Aus dem
Institut für Kulturpflanzenwissenschaften
Universität Hohenheim
Fachgebiet Bioinformatik
Prof. Dr. Hans-Peter Piepho

Optimizing the prediction of genotypic values accounting for spatial trend and population structure

Dissertation
zur Erlangung des Grades eines
Doktors der Agrarwissenschaften

vorgelegt der
Fakultät Agrarwissenschaften
der Universität Hohenheim

von
Master of Science
Bettina Ulrike Müller
aus Ostfildern/Ruit

2010

Die vorliegende Arbeit wurde am 22.12.2010 von der Fakultät Agrarwissenschaften der Universität Hohenheim als "Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften" angenommen.

Tag der mündlichen Prüfung:	13.01.2011
1. Prodekan:	Prof. Dr. A. Fangmeier
Berichterstatter, 1. Prüfer:	Prof. Dr. H.-P. Piepho
Mitberichterstatter, 2. Prüfer:	Prof. Dr. A.E. Melchinger
3. Prüferin:	Prof. Dr. S. Graeff-Hönninger

CONTENT

[1] Müller, B.U., Kleinknecht, K., Möhring, J., and H.P. Piepho, 2010, Crop Science, **50**, 794-802.
[2] Müller, B.U., Schützenmeister, A., and H.P. Piepho, 2010, Plant Breeding, **129**, 581 - 589.
[3] Knörzer, H., Müller, B.U., Guo, B., Graeff-Hönninger, S., Piepho, H.P., Wang, P., and W. Claupein, 2010, Agronomy Journal, **102**, 1023-1031.
[4] Müller, B.U., Stich, B., and H.P. Piepho, 2011, Heredity, DOI: 10.1038/hdy.2010.125; in press.

1. General Introduction

The rapid increase of the world population to 6.909 million in 2010 up to 7.302 million in 2015 (UNO, 2008) requires an increased crop production, which can be achieved by (1) extending the area of land under cultivation, (2) an increase in the yield per hectare per crop, (3) an increase in the number of crops per hectare per year, or (4) a replacement of lower yielding genotypes by higher yielding genotypes (Evans, 1993). The increase in population leads to a further urban development and hence to loss of arable land. Therefore an enhancement of the crop production is only possible by higher yields, which can be the result of intensification of cropping, improvement of cultivation practices, or by success of plant breeding. The intensification of cropping can be achieved by harvesting more crops at the same time or at different times on the same piece of land. When crops are cultivated simultaneously on the same area, then it will be of interest to breed crops which show the same performance as intercrop as like as monocrop (Davis and Woolley, 1993; Nelson and Robichaux, 1997; Padi, 2007).

The progress of breeding programs in the last century was achieved by adaptation of the breeder's aim to the changing needs and as well as by application of new breeding techniques and new methods to accelerate the breeding cycle, like marker based-selection and application of double haploid (DH) lines. For this reason breeders could provide the farmers with new stable genotypes, which were adapted to the relevant requests (Fischbeck, 2009). The yield improvements based on breeding, technical, and agronomic progress for wheat, barley, maize, rapeseed, and rye in the years 1961 to 2007 are represented in Figure 1.

