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Theoretical and experimental investigations on the exploitation of heterosis in hybrid breeding

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1. General Introduction

Hybrid breeding is being practiced in a number of crops as hybrids perform significantly better than the alternative cultivars. The concept of hybrid breeding traces back to maize (*Zea mays* L.) (Beal 1880; East 1908; Shull 1908, 1909). Shull and East carried out selfing of maize plants and crossing the resultant inbred lines, and observed a substantial decrease in vigor and grain yield after selfing and restoration of the same on crossing. Further, F₁ hybrids of inbred parents often exceeded the mean of the parents. On this basis, Shull (1909) proposed the 'pure-line method of corn breeding'. Commercial cultivation of hybrid maize started in the USA in 1920s and it occupied appreciable acreage in 1930s (Crow 1998). Maize grain yield in the US Cornbelt increased dramatically as hybrids replaced open-pollinated varieties (OPVs).

Hybrid maize cultivation started in Germany in the 1950s (Schnell 1992). A heterotic pattern comprising high yielding US Dents and adapted European Flints was exploited in hybrid maize breeding in Germany and Central Europe. The Flint heterotic group traces back to mainly the three OPVs namely Lacaune, Lizargarote, and Gelber Badischer Landmais (Reif et al. 2009). The Dent heterotic group was developed from US inbreds. Another important crop in which hybrid breeding has been successful in Central Europe is rye (*Secale cereal* L.). Research on rye hybrid breeding was started in 1970 at the University of Hohenheim (see Geiger and Miedaner 1999). The hybrid of populations Carsten and Petkus, which were widely used in rye breeding, showed excellent performance for grain yield and these populations served as heterotic groups (Hepting 1978). This heterotic pattern has since then been extensively used in hybrid rye breeding in Central Europe.

1.1 Concept of heterotic groups and patterns

The concept of heterotic groups and patterns developed as hybrid maize breeding progressed so as to systematically exploit heterosis. Melchinger and Gumber (1998) defined a heterotic group as “a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from genetically distinct germplasm groups”. By comparison, the term heterotic pattern refers to a specific pair of two heterotic groups, which express high hybrid performance and heterosis in their cross. Heterotic groups have been developed in crops like maize and rye. In some crops like oilseed rape (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.), development of heterotic groups has recently been initiated. But in triticale (\times *Triticosecale* Wittm.) no heterotic groups are available.

To develop heterotic groups at least two divergent germplasm are identified and improved by inter-population recurrent selection, generally accompanied by introgression of new germplasm (Hallauer et al. 1988). New inbreds are generated within each heterotic group and are evaluated in inter-group testcrosses. The superior inter-group crosses are further tested to identify hybrids for their commercial exploitation. Generally, inbreds with superior testcross performance are also intermated within-groups to develop an improved version of the heterotic group.

Development of divergent heterotic groups maximizes the expression of heterosis and hybrid performance (Falconer and Mackay 1996). It also has the advantages of a low ratio of variance due to specific (σ^2_{SCA}) versus general combining ability (σ^2_{GCA}) (Melchinger 1999). Further, the choice of testers to evaluate the combining ability of newly developed inbreds and the identification of inbred parents for line development of the improved version of heterotic groups are simplified.

1.2 Establishment of heterotic groups

Heterotic groups and patterns in maize have been developed on the basis of agronomic performance of F₁ hybrids and expression of heterosis (Hallauer and Miranda 1988). In many studies two heterotic populations or testers have been identified and used to develop heterotic groups and then enriched through reciprocal recurrent selection (Dhillon et al. 1997; Melchinger 1999; Geiger and Miedaner 1999). However, on the basis of a simulation study, Cress (1967) suggested to combine all genetic materials into one synthetic population and establish heterotic groups by randomly sampling genotypes from this synthetic. In autogamous crops with a complex population structure, such as triticale, heterotic groups have not been established and this is a challenging task. In these crops, the magnitude of heterosis is low, pollination control is difficult, and σ^2_{SCA} for grain yield is of greater importance than σ^2_{GCA} (Oury et al. 2000; Oettler et al. 2003). An alternative to deal with such crops is to utilize the data on F₁ performance, heterosis, combining abilities as well as molecular markers, and apply the novel model-based new clustering approach (Pritchard et al. 2000; Falush et al. 2003). Many workers successfully used molecular markers to identify genetically divergent subgroups (e.g., Menkir et al. 2004; Reif et al. 2003; Tams et al. 2004; Xia et al. 2004). However, in most studies, no or only weak correlations were observed between inbreds belonging to divergent heterotic groups (Melchinger 1999). The new model-based clustering methods, which are implemented with the software STRUCTURE, are powerful tools to unravel the genetic structure and identify diverse groups of genotypes, and they have been successfully applied in maize (e.g., Liu et al. 2003; Stich et al. 2005). In addition to these avenues, an algorithm that explores the entire space to identify diverse heterotic germplasm may be helpful in identifying desired germplasm.

1.3 Broadening heterotic groups

Selection over time is expected to narrow the genetic base of the heterotic groups and the elite germplasm. Therefore, to ensure medium and long-term selection gains, heterotic groups should not be treated as closed populations and their genetic base should be broadened by continuously enriching them with introgression of new germplasm. Exotic germplasm is regarded as a worthwhile source to enhance the genetic diversity of the elite maize breeding germplasm (for review see Ron Parra and Hallauer 1997; Goodman et al. 2000). For temperate areas, possible exotic sources are tropical, subtropical, and unadapted temperate germplasm, and in the European maize germplasm, generally US Cornbelt germplasm has been introgressed. In rye breeding programs in Central Europe, OPVs from Eastern Europe and Near East, and landraces from Asia and South America are promising sources of exotic germplasm (Falke et al. 2008).

