in LD in a random sample of 15 lines decreased in the dent and flint inbreds from Period A to C with a slight increase from Period C to D.

Discussion

Two events in the recent history of maize breeding had a major impact on the genetic diversity among and within cultivated varieties in Central Europe (Schnell 1992). First, the transition from OPVs to hybrids occurred in the 1950s. Second, during the 1980s there was a shift in cultivation of top- or double-cross hybrids to three-way- or single-cross hybrids. Production of the latter became economic due to an improved per se performance of inbred lines and was driven by (1) the major breeding goal to develop higher yielding hybrids and (2) farmer demands for increased uniformity of varieties (Hallauer et al. 1988). Other important breeding goals included selection for earliness and tolerance to high plant densities as a consequence of changing agricultural practices. In the starting phase of hybrid breeding in Central Europe, a few prominent public lines dominated as parents of hybrids (Messmer et al. 1992). Subsequently, the public lines were replaced by proprietary inbreds. Furthermore, at the beginning, the main focus was on grain usage while silage use became predominant later on. The aim of this study was to investigate the impact of these trends in plant breeding on the level and structure of genetic diversity within and among maize varieties of economic importance in Central Europe.

Genetic diversity loss during the transition from OPVs to hybrids

The significantly lower average RD between individual genotypes within hybrids than within OPVs reflects the reduced diversity within hybrid cultivars. This can be explained by the small number of individual genotypes used to generate hybrids (Messmer et al. 1992) but also by directional selection. With increasing genetic homogeneity of varieties, a higher yield is expected but also an increased genetic vulnerability due to a reduced population buffering (Allard and Bradshaw 1964; Hallauer et al. 1988). Considering the total population of cultivated cultivars during a certain period, the reduction in genetic diversity within varieties could have been counterbalanced by an increased diversity between hybrids. However, the RD between genotypes of different varieties decreased from OPVs to hybrids. In addition, the restricted number of five OPVs analyzed in this study presumably led to an underestimation of the diversity present in OPVs, and, thus, a considerable loss of

Table 4 Percentage of SSR loci pairs in linkage disequilibrium (LD%) and percentage of SSR loci pairs in linkage disequilibrium of a random sample of 15 individuals (S-LD%) at probability level $P = 0.01$ for unrelated dent and flint lines based on 55 SSRs. Test results were separated into LD between loci on the same chromosome (Intra) and LD between loci on different chromosomes (Inter). NT refers to the number of tests

Period	Inter			Intra			Total		
	NT	LD (%)	S-LD (%)	NT	LD (%)	S-LD (%)	NT	LD (%)	S-LD (%)
82 Dent lines									
A	1,299	19.9	16.0	121	17.4	13.2	1,400	20.0	15.7
B	1,357	16.2	13.0	126	11.1	10.7	1,483	15.8	12.8
C	1,345	14.1	11.4	126	9.5	9.9	1,471	13.7	11.3
D	1,261	13.4	12.5	117	14.5	12.1	1,378	13.5	12.5
Total	1,357	20.7		126	14.3		1,483	20.2	
66 Flint lines									
A	1,253	19.9	16.3	116	20.7	17.0	1,369	19.9	16.3
B	1,353	10.4	10.4	126	10.3	10.3	1,479	10.3	10.3
C	1,304	7.4	7.3	121	5.8	5.7	1,425	7.3	7.2
D	1,213	9.6	9.6	111	9.0	7.7	1,324	9.5	9.4
Total	1,357	17.6		126	12.7		1,483	17.2	

diversity was observed within but also between varieties during the transition from OPVs to hybrids.

The large proportion of alleles of the OPVs (28%), that were not recovered in the flint lines clearly indicates a reduction in the allelic diversity during the establishment of elite flint inbreds used in hybrid breeding. Apart from exotic germplasm, adapted OPVs are a promising source to broaden the genetic base of the elite flint breeding pool. Population Mahndorfer had the highest number of unique alleles (35) among the OPVs, which were absent in the flint lines. This suggests that the germplasm contribution of Mahndorfer to the elite flint pool was low. Consequently, this OPV is a very promising source for untapped allelic variation.

Genetic diversity trends in hybrids and their parental dent and flint components

The PCoA of the hybrids revealed a clear shift in allele frequencies from Period A to D (Fig. 1). This was corroborated by the PCoA of the dent and flint lines (Fig. 2). The dent and flint lines evolved in a parallel way with respect to PC2, reflecting that dent and flint inbreds have undergone similar selection pressures. Drift and introgression of novel plant material could also have caused a shift in allele frequencies, but then a parallel evolution of both sets of lines is unexpected.

We observed a monotonic decrease in genetic heterogeneity within hybrids from Period A to D (Table 3). This reflects the change in the types of hybrid (Table 1). The transition in types of hybrid is also most likely the major cause for the decrease of n_A from Period A to D (Table 2) and is in close agreement with results reported by Duvick et al. (2004a), who investigated 51 US hybrids with 100 SSRs. The trend in n_A is of interest particularly for Periods A and B, because it decreased drastically for the flints but increased for the dents. The decrease in n_A for the flints can be mainly explained by the use of OPVs as parental component of the top-cross hybrids Hymador and Perdux in Period A, and the disappearance of top-cross hybrids in Period B (Table 1). The increase in n_A for the dent lines suggests an augmented introgression of novel germplasm from the US in the 1970s, as indicated also by n_N.