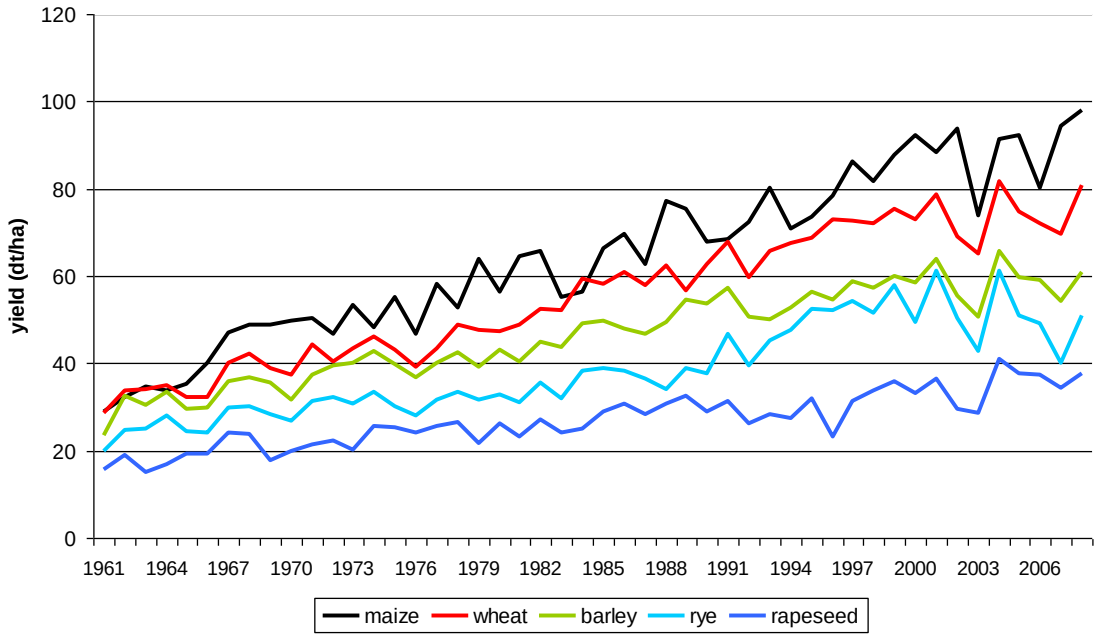

Figure 1: Changes of yield (dt/ha) for maize (black line), wheat (red line), barley (green line), rye (blue line), and rapeseed (dark blue line) between the years 1961 to 2007 in Germany (FAOSTAT, 2010).

For the breeding progress it is important to know how two genetically similar genotypes react within the different environments or within different cropping systems, and how large these non-genotypic variations are. For the selection process of these new genotypes, it is important to differentiate between the genotypic variation and all non-genotypic sources of variation, which are affecting the phenotypic value of the genotypes.

Non-genotypic variation has different sources at one environment: trial layout, agricultural technique, competition effects between neighbouring plots, climatic

influences, spatial influences, soil, and many more. Also a source for variation of the genotypes is the interaction of genotypes within different environments (Piepho, 1998) or the interaction of genotypes within different cropping systems (Davis and Woolley, 1993; Nelson and Robichaux, 1997; Padi, 2007). The estimation of genotypic values of agronomic traits like yield is affected by the influence of all of these non-genotypic sources of variation. Therefore, various field designs and statistical methods were developed to separate the error from non-genotypic variance, which is influencing the phenotypic value.

At the beginning of the 20th century there was a development to use small and more complex block designs instead of using large heterogeneous replicates. Different field designs, like lattice designs or incomplete Latin square designs, were proposed by Fisher (1925) and Yates (1936a, b). Advanced field designs like augmented designs (Federer and Raghavarao, 1975) or α- designs (Patterson et al., 1978) were developed in the following years to adjust for non-genotypic errors, especially spatial trend. Also in the beginning of the 20th century statistical models were proposed of Papadakis (1935), which account for spatial trend effect. These spatial models were subsequently extended and refined (see Piepho et al., 2008b for a review).

All methods mentioned above can be based on a general linear mixed model. The primary goal of the mixed model analysis is to estimate variance components. One characteristic of the mixed model analysis is that an effect can be a fixed effect or random effect. The presence of both fixed and random effects leads to a mixed model. Fixed effects are estimated as best linear unbiased estimation (BLUE). Random effects

are estimated by best linear unbiased prediction (BLUP) and have, in contrast to fixed effects, a covariance structure. Genetic effects, such as general and specific combining ability of a breeding population, can be represented by their covariance structure (Bernardo, 2002), if modelled as random. Also the covariance structure of the spatial trend as well as the structure of field design, such as the block effect, can be modelled as a random effect. All model components can be analysed by a mixed model using Restricted Maximum Likelihood (REML) (Patterson and Thompson, 1971; Gilmour et al., 1995). For getting a more accurate prediction of the genotypic value the mixed model analysis can be further extended by pedigree and marker information (Piepho et al., 2008a). One application of molecular markers in plant breeding is the detection of quantitative trait loci (QTL) to understand the relation between marker and genotype for a specific trait. Linkage and association mapping are methods for detecting such marker-trait associations. In these applications genetic effects are usually modelled as both fixed (regression on markers) and random (unexplained residuals) simultaneously (Piepho, 2005).