To identify possible heterotic patterns and appropriate germplasm for introgression from exotic germplasm, performance of inter-heterotic groups and performance *per se* in field trials in the target region are important. Field evaluation of hybrid performance and heterosis can be complemented with genetic distances based on molecular markers. Initially molecular marker technology was considered very promising for the prediction of heterosis but the results were not consistent. Melchinger (1999) summarized the results of such studies. He distinguished three situations: (1) crosses among unrelated lines, (2) intra-group crosses among unrelated lines, and (3) inter-group crosses. For the first situation, there was a strong positive relationship between heterosis and parental genetic distance. In the second situation, there is also a linear relationship, but of moderate magnitude. When parents originated from different heterotic groups, heterosis at the best showed only weak correlation with genetic distance.

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In hybrid breeding it is important to predict the performance of hybrids, and identify the superior ones for their field evaluation, as the number of possible hybrids increases very rapidly with an increase in the number of parents. Melchinger et al. (1987) emphasized the importance of σ^2_{SCA} , σ^2_{GCA} , and their ratio in the prediction of hybrid performance. Further, Reif et al. (2007) showed that increased genetic divergence between heterotic groups leads to a larger contribution of σ^2_{GCA} compared with σ^2_{SCA} . This predominance of σ^2_{GCA} improves prediction. Thus, enhanced divergence between heterotic groups is important for improving and reliably predicting hybrid performance. A predominance of σ^2_{GCA} over σ^2_{SCA} was reported in several studies in maize (Parriseseaux and Bernardo 2004; Schrag et al. 2006).

Selection changes gene frequencies, which is expected to adversely affect the magnitude of genetic variance components (Falconer and Mackay 1996). On the other hand the presence of genetic variability is an indispensable requirement for selection. Thus, it is important to have information on temporal changes in σ^2_{GCA} and σ^2_{SCA} in applied breeding programs. However, there are not many studies on the effect of selection on genetic variance in a population and still fewer on inter-population crosses (e.g., Moll 1991; Schnicker and Lamkey 1993). Moreover, these studies are based on closed populations. There seems to be no published report on temporal trends of genetic variance in heterotic groups undergoing inter-population improvement accompanied by germplasm introgression which is typical in an applied breeding program.

1.4 Objectives

The overall goal of my thesis research was to conduct theoretical and experimental investigations on the maximization of the exploitation of heterosis in hybrid breeding in maize, rye, and winter triticale. The specific objectives in the different crops were:

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Maize

1. To investigate the heterotic relationships between the Central European and US Cornbelt heterotic groups based on agronomic performance of hybrids in different mega-environments and SSR markers in inbred parents;
2. To estimate the temporal changes over 30 years in the magnitude of σ^2_{GCA} , σ^2_{SCA} , and their ratio in an applied hybrid maize breeding program, and critically analyze the estimates of variance components in relation to the expected trends; and
3. To develop a strategy to broaden the genetic base of the Central European heterotic groups based on their relationships with US heterotic groups.

Rye

1. To examine the genetic diversity in Eastern European OPVs and Central European heterotic groups based agronomic performance and SSR markers;
2. To investigate the heterotic relationship of the OPVs with the heterotic groups; and
3. To develop a strategy for broadening the genetic base of the Central European heterotic groups with germplasm from Eastern Europe.

Winter triticale

1. To evaluate agronomic performance of inbred lines and their combining ability in diallel crosses;

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2. To examine the relationship of SSR marker-based genetic divergence with the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio; and
3. To optimize *per se* performance, heterosis, and $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio in hybrid populations by exploring the entire space through an algorithm and identifying different combinations with variable number of parental lines for the development of heterotic groups.

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Xia X.C., J.C. Reif, D.A. Hoisington, A.E. Melchinger, M. Frisch, M.L. Warburton, 2004 Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: I. Lowland tropical maize. *Crop Sci.* 44: 2230-2237.

2. Impact of genetic divergence on the ratio of variance due to specific versus general combining ability in winter triticale

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Abstract. The objective of our study was to examine the influence of genetic divergence on the ratio of the components of variance for specific (σ^2_{SCA}) and general (σ^2_{GCA}) combining ability using experimental data in triticale (*× Triticosecale* Wittm.). In total, 21 lines and their 210 crosses were evaluated for grain yield in field trials. Published molecular data were reanalyzed indicating an optimum of two subgroups. The estimates of σ^2_{SCA} and σ^2_{GCA} were determined for the total diallel and between the two subgroups. The ratio of σ^2_{SCA} versus

σ^2_{GCA} tended to be lower for crosses between than within genetically distinct groups. Our experimental findings can be interpreted as an indicator of a more favorable ratio of σ^2_{SCA} versus σ^2_{GCA} in situations with two genetically distinct populations compared with situations with populations that are not genetically distinct.

3. Development of heterotic groups in triticale

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Abstract. Heterotic groups are an essential prerequisite for efficient hybrid breeding. The present study in winter triticale was carried out to (1) evaluate the magnitude of heterosis, (2) investigate the efficiency of the prediction of hybrid performance based on midparent value or general combining ability (GCA) effects, and (3) identify two heterotic groups by employing principle coordinate analysis and an enumeration algorithm for maximizing F_1 -performance, midparent heterosis, and $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio for grain yield. Twenty-one inbred lines and their 210 diallel crosses were field-evaluated for grain yield at five agroecologically diverse locations in Germany. On the average, the hybrids yielded 8.6% higher than the

midparent performance, and the maximum superiority was 12.4%. Hybrid performance was predicted more reliably with GCA effects rather than with midparent performance. An enumeration algorithm based on optimum allocation of parents to heterotic groups, improved all three criteria. F₁-performance, midparent heterosis, and $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio increased by about 3, 24, and 70%, respectively, as compared with the base situation of having no heterotic groups. These improvements substantiate the potential advantages, when heterotic groups are developed in hybrid breeding.