The average RD between individual genotypes of different hybrids is influenced by (1) the RD between dent lines, (2) the RD among flint parental components, and (3) the RD between dent and flint parental components. The average RD of all the three groups increased from Period A to B and, consequently, caused an increase in the average RD between individual genotypes of different hybrids (Table 3). The higher RD among dents in Period B than A can be explained by the reduced use of identical parental components in different hybrids (Table 2). In addition, the enhancement can be due to a broadening of the genetic base of dent lines by adapting new germplasm from the US, as indicated by the high value for n_N found in Period B (Table 2). The

higher average RD among flints of Period B than A can be explained by the dominance of a few public inbred lines at the beginning of hybrid breeding (Messmer et al. 1992). Especially, the cross of the two French lines $F2\times$ $F7$ served in 30% of the hybrids of Period A as parental component (data not shown).

The average RD between individual genotypes of different hybrids decreased from Period C to D, which was mainly caused by a reduction of the diversity in the dent pool (Table 3). On the one hand, the decrease of diversity in the dent pool can be explained by an increased use of certain individual lines as parental component of various hybrids by breeders in Period D (Table 2). On the other hand, one could speculate that intensive selection and focusing on a small number of key parents for the development of new lines have caused a reduction in the diversity of the dent lines. To verify whether a reduction in the diversity of the dent pool is due to a multiple use of (1) inbreds as parents of several hybrids or (2) certain inbreds as key parents for the development of new inbreds, further investigations are required with fingerprinting of germplasm from the individual breeding programs and examining the pedigree relationships among the inbred lines.

We found a significantly higher average RD between dent than between flint lines, which is in accordance with previous studies with germplasm representing the diversity within breeding pools (Messmer et al. 1992; Lübberstedt et al. 2000). The higher diversity in the dent than in the flint pool can be explained by the genealogy of the germplasm. The Central European dent germplasm encompasses a wide range of materials of North American origin with genetic background of different heterotic groups such as Stiff Stalk and Non-Stiff Stalk. By comparison, the Central European flint germplasm is genetically narrower due to (1) a bottleneck during the introduction of maize into Europe and/or (2) a loss of genetic diversity during the selective adaptation to European conditions (Rebourg et al. 2003).

Genetic diversity trends between dents and flints

The high number of unique alleles, which are exclusively present in either the dent or flint lines (35%), reflects the large divergence between the allelic profiles of both heterotic groups. The differences between the dent and flint heterotic groups existed already at the beginning of hybrid breeding in Germany (Fig. 2; Table 3). This can be explained by the long isolation between the parental germplasm sources used to establish these heterotic groups: the US dent and the European flint pool (Rebourg et al. 2003). The clear divergence of the heterotic groups at the beginning of hybrid breeding in Central Europe is in contrast to results for US germplasm. Duvick et al. (2004b) found that the US germplasm was at the starting phase of hybrid breeding rather unstructured but diverged with ongoing reciprocal recurrent selection.

Trends in linkage disequilibrium

The absence of significant LD in the OPVs (Reif et al. 2005) suggested that LD was either absent or the applied marker density was not high enough to detect LD caused by linkage within populations of the pre-hybrid era, which served as the germplasm base for the elite flint pool. In contrast to the OPVs, LD between SSR marker pairs was observed in Period A in the dents and flints (Table 4). This LD can be caused by admixture of inbred lines originating from populations with different gene frequencies and/or from drift in small populations. In addition, this LD can also be caused by epistatic interactions or suppressed recombination. For the dent and flint pool, both causes are very likely because (1) the dent pool was established with limited number of US dent lines from distinct heterotic groups (Messmer et al. 1992) and (2) the flint pool was developed with a limited number of inbreds originating from selfing in European OPVs with different allele frequencies (Reif et al. 2005).

The standardized fraction of loci pairs in LD decreased for the dents and flints from Period A to C (Table 4). This can be explained by the successive generations of inter-mating since the establishment of both germplasm groups. The slight increase in the standardized fraction of loci pairs in LD from Period C to D for the dents and flints can be due to (1) a high selection intensity for favorable epistatic interactions of alleles from different loci (Falconer and Mackay 1996) and/or (2) drift in breeding populations with a small effective population size. Consequently, one could speculate that the number of different allele combinations increased during the first three time periods but decreased in the last period in Central European maize germplasm. On one hand, this increased extent of LD could be beneficial for plant breeding as it would allow to use additive × additive effects more efficiently. On the other hand a reduced number of different allele combinations could also cause a reduction in selection response (Mackay and Gibson 1993).