In the next subsections field designs and statistical mixed model approaches will be presented which have the goal to separate the genotypic value from the phenotypic value, and therefore, lead to a more accurate prediction of the genotypic value. Such a mixed model analysis is also further extendable by mixed model components for association mapping and for genomic selection. In this thesis the genotypic values were estimated mostly by a fixed genotype effect, because single trials were analysed, where the aim of analysis was to determine the differences between specific pairs of varieties

(Chapter 2, 3, and 4). Chapter 5, which deals with association mapping, uses both fixed and random genetic effects.

The studies of this thesis are part of the GABI GAIN project at the University of Hohenheim (http://gabi.de), which is supported by the BMBF (Bundesministerium für Bildung und Forschung). The project has the objective to develop biometrical and bioinformatics tools for genomic based plant breeding. The objectivities of this thesis are part of the workpackage C. Goal of the workpackage C is at the one hand to extend the pedigree-based BLUP by a Bayesian approach and on the other hand to increase the efficiency of a single trial analysis by using spatial models, which are applicable in a routine analysis. The second objective of this workpackage was treated in this thesis.

1.1 Field designs for crop and plant breeding trials

Before Fisher (1925) proposed the randomised complete block design and the analysis of variance, German plant breeders commonly used field designs such as “Langparzellenanlage” of Zade, which is better known as “Standardanlage”, and the design of Lindhard-Mitscherlich (Thomas, 2006). In the first design control plots are added systematically to the tested plots, in the latter design the varieties are laid out several times in the same order with the aim to correct the plot yields for the spatial trend.

In 1925 Fisher published his work about “Statistical methods for research workers”. In this work he explained the principles of the construction of trials and specified the analysis of variance as a tool for analysing the data. The three main principles for the

construction of trials are replication, randomisation, and control of variation between plots by blocking (Kempton and Fox, 1997; Thomas, 2006).

Different designs for plant breeding trials were developed in the following years, which are based on the three principles of Fisher (1925). All these designs have the aim to minimise the influence of position effect, time effect, and effects caused by human and technique, and thereby to reduce the influence of the non-genotypic variation. The results of small plot trials are conducted under realistic practical conditions to demonstrate the transfer of the trial results to the practice of plant production. A design, which is used in plant production, is the "Grossflächenstreuversuch" (Thomas, 2006). This type of experiment is laid out at the same time on different locations and each location represents a complete block of tested factors.

Two specific kinds of trials are on the one hand unreplicated trials for plant breeding trials, and on the other hand intercropping trials for plant production. Both kinds of trial have in common that they violate one of Fisher´s (1925) principles. Unreplicated trials are trials which are used for early generations in plant breeding programs. This trial violates the principle of replication. Because in the first generation there is only a low amount of seed available, it is not usually feasible to replicate the entries at all locations. Replicated check genotypes, which are often well established varieties, measure the variation of the yield within the trial. The unreplicated genotypes are adjusted based on the replicated check plots. Often the plant breeder prefers a systematic arrangement of the check plots, i.e. each 5^{th} or 6^{th} plot is a check. When spatial methods are to be used it should be of interest if there are more than two different distances between checks

within blocks in order to reliably estimate the spatial covariance model, and whether it is desirable to choose different arrangements of check plots within the blocks. This means that it is important to know if it is better to arrange the check plots systematically or non-systematically within the trials. This question is addressed in the present thesis.

Intercropping trials are experiments, in which two or more different crops are grown simultaneously or sequentially on the same area of land (Federer, 1993). Mainly, these experiments are laid out as strip intercropping trials, in which two or more crops grow simultaneously in alternating strips wide enough to permit independent cultivation but narrow enough for the crops to interact agronomically (Knoerzer et al., 2009). This design violates one of the three principles of Fisher (1925), randomisation, by restricting randomisation or making randomisation impossible. A key issue in the analysis of intercropping trials is that there is no possibility to randomise the position and the crop effects. The position effect reflects the position of a crop row within a plot, i.e. whether the crop row is laid out on the border of two crops as intercrop or within the plot as monocrop. Of agricultural interest in this cultivation practice is the exploitation of the competition effect between the different intercrops, which is measurable by a comparison of monocrop and intercrop yields. To answer these questions, statistical analysis must account for lack of randomisation, and as will be shown in this thesis, this is possible by spatial methods.