4. Broadening the genetic base of European maize heterotic pools with US Cornbelt germplasm using field and molecular marker data

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Abstract. Maize (*Zea mays* L.) breeders are concerned about the narrowing of the genetic base of elite germplasm. To reverse this trend, elite germplasm from other geographic regions can be introgressed, but due to lack of adaptation it is difficult to assess their breeding potential in the targeted environment. The objectives of this study were to (1) investigate the relationship between European and US maize germplasm, (2) examine the suitability of different mega-environments and measures of performance to assess the breeding potential of exotics, and (3) study the relationship of genetic distance with mid-parent heterosis (MPH). Eight European inbreds from the Dent and Flint heterotic groups, 11 US inbreds belonging to Stiff Stalk (SS), non-Stiff Stalk (NSS), and CIMMYT Pool 41, and their 88 factorial crosses in F₁ and F₂ generations were evaluated for grain yield (GY) and dry matter concentration (DMC). The experiments were conducted in three mega-environments: Central Europe (target mega-environment), US Cornbelt (mega-environment where donor lines were developed), and Southeast Europe (an

intermediate mega-environment). The inbreds were also fingerprinted with 266 SSR markers. Suitable criteria to identify promising exotic germplasm were F_1 hybrid performance in the targeted mega-environment and F_1 and parental performance in the intermediate mega-environment. Marker-based genetic distances reflected relatedness among the inbreds, but showed no association with MPH. Based on genetic distance, MPH, and F_1 performance, we suggest to introgress SS germplasm into European Dents and NSS into European Flints, in order to exploit the specific adaptation of European flint germplasm and the excellent combining ability of US germplasm in European maize breeding programs.

5. Molecular marker assisted broadening of the Central European heterotic groups in rye with Eastern European germplasm

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Abstract. Broadening the genetic base of heterotic pools is a key to ensure continued genetic gains in hybrid breeding and extend hybrid cultivation to new areas. In the present study two Central European heterotic pools (Carsten and Petkus) and five Eastern European open-pollinated varieties (OPVs, Pop-1 to Pop-5) were studied with the objectives to: (1) investigate the genetic diversity in OPVs and the heterotic pools using molecular and field data, (2) evaluate the molecular diversity among OPVs, (3) examine the combining ability of the OPVs when crossed with testers in field trials for grain yield, (4) and develop a strategy for targeted introgression of OPV germplasm into the heterotic pools. In total, 610 S_0 clones, 347 from OPVs and 263 from heterotic pools, were developed. The clones

of OPVs were crossed with two testers belonging to each heterotic pool, while clones of heterotic pools were crossed with only the opposite tester. Testcrosses were evaluated for grain yield in multi-location trials. In addition, 589 S₀ clones were fingerprinted with 30 SSR markers. The data revealed that the Carsten pool has a narrow genetic base and should be the primary target for broadening the established heterotic pattern. Mean and genetic variance suggested that Pop-2 and Pop-4 are good candidates for introgression in Petkus pool and Pop-5 in Carsten pool. Nevertheless, introgression of Pop-5 in Carsten could reduce the genetic diversity between heterotic pools. Therefore, we suggest that either selected clones of Pop-5 should be introgressed or large Eastern European germplasm should be fingerprinted and field evaluated to identify promising germplasm for broadening the established heterotic pattern.

6. Trends in genetic variance components during 30 years of hybrid maize breeding at the University of Hohenheim

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Abstract. The ratio of variance due to specific versus general combining ability ($\sigma^2_{SCA}:\sigma^2_{GCA}$) is of central importance for predicting hybrid performance from GCA effects. The objectives of our study were to (1) analyze the changes in estimates of σ^2_{GCA} , σ^2_{SCA} , and their ratio during 30 years of hybrid maize breeding and (2) compare the observed trends in genetic variances with those expected under a simple genetic model. We analyzed multilocation yield trials based on the North Carolina Design II conducted in the maize breeding program of the University of Hohenheim from 1975 to 2004 for grain yield (GY) and dry matter content (DMC). GY showed a significant ($P < 0.05$) annual increase of 0.17 Mg ha^{-1} , but no linear trend was found for DMC. Since the beginning of hybrid breeding at the University of Hohenheim, the sum of estimates of σ^2_{GCA} of the flint and dent heterotic groups

were higher than the estimates of their σ^2_{SCA} . This predominance did not change with ongoing inter-population improvement. Consequently, superior hybrids can be identified and selected mainly based on their prediction from GCA effects.

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7.1 Establishment of heterotic groups in winter triticales

Hybrid breeding has several advantages compared to line breeding like (i) higher productivity and desirable performance for other traits, (ii) stable performance under stresses, and (iii) exploitation of heterosis. At present only line varieties are cultivated in triticales. Hybrid breeding has not been successful even though an effective CMS system is available. This lack of success is due to low heterosis. An avenue to enhance heterosis is the development of diverse heterotic groups. It is added that from 1996 to 2000, 65 to 70% of the triticales acreage in Germany was planted with new seed of line varieties every year (Schachschneider 2000). This indicates that there are a large number of farmers willing to purchase new hybrid seed every year, and this should facilitate a quick transfer from line varieties to hybrids. Further, depending on the superiority of hybrids over line varieties, hybrids should occupy all 100% of triticales acreage.

For the establishment of heterotic groups generally suitable populations have been identified and these or appropriate testers have been crossed with diverse germplasm to identify promising material for hybrid breeding. Many breeders used this approach in different crops like maize (Hallauer and Miranda 1988; Dhillon et al. 1997; Melchinger 1999; Reif et al. 2003a), rye (Geiger and Miedaner 1999), and triticales (Tams et al. 2004). On the other hand Cress (1967), based on a simulation study, suggested to generate a synthetic population by putting together all genetic material and randomly choose genotypes from the synthetic to form two heterotic groups. There is no published report in the literature on the use of Cress's approach. Reif et al. (2007) proved with quantitative genetic theory assuming (i) a

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biallelic situation at all loci and (ii) linkage equilibrium, that the first approach is superior to the second.

We attempted to develop two genetically divergent heterotic groups using 128 inbred lines of winter triticale. From these 128 lines, 21 were chosen based on genetic distances computed from molecular markers (Fischer et al. 2009a). These 21 inbred lines were then evaluated in diallel crosses. The parents and their 210 diallel crosses (the largest diallel reported in the literature) were field tested and subjected to combining ability analysis following method 4 model 2 of Griffing (1956). The statistical model of Griffing was extended and we used a completely unstructured covariance between GCA effects of hybrids and *per se* performance (Fischer et al. 2009a). This extended model provided estimates of the variance components of the diallel crosses. We also computed the correlation coefficients (r) between *per se* performance and GCA effects of parents.