Conclusions

The genetic diversity within varieties decreased monotonically during the past 50 years. Consequently, the diversity among varieties is of increased importance, but decreased slightly during the last time period. The major cause for this decrease is the multiple use of elite lines as parents for various hybrids. While the multiple use of lines enables breeders to optimally exploit their elite germplasm, it leads inevitably to a reduced diversity among hybrids.

One important criterion for the eligibility of newly bred varieties is that they are in at least one trait (disease resistance, yield, quality) better than the already registered varieties. Adding a criterion 'positive contribution toward an increased genetic diversity of the available varieties', which could be measured with molecular markers, would encourage innovative breeding. Thus, the suggested modification of the registration procedure and changes in the intellectual property practices encouraging plant breeders to evaluate new exotic diversity, could help to prevent a further potential erosion in genetic variation among commercial hybrids.

References

Allard RW, Bradshaw AD (1964) Implications of genotype-environment interactions in applied plant breeding. Crop Sci 4:503–508

Comstock RE, Robinson HF, Harvey PH (1949) A breeding procedure designed to make maximum use of both general and specific combining ability. J Am Soc Agron 41:360–367

Duvick DN, Smith JSC, Cooper M (2004a) Changes in performance, parentage, and genetic diversity of successful corn hybrids, 1930–2000. In: Smith CW, Betran J, Runge ECA (eds) Corn: Origin, History, Technology, and Production. Wiley, New York, pp 65–97

Duvick DN, Smith JSC, Cooper M (2004b) Long-term selection in a commercial hybrid maize breeding program. Plant Breed Rev 24:109–151

Falconer DS, Mackay TF (1996) Introduction to Quantitative Genetics, 4th edn. Longman Group Ltd, London

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325–338

Hallauer AR, Russell WA, Lamkey KR (1988) Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and Corn Improvement, 3rd edn. Agron Monogr 18, ASA, CSSA, and SSSA, Madison, WI, pp 463–564

Hartigan JA, Wong MA (1979) A K-means clustering algorithm. Appl Stat 28:100–108

Hollander M, Wolfe DA (1973) Nonparametric statistical inference. Wiley, New York, pp 139–146

Ihaka R, Gentleman R (1996) A language for data analysis and graphics. J Comput Graph Stat 5(3):299–314

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

Lübberstedt T, Melchinger AE, Dussle C, Vuylsteke M, Kuiper M (2000) Relationships among early European maize inbreds: IV. Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. Crop Sci 40:783–791

Mackay IJ, Gibson JP (1993) The effects of gametic-phase disequilibrium on the prediction of response to recurrent selection in plants. Theor Appl Genet 87:152–160

Maurer HP, Melchinger AE, Frisch M (2004) Plabsoft: Software for simulation and data analysis in plant breeding. XVIIth EUCARPIA General Congress 2004, Tulln, Austria, pp 359–362

Messmer MM, Melchinger AE, Boppenmaier J, Brunklaus-Jung E, Herrmann RG (1992) Relationships among early European maize inbreds: I. Genetic diversity among flint and dent lines revealed by RFLPs. Crop Sci 32:1301–1309

Oettler G, Schnell FW, Utz HF (1976) Die westdeutschen Getreide- und Kartoffelsortimente im Spiegel ihrer Vermehrungsflächen. In: Alleweldt G (ed) Hohenheimer Arbeiten. Schriftenreihe der Universität Hohenheim Reihe: Pflanzliche Produktion. Verlag Eugen Ulmer, Stuttgart, Germany

Rebourg C, Gouesnard B, Welcker C, Dubreuil P, Chastanet M, Charcosset A (2003) Maize introduction into Europe: the history reviewed in the light of molecular data. Theor Appl Genet 106:895–903

Reif JC, Hamrit S, Heckenberger M, Schipprack W, Bohn M, Melchinger AE (2005) Genetic structure and diversity among European flint maize populations determined with SSRs. Theor Appl Genet (in press)

Roger JS (1972) Measures of genetic similarity and genetic distance. Studies in genetics. VII. Univ Tex Publ 7213:145–153

Saghai-Maroof MA, Soliman KM, Jorgenson R, Allward RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Schnell FW (1992) Maiszüchtung und die Züchtungsforschung in der Bundesrepublik Deutschland. Vorträge Pflanzenzüchtung 22:27–44

Stojsin D, Kannenberg LW, Rajnpreht J, Pauls PK, Stojsin R (1996) Genetic relationships among commercial corn hybrids and parents based on RAPD analyses of pericarp and embryo DNA. Genetika 28:137–150

UPOV (2002) General introduction to the examination of distinctness, uniformity and stability and the development of harmonized descriptions of new varieties of plants. TG/1/3. Geneva

Wang J, Zhong GY, Chin ECL, Register JC, Riley RD, Niebur WS, Smith JSC (2002) Identification of parents of F1 hybrids through SSR profiling of maternal and hybrid tissue. Euphytica 124:29–34

Weir BS (1996) Genetic Data Analysis II, 2nd edn. Sinauer Associates Inc, Sunderland, MA

Wright S (1978) Evolution and Genetics of Populations, vol IV. The Univ of Chicago Press, p 91