1.2 Spatial analysis

After Fisher had introduced the principles of field designs and therewith the randomised complete block design, two further approaches were proposed to remove the non-

genotypic variation caused by soil differences. Papadakis (1935) introduced a method which adjusts the yield of the field experiments by analysis of covariance with respect to corrected yields of adjacent plots. The study of Wilkinson et al. (1983) led to further development of spatial analysis by introducing the 'trend plus error' model for field experiments (Besag and Kempton, 1986). The 'trend plus error' model assumes that the plot error consists of a local trend, which can be removed by differencing, and a residual error, which is assumed to be uncorrelated among plots. Computer programs for spatial analysis popular in plant breeding are ANOFT (Schwarzbach, 1984), which is used by some German plant breeding companies, and Agrobase (Schwarzbach et al., 2007). These programs analyse the data by differencing of yields from neighboured plots (Papadakis, 1935; Wilkinson et al., 1983).

A large number of different spatial models, applicable for a mixed model analysis, were proposed by Gleeson and Cullis (1987), and Schabenberger and Gotway (2005). These spatial models differ in their characteristics of correlation. All these approaches are based on the law of Tobler (1970): „Everything is related to everything else, but near things are more related than distant things". All the spatial models assume that plots which are closer together have higher correlations than plots which are farer away.

Plant breeding companies in Germany repeatedly requested to move from older stand-alone software for the method of Papadakis (1935) and Wilkinson et al. (1983) to mixed model-based analysis, which includes a component for spatial trend. A spatial model is needed, which is robust and can be routinely applied (Martin et al., 1993; Cullis et al., 2006; Martin et al., 2006; Piepho et al., 2008b). Before a spatial model can be used in a

routine analysis, it should be tested and the precision of different spatial models should be compared for their efficiency.

Spatial analysis is sometimes considered as an alternative to the use of classical field designs. Gilmour et al. (1997) and Smith et al. (2001) proposed a strategy to identify the sources of variation in field experiments, modeling the global trend by polynomial and smoothing splines, the extraneous variation by design effects and the local trend by spatial models. These studies propose to start off with a spatial model as baseline model and to extend these models by polynomial and smoothing splines and neglecting therefore the classic field designs. Different attempts were made to find optimal designs which are based on a spatial model (Cullis et al., 2006; Martin et al., 2006; Butler et al., 2008). Williams et al. (2006) proposed the resolvable spatial row-column designs. These designs offer the possibility that the design is based on a spatial model but it also keeps the classic field design principles in mind. In case the spatial component does not lead to an improvement, there is a further possibility, that randomization-based classical model is best suited for analysis. For application of spatial models as a routine method, it is therefore worth considering, if it is better to start the analysis of field trials by using a randomization-based baseline model. The baseline model accounts for row and column effects and is then extended by spatial models. Thereby it can be checked whether the model fit and therefore the adjustment of genotypic values can be improved or not.

1.3 Association mapping

Plant breeders test genotypes in multiple environments in order to obtain estimates of yield and other traits. By a mixed model analysis the structure of the experimental design as well as the spatial covariance are often modelled separately or together as a random effect. The genotypic value can be either estimated as fixed effect (Best Linear Unbiased Estimation - BLUE), or they are predicted as random effect (Best Linear Unbiased Prediction - BLUP). The interaction of the genotype and environment are often modelled as random. A fully efficient method is a one-step approach, in which the phenotypic and genotypic data are analysed at the plot level by one mixed model analysis. When the number of genotypes and environments is large, it is either impossible or impractical to analyse the plot data due to excessive computing time. In studies of Piepho and Möhring (2007) as well as Möhring and Piepho (2009) a weighted two-step approach were suggested to overcome this problem. In the first step each environment is analysed considering the genotypic effect as fixed effect. To achieve a more accurate prediction, spatial covariance and experimental design can be modelled in this step as an additional random effect. In the second step the adjusted genotypic means of each environment are used to compute means for genotypes across environments, using standard errors of means as weights. Further extensions of this step are to integrate pedigree and marker information. For example, marker information can be used either to estimate the genomic breeding values using the genomic selection approach (Meuwissen, 2009) or to detect QTL. QTL can be detected in plants by

linkage mapping, which is routinely applied for plants (Stuber et al., 1999), and by association mapping (Yu et al., 2006; Stich et al., 2008).