In the analysis of the diallel crosses, we observed a predominance of GCA over SCA, and the estimate of σ^2_{GCA} was about three times larger than the estimate of σ^2_{SCA} (Fischer et al. 2009a). This indicates that hybrid performance could be reliably estimated based on GCA effects. These results are in agreement with those of Reddy (1976) who analyzed a 7×7 diallel cross of triticale inbred lines from the CIMMYT breeding program. However, the $\sigma^2_{\text{SCA}} / \sigma^2_{\text{GCA}}$ ratio in winter triticale was higher in comparison with those in allogamous crops like maize (Schrag et al. 2006; Fischer et al. 2008) and rye (Tomerius 2001; Miedaner et al. 2005).

Molecular markers can serve as a tool to facilitate a systematic search for divergent germplasm. Many workers successfully used molecular markers to identify genetically divergent subgroups (e.g., Menkir et al. 2004; Reif et al. 2003b; Tams et al. 2004; Xia et al. 2004). However, in most of initial studies, no or only weak correlations were observed between inbreds belonging to divergent heterotic

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pattern (Melchinger 1999). But the lack of groups within germplasm does not necessarily mean that there are no heterotic materials available. Success depends on the germplasm under study and the applied biometrical approaches. Possible heterotic relationships may have remained undetected because of the limited power of the multivariate techniques used in those studies such as principal coordinate analysis or cluster analysis. However, we used a newly developed novel model-based clustering method, which is implemented in STRUCTURE (Pritchard et al. 2000). Further, we applied the *ad hoc* statistic of Evanno et al. (2005) to the results obtained with STRUCTURE to determine the number of possible groups within our germplasm (Fischer et al. 2009a). With these analyses of marker-based genetic distances, we identified two heterotic groups comprising 10 and 11 inbreds.

Reif et al. (2007) showed that with an increase in genetic divergence between two populations, the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio in the hybrid population decreased. In our study we allocated the 21 inbred lines to two divergent groups based on genetic distances. Based on that, we divided the diallel crosses of 21 inbreds into one factorial of 10 × 11 inter-group crosses and into two subdiallels of 10 and 11 inbred lines having the remaining intra-group crosses. To perform combining ability analyses of these factorial and diallel crosses, we extended our statistical model (Fischer et al. 2009a). The identification of two divergent groups of inbred parents based on molecular markers, resulted in a lower $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio in the 10 x 11 factorial crosses compared to the diallel crosses of 21 parents. Thus, our experimental finding demonstrated that the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio could be decreased with two genetically divergent heterotic groups. These results on the relative importance of the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio in inter- compared to intra-group crosses, are in accordance with those reported earlier in maize (Dudley et al. 1991; Dhillon et al. 1993; Melchinger and Gumber 1998). These studies in combination with our results on the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio indicate that the development of divergent heterotic groups is advantageous to maximize heterosis and facilitate hybrid breeding.

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The $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio in the factorial crosses in our study was still higher than that in allogamous species (Schrag et al. 2006; Fischer et al. 2008; Tomerius 2001; Miedaner et al. 2005), i.e., the ratio is unfavorable for reliable prediction of hybrid performance in triticale compared to maize and rye. However, it is important to ponder whether the similar ratio could be expected in autogamous and allogamous crops. One possible reason for dissimilarity may be the relative importance of epistasis, which is expected to be higher in autogamous crops. In autogamous species such as triticale, it is expected that additive \times additive epistatic variance (σ^2_{AA}) is of great importance (e.g., Cockerham 1954; Melchinger et al. 2007). As σ^2_{AA} equally contributes to σ^2_{SCA} and σ^2_{GCA} (Wricke and Weber 1986), an increase in the estimates of σ^2_{AA} leads to a higher $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio. Nevertheless, σ^2_{AA} , like other genetic variance components, is a function of allele frequencies of the populations (Schnell 1965) and, therefore, genetic divergence also influences the relative importance of σ^2_{AA} compared to the additive genetic variance (σ^2_A) and dominance variance (σ^2_D). Another factor may be the higher degree of relatedness in triticale due to common ancestors and the bottleneck caused by the use of a limited number of rye and wheat lines while synthesizing primary triticale (Kuleung et al. 2006).

It is more difficult to establish heterotic groups in autogamous crops compared to allogamous ones. The reasons are lower magnitude of heterosis, difficult pollination control, and a more complex population structure. Therefore, most autogamous crops including triticale have no established heterotic groups. We endeavoured to augment the approach commonly used in allogamous crops for its application in autogamous crops (Fischer et al. 2009b).

In addition to the molecular marker-based allocation of the 21 inbred parents to two diverse groups, we employed an enumeration algorithm which optimized different criteria (F_1 performance, midparent heterosis, and $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio) for grain yield

to establish two diverse heterotic groups of the same inbreds (Fischer et al. 2009a, b), with a restriction that a heterotic group has at least five inbreds. We could enhance F_1 performance and midparent heterosis and decrease the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio with this algorithm as compared to the various estimates obtained in the 10 x 11 factorial based on molecular diversity (Fischer et al. 2009a). Though, F_1 performance and heterosis are more important criteria, we obtained the highest improvement of 60.5% in $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio (Fischer et al. 2009b). Therefore, we suggest developing an index based on F_1 performance, heterosis, and $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio. However, this approach has to be validated with larger and more diverse germplasm having a higher level of heterosis. Once established in triticale, the approach can be extended to other crop species.

7.2 Broadening the genetic basis of heterotic groups

During the 20th century, landraces of most crop plants have been continuously replaced by modern crop varieties, which were bred with a limited number of germplasm in their pedigree. Therefore, they contained less genetic diversity compared to the landraces (Frankel 1970). This decrease in genetic diversity may have consequences on the vulnerability of crops to pests and on their ability to respond to changes in climate or agricultural practice (FAO 1998), besides response to selection for the development of varieties in the long run.