Association mapping is a method (Thornsberry et al., 2001; Ersoz et al., 2008), which was successfully used at first in human genetics (Corder et al., 1994; Kerem et al., 1989), and which is applied now also in plant genetics. When non-random association should be detected between genotypes and phenotypes of well-known parents (Flint-Garcia et al., 2003; Ersoz et al., 2008), linkage mapping and association mapping are favourable methods for detecting QTL. In contrast to linkage mapping, association mapping offers the possibility to study association with genotypes and phenotypes of diverse populations. More opportunities for recombination of the alleles are present in a species over several generations. Therefore, association mapping has a higher mapping resolution to detect QTL but lower power than linkage mapping (Ersoz et al., 2008). A major drawback of association mapping studies is the low power of the method. False positives will occur if the incorrect null hypothesis is rejected or in cases, when the statistical test is suitable but there is no association with the trait of interest but rather an association with the population structure. Linkage disequilibrium caused by population structure and relatedness can lead to false positive results (Pritchard et al., 2000; Yu et al., 2006). For association mapping and linkage mapping multiple testing is performed and so methods are needed to control the genome-wide Type I error rate (GWER). Methods for controlling the GWER are the Bonferroni correction and the permutation test (Churchill and Doerge, 1994). Both methods have their disadvantages: The Bonferroni correction is a too conservative approach for multiple testing, and the

permutation test does not account for the population structure when applied in association mapping. Several other alternative analytical methods have been proposed to control the GWER in linkage mapping (Davies, 1977; Lander and Botstein, 1989; Feingold et al., 1993; Rebai et al., 1994; Dupuis and Siegmund, 1999; Piepho, 2001; Li and Ji, 2005). To our knowledge no established method is available which uses the population structure for detecting association of genotypes and phenotypes of distant related individuals.

1.4 Objectivities of the thesis

The overall goal of the thesis was to develop and test methods for minimising the influence of the phenotypic variance for plant production and plant breeding trials of different crops (barley, wheat, sugar beet, pea, maize, and rapeseed) using different appropriate biometric approaches. In particular, the objectives were to:

1. Compare the precision of different spatial models against a baseline model in replicated plant breeding trials for sugar beet and barley trials by using different evaluation criteria, like Akaike Information Criterion and phenotypic correlation (Chapter 2).

2. Check the systematic arrangement against a non-systematic arrangement of check plots in augmented designs. Different unreplicated trials were laid on uniformity trials of winter wheat and barley with different block sizes and

number of used check plots. All generated unreplicated trials were analysed using spatial models and a baseline model (Chapter 3).

3. Check the influence of the intercropping effect against the monocropping effect within intercropping trials of maize, pea, and wheat and to apply different spatial models and a baseline model to these intercropping trials of China and Germany (Chapter 4).

4. Develop an approach to control the genome-wide Type I error rate for association mapping and linkage mapping, which accounts to the population structure (Chapter 5).

References

BERNARDO, R., 2002: Breeding for quantitative traits in plants. Stemma Press. Woodbury.

BESAG, J., and R. KEMPTON, 1986: Statistical analysis of field experiments using neighbouring plots. Biometrics, **42**, 231-251.

BUTLER, D.G., J.A. ECCLESTON, and B.R. CULLIS, 2008: On an approximate optimality criterion for the design of field experiments under spatial dependence. Australian and New Zealand Journal of Statistics, **50**, 295-307.

CHURCHILL, G.A., and R.W. DOERGE, 1994: Empirical threshold values for quantitative trait mapping. Genetics, **158**, 963-971.

CORDER, E.H., A.M. SAUNDERS, N.J. RISCH, W.J. STRITTMATTER, D.E. SCHMECHEL et al., 1994: Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nature Genetics, **7**, 180-184.

CULLIS, B.R., A.B. SMITH, and N.E. COOMBES, 2006: On the design of early generation variety trials with correlated data. Journal of Agricultural, Biological and Environmental Statistics, **11**, 1-13.

DAVIS, J.H.C., and J.N. WOOLLEY, 1993: Genotypic requirement for intercropping. Field Crops Research, **34**, 407-430.

DAVIES, R.B., 1977: Hypothesis testing when a nuisance parameter is present only under the alternative. Biometrika, **64**, 247-254.