To counterbalance a loss in genetic diversity, plant breeders should continuously enrich their breeding material with new germplasm. In hybrid breeding, the genetic divergence between heterotic groups is to be optimised and genetic variance within groups increased with the objectives to enhance hybrid performance and heterosis and decrease the $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio so as to ensure long-term selection response and have a wider genetic base of the cultivars on farm. To undertake introgression into established heterotic groups, it is necessary to identify genotypes with high

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combining ability with the opposite heterotic group of the heterotic pattern, and acceptable *per se* performance.

Possible germplasm sources for introgression could be landraces, elite germplasm, exotic germplasm, related wild species, etc. For broadening the genetic base of Central European maize heterotic groups, the promising sources are germplasm from Mexico (the centre of origin), elite germplasm from US Cornbelt and other temperate regions, unadapted temperate germplasm, elite subtropical and tropical germplasm, and landraces (*cf.* Ron Parra and Hallauer 1997; Goodman et al. 2000; Šimić et al. 2003). In rye, for Central European breeding programs possible sources are Eastern European OPVs, landraces from Asia or South America, and primitive populations from the Near East (Falke et al. 2008).

To identify candidate germplasm for introgression, diverse germplasm should be tested in cross combination with testers to evaluate F_1 performance for important agronomic traits. Additionally, an assessment of the parents is also required for estimation of heterosis and examines the economics of commercial seed production. Adaptation problems, if any, are identified in field trials. If there are adaptation problems or the direct introduction is not of interest for other reasons, some genes or traits of interest could be used with backcrossing to the adapted heterotic groups.

Molecular markers are a promising tool to complement field trials. If genotypes are closely related at molecular level, they are expected to have a low magnitude of heterosis in their cross combination. With the use of molecular markers these poor crosses could be avoided. It would be time- and resource-saving if molecular marker data on different germplasm generated over years in a program or even in different programs are available.

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We carried out studies on broadening the Central European heterotic groups in maize, namely Flint and Dent (Reif et al. 2009) and in rye, Carsten and Petkus (Fischer et al. 2009c). In maize we used 11 inbred lines belonging to the US Cornbelt heterotic groups (Stiff Stalk, non-Stiff Stalk) and derived in the USA from CIMMYT's temperate material (Pool 41). In addition, we used eight lines belonging to the Central European heterotic groups Flint and Dent.

In maize 19 parents and 88 factorial crosses (European inbreds x US inbreds) were evaluated in F_1 and F_2 generations in multi-environment (13 location-year combinations) trials conducted in three mega-environments: Central Europe as target environment, US Cornbelt as source environment, and Southeast Europe as intermediate environment. Data were recorded for grain yield and dry matter concentration and midparent heterosis and inbreeding depression were also computed. In rye testcrosses of 610 S_0 clones of the two Central European heterotic groups and five Eastern European OPVs were evaluated at eight locations in Germany for grain yield. Maize inbreds and rye S_0 clones were also fingerprinted with 266 and 30 SSR markers, respectively.

We found gene diversities from 0.33 (Pool 41) to 0.48 (Stiff Stalk, non-Stiff Stalk). The Central European heterotic groups Flint and Dent had lower gene diversity compared to the US Cornbelt Stiff Stalk and non-Stiff Stalk material. In rye we used a different approach and examined five OPVs of Eastern Europe (Pop-1 to Pop-5) i.e., exotic germplasm expected to have good adaptation. We found higher mean gene diversity among the five Eastern European OPVs (0.56) than in the established Central European heterotic groups Carsten (0.43) and Petkus (0.53). In both crops, compared to the established European heterotic groups, the exotic germplasm evaluated for possible introgression to broaden the genetic base of these groups, had larger gene diversity.

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The maize study indicated that the focus should be on hybrid evaluation in the target environment. However, if there is enough seed available the genotypes should also be evaluated in the intermediate environment to obtain more reliable estimates. Based on phenotypic and molecular data, we concluded to introgress the non-Stiff Stalk germplasm into the Flint group and the Stiff Stalk germplasm into the Dent group (Reif et al. 2009). In rye, the Carsten group has narrow genetic base and should, therefore, be the primary target for broadening. Nevertheless, all five OPVs are genetically closer to Petkus. Pop-2 and Pop-4 were identified as good candidates for introgression into Petkus and Pop-5 into Carsten. As the introgression of Pop-5 in Carsten may reduce the genetic diversity between two heterotic groups, we may introgress only selected clones of Pop-5. Alternatively, a large number of exotic germplasm should be fingerprinted, diverse material selected on that basis and field evaluated to identify promising populations for broadening the established heterotic pattern in rye.

7.3 Temporal trends in genetic variance in an applied maize breeding program

Genetic variance within heterotic groups is expected to decrease as selection cycles progress (Tanksley and McCouch 1997; Yu and Bernardo 2004). Some snapshots on the relative magnitude of the components of genetic variance (σ^2_A vs. σ^2_D , σ^2_{SCA} vs. σ^2_{GCA}) in heterotic populations were available in the literature (for review, see Fischer et al. 2008). However, there are only a few reports on temporal trends. Monitoring temporal changes in the relative contribution of these variances is important to have a thorough understanding of the possible changes due to recurrent selection and to ensure response to selection in the medium and long term. In applied hybrid breeding programs, data from agronomic performance trials are available over decades. In our study we estimated trends in σ^2_{SCA} and σ^2_{GCA} using data generated over 30 years in the maize hybrid breeding program of the

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University of Hohenheim. During this period hybrid breeding was undertaken with intensive selection accompanied by introgression of new germplasm in the heterotic groups. Further, there has been no germplasm flow between two heterotic groups.

The estimates of σ^2_{GCA} were significantly higher than those of σ^2_{SCA} for grain yield and dry matter concentration (Fischer et al. 2008). We found no significant changes in the estimates of σ^2_{GCA} , σ^2_{SCA} , and their ratio. However, Betrán and Hallauer (1996) reported decreasing $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio for grain yield and grain moisture. Our study demonstrated that intensive selection did not reduce genetic variance. The reason for such results may be that continuous introgression of new germplasm has off-set the losses in genetic variance due to intensive selection. Therefore, we expect continuous response to selection in the Flint \times Dent heterotic pattern on which the hybrid breeding is based at the University of Hohenheim. Further, the magnitude of selection gain in the future is expected to be similar as in the past decades.