DUPUIS, J., and D. SIEGMUND, 1999: Statistical methods for mapping quantitative trait loci from a dense set of markers. Genetics, **151**, 373-386.

ERSOZ, E.S., J. YU, and E.S. BUCKLER, 2008: Applications of linkage disequilibrium and association mapping in maize in Molecular Genetic Approaches to Maize Improvement edited by A. Kriz and B. Larkins. Springer Verlag, Berlin.

EVANS, L.T., 1993: Crop evolution, adaptation and yield. Cambridge University Press, Cambridge.

FAOSTAT, 2010: http://faostat.fao.org/site/567/default.aspx#ancor (last accessed on 30 July 2010)

FEDERER, W.T., and D. RAGHAVARAO, 1975: On augmented designs. Biometrics, **31**, 29-35.

FEDERER, W.T., 1993: Statistical design and analysis for intercropping experiments. Volume 1. Springer Verlag, Berlin.

FEINGOLD, E.P., P.O BROWN, and D. SIEGMUND, 1993: Gaussian models for genetic linkage analysis using complete high-resolution maps of identity by descent. American Journal of Human Genetics, **53**, 234-251.

FISCHBECK, G., 2009: Veränderungen in der pflanzenbaulichen Nutzung des Ackerlandes, In: Die Entwicklung der Pflanzenzüchtung in Deutschland (1908-2008), ed. By Röbbelen, G., Gesellschaft für Pflanzenzüchtung, Göttingen.

FISHER, R.A., 1925: Statistical Method for research workers. Oliver and Boyd. Edinburgh, U.K.

FLINT-GARCIA, S.A., J.M. THORNSBERRY, and E.S. BUCKLER, 2003: Structure of linkage disequilibrium in plants. Annu. Rev. Plant Biol, **54**, 357-374.

GILMOUR, A.R., R. THOMPSON, and B.R. CULLIS, 1995: Average Information REML: An efficient algorithm for variance parameter estimation in linear mixed models. Biometrics, **51**, 1440-1450.

GILMOUR, A.R., B.R. CULLIS, and A.P. VERBYLA, 1997: Accounting for natural and extraneous variation in the analysis of field experiments. Journal of Agricultural, Biological, and Environmental Statistics, **2**, 269-293.

GLEESON, A.C., and B.R. CULLIS, 1987: Residual maximum likelihood (REML) estimation of a neighbour model for field experiments. Biometrics, **43**, 277-288.

KEMPTON, R.A., and P.N. FOX, 1997: Statistical methods for plant variety evaluation. Chapman & Hall, London.

KEREM, B., J.M. ROMMENS, J.A. BUCHANAN, D. MARKIEVICZ, D.K. COX et al., 1989: Identification of the cystic fibrosis gene: genetic analysis. Science, **245**, 1073-1080.

KNOERZER, H., S. GRAEFF-HÖNNINGER, B. GUO, P. WANG, and W. CLAUPEIN, 2009: The rediscovery of intercropping in China: a traditional cropping system for future Chinese agriculture. In: Sustainable Agriculture Reviews 2: „Climate change, intercropping, pest control and beneficial microorganisms“, ed. by Eric Lichtfouse, Springer Science+Business Media, Berlin, pp. 13-44.

LANDER, E.S., and D. BOTSTEIN, 1989: Mapping mendelian factors underlying quantitative traits using RFLP markers. Genetics, **121**, 185-199.

LI J., and L. JI, 2005: Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity, **95**, 221-227.

MARTIN, R.J, J.A. ECCLESTON, and A.C. GLEESON, 1993: Robust linear block designs for a suspected LV model. Journal of Statistical Planning and Inference, **34**, 433-450.

MARTIN, R.J., J.A. ECCLESTON, N. CHAUHAN, and B.S.P. CHAN, 2006: Some results on the design of field experiments for comparing unreplicated treatments. Journal of Agricultural, Biological and Environmental Statistics, **11**, 1-17.

MEUWISSEN, T.H.E, 2009: Accuracy of breeding values of 'unrelated' individuals predicted by dense SNP genotyping. Genetics Selection Evolution, **41**, 35.

MÖHRING, J., and H.-P. PIEPHO, 2009: Comparison of weighting in two-stage analysis of plant breeding trials. Crop Science, **49**, 1977-1988.