The estimates of σ^2_{GCA} and σ^2_{SCA} are equal to σ^2_A and σ^2_D , respectively, assuming (i) parents are homozygous inbred lines with an inbreeding coefficient of one, (ii) there is no epistasis, (iii) and there is no linkage (Wricke and Weber 1986). With this simplified model and assuming variable degree of dominance and gene frequency, we simulated the expected trends in the ratio of σ^2_A vs. σ^2_D under selection (Fischer et al. 2008). We observed that the ratio decreased or increased when there was partial dominance, monotonically increased under complete dominance, and monotonically decreased under overdominance. We compared the simulations with the ratio estimated in the heterotic groups undergoing selection. We concluded that there is either (i) partial dominance or (ii) a mixture of complete dominance and overdominance in our materials. As per quantitative genetic theory, genetic divergence between heterotic groups leads to a predominance of σ^2_{GCA} over σ^2_{SCA} (Reif et al. 2007). In such a situation, the

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performance of hybrids can be reliably predicted based on GCA effects of the parents and, thereby, promising hybrids can be short-listed for field evaluation. To ensure genetic gain in the long run, it would be desirable to regularly monitor the magnitude of genetic variance components in the heterotic groups.

Earlier studies of Betrán and Hallauer (1996) and Yu and Bernardo (2004), who reported a decrease in genetic variance, were based on closed populations. Thus, these studies did not reflect the actual situation in applied maize breeding programs. Maize breeders generally would use open populations and would introgress germplasm into them. Thus, our results are more relevant to maize breeders (Fischer et al. 2008).

It is mandatory to guard against adverse effects of germplasm introgression on the diversity between and within heterotic groups and performance of the hybrid population and heterotic groups. There should be augmentation of genetic distance between heterotic groups, F_1 performance, heterosis, and genetic variance within-groups, and preferably also the *per se* performance of the heterotic groups. Care should be taken not to introgress germplasm from the same source into both heterotic groups of one heterotic pattern, as that will decrease the genetic divergence between them. The establishment and enrichment of the heterotic groups should be based on field evaluation which can be complemented with molecular markers to screen germplasm on a large scale. It is added that the germplasm used in the establishment and for introgression into heterotic groups should be properly documented as it is very important for the efficiency of the breeding program in the future.

Conclusion and outlook

In winter triticale we observed an appreciable magnitude of heterosis in some crosses. The performance of these crosses and the expression of heterosis therein indicated that it should be promising to begin a hybrid breeding program. Based on

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hybrid performance, heterosis, $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio, molecular marker based diversity, and employing an enumeration algorithm that examined the entire space of the diallel crosses among 21 inbreds, we identified the most promising pair of heterotic groups with five inbreds belonging to one heterotic group. Further studies with larger germplasm are required to identify more diverse germplasm and enhance F_1 performance and heterosis.

In rye Eastern European OPVs are a good source to broaden the already established Central European heterotic groups Carsten and Petkus. Our study revealed that the heterotic group Carsten should be the first target for broadening the genetic base. Among the OPVs studied, Pop-5 is promising for that purpose. In view of the relationship of the OPVs studied with Petkus, we suggest that more exotic germplasm should be evaluated.

In the studies on maize, US Cornbelt germplasm was observed to be a good source germplasm for enhancing the genetic base of the Central European heterotic groups Flint and Dent. The study showed that Stiff Stalk germplasm should be introgressed into the Dent group and non-Stiff Stalk into the Flint group. In the evaluation of genetic variance in maize heterotic groups being used in hybrid breeding by the University of Hohenheim over a period of 30 years, it was observed that the ratio of variance components due to SCA vs. GCA did not change. This was attributable to the continuous enrichment of the heterotic groups.

Our studies showed that field evaluation of hybrid performance should basically guide the establishment and enrichment of the heterotic groups. It can be complemented with molecular markers. Further, elite exotic germplasm from agro-climatic regions similar to Central Europe is a good source, and these germplasm should be studied on a larger scale to identify promising germplasm for introgression. The $\sigma^2_{SCA}/\sigma^2_{GCA}$ ratio in heterotic groups in an applied maize hybrid breeding did not change over a period of 30 years. This was possibly due to

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continuous enrichment of the heterotic groups. It highlighted the utility of germplasm introgression. However, it must be ensured that the introgression leads to an enhancement of genetic variance between and within heterotic groups, F_1 performance, and heterosis, and preferably also to upgrade the *per se* performance of the groups.

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8. Summary

Hybrid breeding, based on heterotic groups, has played a key role in the improvement of the productivity of many crops. Genetic variability, an essential prerequisite in plant breeding, is expected to decrease in heterotic groups as a result of continuous breeding efforts. The consequences of the narrowing of genetic variability are a decrease in selection gain and an increase in the susceptibility of cultivars to biotic and abiotic stresses. Thus, establishment of heterotic groups and broadening the genetic base of established heterotic groups are very important research topics in hybrid breeding.

The present work consists of five studies with triticale, maize, and rye. Our objectives were to (1) evaluate heterosis in winter triticale and identify heterotic groups based on field and SSR marker data, (2) investigate by field evaluations and SSR markers the heterotic relationships between the Central European heterotic groups in maize and rye and exotic germplasm from the US and Eastern Europe, respectively, and (3) monitor temporal changes over 30 years in the magnitude of variances due to σ^2_{GCA} (variance due to general combining ability) and σ^2_{SCA} (variance due to specific combining ability) in an applied hybrid maize breeding program.

Triticale has in general low heterosis and, therefore, no hybrid cultivars have been developed so far, although an effective CMS system is available. Twenty-one lines and their 210 diallel crosses were field-evaluated for grain yield at five locations in Germany. Molecular data on 95 SSR markers obtained from an earlier study were reanalyzed for identifying two diverse subgroups. Hybrid performance, midparent heterosis, and estimates of σ^2_{GCA} and σ^2_{SCA} were determined in a diallel, a 10×11 factorial, and the remaining two sub-diallels with 10 and 11 parents. In addition, we applied an enumeration algorithm, which explored the entire sample space to identify diverse heterotic groups and optimize different criteria (F_1 performance, midparent heterosis, and/or $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio) in this context. The $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio

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was higher for factorials between groups of parents than in diallels and subdiallels within groups. The analyses indicated a more favorable $\sigma^2_{\text{GCA}}/\sigma^2_{\text{SCA}}$ ratio in situations with genetically distinct populations compared to situations with genetically less distinct populations. Application of the enumeration algorithm improved all three criteria. F_1 performance followed by heterosis were the most important criteria for development and enrichment of heterotic groups. The ratio $\sigma^2_{\text{GCA}}/\sigma^2_{\text{SCA}}$ strongly affected the power to predict hybrid performance from GCA effects of the parents.

Introgression of new germplasm to broaden the genetic base of heterotic groups is required to ensure continued genetic gains in hybrid breeding. In maize, we evaluated 19 inbreds belonging to two Central European heterotic groups (Flint and Dent) and US heterotic groups [Stiff Stalk (SS), non-Stiff Stalk (NSS), and CIMMYT Pool 41] and their factorial crosses (8 x 11) in F_1 and F_2 generations for grain yield and dry matter concentration. The parental inbreds were additionally fingerprinted with 266 SSR markers. Multi-environment (13 location-year combinations) evaluation was performed in three mega-environments: Central Europe as target environment, US Cornbelt as source environment, and Southeast Europe as intermediate environment. We found higher genetic diversity in the exotic germplasm than in the Central European heterotic groups. Based on F_1 performance and heterosis, we conclude that NSS germplasm should be introgressed into the Flint group and the SS germplasm into the Dent group. This strategy exploits the specific adaptation of the European Flint germplasm to the cold climatic conditions in Central Europe as well as the excellent combining ability of the US germplasm.

In rye, we evaluated testcrosses of 610 S_0 clones belonging to the two Central European heterotic groups (Carsten and Petkus) and five East European open-pollinated varieties (OPVs, Pop-1 to Pop-5). S_0 clones were also fingerprinted with 30 SSR markers. The experiments were laid out at eight German locations and grain yield was measured. We found higher genetic diversity in the OPVs compared to the Central European heterotic groups. The Carsten group had a

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narrow genetic base and should, therefore, be the primary target for genetic broadening. Nevertheless, all five OPVs were genetically closer to Petkus than Carsten. Pop-2 and Pop-4 were identified as good candidates for introgression into Petkus and Pop-5 into Carsten. We suggest to use selected clones of these populations for introgression.

Continuous selection is expected to narrow the genetic base of heterotic groups over time. We studied the nature and magnitude of genetic variability in the breeding materials of the maize program of the University of Hohenheim, which is based on two heterotic groups with continuous enrichment by other germplasm. The data generated in multilocation field trials based on inter-group factorial designs conducted from 1975 to 2004 for grain yield and dry matter concentration were analyzed. Grain yield showed a significant annual increase of 0.17 Mg ha^{-1} . There was neither a decrease in the magnitude of genetic variance nor a change in the predominance of σ^2_{GCA} (sum of σ^2_{GCA} of the Flint and Dent heterotic groups) over σ^2_{SCA} . Consequently, for avoiding the adverse effects of selection on genetic variation and for ensuring medium and long-term selection gains, heterotic groups should not be treated as closed populations, but should be continuously enriched by introgression of new germplasm.

The studies in all three crops showed that field evaluation is mandatory to investigate heterotic relationships among germplasm for the establishment, broadening, and monitoring of heterotic groups. Furthermore, information on molecular markers can complement field evaluation studies for these breeding tasks.

9. Zusammenfassung

Für die Verbesserung der Produktivität vieler Kulturpflanzen spielt die Hybridzüchtung, basierend auf heterotischen Gruppen, eine Schlüsselrolle. Grundsätzlich ist genetische Varianz eine wichtige Voraussetzung für Pflanzenzüchtung, welche jedoch, bedingt durch den kontinuierlichen Züchtungsfortschritt in heterotischen Gruppen, abnimmt. Die Konsequenzen dieser Einengung der genetischen Varianz sind ein sinkender Selektionsgewinn und steigende Anfälligkeit der Sorten gegen biotische und abiotische Stressfaktoren. Aus diesem Grund sind die Etablierung heterotischer Gruppen und die Erweiterung der genetischen Basis etablierter heterotischer Gruppen sehr wichtige Fragestellungen in der Hybridzüchtung.

Die vorliegende Arbeit besteht aus fünf Triticale-, Mais- und Roggenstudien, und hat folgende Zielstellungen: (1) Bewertung der Heterosis in Inzuchtlinien von Wintertriticale und Bestimmung heterotischer Gruppen basierend auf Feld- und SSR-Markerdaten, (2) Untersuchung der heterotischen Beziehungen zwischen den mitteleuropäischen heterotischen Gruppen bei Mais und Roggen und exotischem Genmaterial aus den USA und Osteuropa basierend auf Ergebnissen von Feldexperimenten und SSR-Markern, sowie (3) Untersuchungen der zeitlichen Veränderung des Ausmaßes an σ^2_{GCA} (Varianz der allgemeinen Kombinationsfähigkeit) und σ^2_{SCA} (Varianz der spezifischen Kombinationsfähigkeit) in einem praktischen Hybridmaiszuchtprogramm über eine Zeitspanne von 30 Jahren.

Im Allgemeinen hat Triticale eine niedrige Heterosis, weshalb bisher keine Hybridsorte entwickelt wurde, obwohl ein effektives CMS System vorhanden ist. In unserer ersten Studie wurden 21 Linien und ihre 210 diallelen Kreuzungen auf Kornertag an fünf Standorten in Deutschland in Feldexperimenten bewertet.

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Molekulare Daten aus einer vorherigen Studie mit 95 SSR-Markern wurden analysiert, um verschiedene Untergruppen zu identifizieren. Hybridleistung, Heterosis (zum Elternmittel) und Schätzwerte für σ^2_{GCA} und σ^2_{SCA} wurden in einem Diallel, einem 10 x 11 faktoriellen Kreuzungsschema und den verbleibenden zwei Unterdiallelen mit 10 und 11 Eltern bestimmt. Zusätzlich wurde ein Aufzählungsalgorithmus verwendet, der alle möglichen Kombinationen bestehend aus zwei disjunkten Untermengen von Linien untersucht, um heterotisches Genmaterial zu identifizieren und die verschiedenen Kriterien (F_1 Leistung, Heterosis, $\sigma^2_{GCA}/\sigma^2_{SCA}$ Verhältnis) in diesem Kontext zu optimieren. Das $\sigma^2_{GCA}/\sigma^2_{SCA}$ Verhältnis war für die faktoriellen Kreuzungen (zwischen Elterngruppen) größer als in diallelen Kreuzungen und Unterdiallelen (innerhalb der Gruppen). Die Ergebnisse zeigten, dass in Situationen mit genetisch verschiedenen Populationen ein günstigeres $\sigma^2_{GCA}/\sigma^2_{SCA}$ Verhältnis erreicht werden kann als mit Populationen, die nicht genetisch verschieden sind. Durch den Aufzählungsalgorithmus konnte eine Verbesserung in allen drei Kriterien erreicht werden (F_1 Leistung gefolgt von Heterosis sind die wichtigsten Kriterien für die Entwicklung und Erweiterung der heterotischen Gruppen). Das $\sigma^2_{GCA}/\sigma^2_{SCA}$ Verhältnis hatte einen starken Einfluss auf die Vorhersagekraft der Hybridleistung mittels GCA-Effekten.

Die Introgression neuen Genmaterials zur Verbreiterung der genetischen Basis von heterotischen Gruppen ist erforderlich, um einen kontinuierlichen Selektionsfortschritt in der Hybridzüchtung sicherzustellen. In einer Maisstudie untersuchten wir 19 Inzuchtlinien aus zwei mitteleuropäischen heterotischen Gruppen (Flint und Dent) und amerikanischen heterotischen Gruppen [Stiff Salk (SS), non-Stiff Stalk (NSS) und CIMMYT Pool 41]. Die faktoriellen Kreuzungen (8 x 11) in den Generationen F_1 und F_2 wurden für die Eigenschaften Kornertrag und Korntrockensubstanzgehalt geprüft. Die elterlichen Inzuchtlinien wurden zusätzlich mit 266 SSR-Markern genotypisiert. Die Prüfglieder wurden in mehreren Umwelten (13 Ort x Jahr Kombinationen) in drei Mega-Umwelten getestet: Mitteleuropa als

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Zielumwelt, US Cornbelt als Herkunft des exotischen Materials und Südosteuropa als klimatisch dazwischen liegende Umwelt. In dem exotischen Genmaterial wurde eine höhere genetische Diversität gefunden als in den mitteleuropäischen heterotischen Gruppen. Aufgrund der F_1 Leistung und Heterosis konnte der Schluss gezogen werden, dass eine Einlagerung von NSS Genmaterial in Flint und SS Genmaterial in Dent empfehlenswert ist. Diese Strategie nutzt sowohl die spezifische Anpassung des europäischen Flintzuchtmaterials an die kühlen mitteleuropäischen Klimabedingungen als auch die exzellente Kombinationsfähigkeit des amerikanischen Genmaterials.

In einer Roggenstudie wurden Testkreuzungen von 610 S_0 Klonen, die den beiden mitteleuropäischen heterotischen Gruppen (Carsten und Petkus) und fünf osteuropäischen offenabblühenden Populationensorten (OPVs, Pop-1 bis Pop-5) angehörten, bewertet. Die S_0 Klone wurden mit 30 SSR-Markern genotypisiert. Die Feldexperimente wurden an acht deutschen Standorten auf Kornertrag geprüft. In den OPVs lag eine höhere genetische Diversität vor als in den mitteleuropäischen heterotischen Gruppen. Carsten hatte eine engere genetische Basis und sollte deshalb vorrangig erweitert werden. Trotzdem waren alle fünf OPVs genetisch näher an Petkus. Pop-2 und Pop-4 wurden als gute Kandidaten für die Erweiterung der genetischen Diversität von Petkus und Pop-5 für die Erweiterung von Carsten eingestuft. Dabei könnten einzelne, besonders leistungsstarke Klone dieser Populationen für die Introgression verwenden werden.

Allgemein wird erwartet, dass Selektion über längere Zeit die genetische Basis der heterotischen Gruppen einengt. Wir untersuchten die Art und das Ausmaß genetischer Varianz im Maiszuchtmaterial der Universität Hohenheim, welches auf zwei heterotischen Gruppen basiert, die kontinuierlich mit anderem Genmaterial erweitert wurden. Es wurden Leistungsprüfungsergebnisse aus den Jahren 1975 bis 2004 für Kornertrag und Korntrockensubstanzgehalt aus mehrortig geprüften faktoriellen Kreuzungen zwischen Flint- und Dent-Inzuchtlinien ausgewertet. Der

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Kornertrag zeigte einen signifikanten jährlichen Anstieg von 0.17 Mg ha^{-1} . Es konnte keine Reduktion der genetischen Varianz und auch keine Änderung im Verhältnis der σ^2_{GCA} (Summe der σ^2_{GCA} der heterotischen Gruppen Flint und Dent) zur σ^2_{SCA} festgestellt werden. Daraus folgt, dass heterotische Gruppen nicht als geschlossene Populationen zu betrachten sind, sondern kontinuierlich durch Introgression von neuem Genmaterial erweitert werden sollten, um nachteilige selektionsbedingte Effekte auf die genetische Varianz zu vermeiden und mittel- und langfristige Selektionsgewinne sicherzustellen.

Die Studien aller drei Kulturarten zeigten, dass Feldversuche unabdingbar sind (1) zur Feststellung der heterotischen Beziehungen zwischen Genmaterial, (2) zur Etablierung heterotischer Gruppen und (3) zur Erweiterung von deren genetischer Basis. Zusätzlich können molekulare Marker als weiteres wichtiges Werkzeug zur Lösung dieser züchterischen Problemstellung eingesetzt werden.

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Stuttgart, im April 2009

Sandra Fischer