NELSON, S.C., and R.H. ROBICHAUX, 1997: Identifying plant architectural traits associated with yield under intercropping: Implications of genotype-cropping systems interactions. Plant Breeding, **116**, 163-170.

PADI, F.K., 2007: Genotype x environment interaction and yield stability in a cowpea-based cropping system. Euphytica, **158**, 11-25.

PAPADAKIS, J., 1935: Methode statistique pour des experiences sur champ. Institut pour l'Ameloriation des Plantes, Salonique (Grèce), Bulletin 23.

PATTERSON, H.D., and R. THOMPSON, 1971: Recovery of inter-block information when block sizes are unequal. Biometrika, **58**, 545-554.

PATTERSON, H.D, E.R. WILLIAMS, and E.A. HUNTER, 1978: Block designs for variety trials. Journal of Agricultural Science Cambridge, **90**, 395-400.

PIEPHO, H.-P., 1998: Methods for comparing the yield stability of cropping systems - review. Journal of Agronomy of Crop Science, **180**, 193-213.

PIEPHO, H.-P., 2001: A quick method for computing approximate thresholds for quantitative trait loci detection. Genetics, **157**, 425-432.

PIEPHO, H.-P., 2005: Statistical tests for QTL and QTL-by-environment effects in segregating populations derived from line crosses. Theoretical and Applied Genetics, **110**, 561-566.

PIEPHO, H.-P., and J. MÖHRING, 2007: On weighting in two stage analyses of series of experiments. Biuletyn Oceny Odmian, **32**, 109-121.

PIEPHO, H.-P., J. MÖHRING, A.E. MELCHINGER, and A. BÜCHSE, 2008a: BLUP for phenotypic selection in plant breeding and variety testing. Euphytica, **161**, 209-228.

PIEPHO, H.-P., C. RICHTER, and E. WILLIAMS, 2008b: Nearest neighbour adjustment and linear variance models in plant breeding trials. Biometrical Journal, **50**, 164-189.

PRITCHARD, J.K., M. STEPHENS, N.A. ROSENBERG, and P. DONNELLY, 2000: Association mapping in structured populations. American Journal of Human Genetics, **67**, 170-181.

REBAI, A., B. GOFFINET, and B. MANGIN, 1994: Approximate thresholds of interval mapping tests for QTL detection. Genetics, **138**, 235-240.

SCHABENBERGER, O., and C. GOTWAY, 2005: Statistical methods for spatial data analysis, CRC Press, Boca Raton.

SCHWARZBACH, E., 1984: A new approach in the evaluation of field trials. Vorträge für Pflanzenzüchtung, **6**, 249-259.

SCHWARZBACH, E., J. HARTMANN, and H.-P. PIEPHO, 2007: Multiplicative main cultivar effects in Czech official winter wheat trials 1976-2005. Czech J. Genet Plant Breed, **43**, 117-124.

SMITH, A., B.R. CULLIS, and R. THOMPSON, 2001: Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. Biometrics, **57**, 1138-1147.

STICH, B., J. MÖHRING, H.-P. PIEPHO, M. HECKENBERGER, E.S. BUCKLER et al., 2008: Comparison of mixed-model approaches for association mapping. Genetics, **178**, 1745-1754.

STUBER, C.W., M. POLACCO, and M.L. SENIOR, 1999: Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Science, **39**, 1571-1583.

THOMAS, E., 2006: Feldversuchswesen, Verlag Eugen Ulmer, Stuttgart.

THORNSBERRY, J.M., M.M. GOODMANN, J. DOEBLEY, S. KRESOVICH, D. NIELSEN et al., 2001: Dwarf8 polymorphisms associate with variation in flowering time. Nature Genetics, **28**, 286-289.

TOBLER, W., 1970: A computer movie simulating urban growth in the Detroit region. Economic Geography, **46**, 234-240.

UNO, 2008: Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat, World Population, Prospects. The 2008 Revision, http://esa.un.org/unpp (last accessed 30 July 2010).

WILKINSON, G.N., S.R. ECKERT, T.W. HANCOCK, and O. MAYO, 1983: Nearest neighbour analysis of field experiments (with discussion). Journal of Royal Statistical Society B, **45**, 151-211.

WILLIAMS, E.R., J.A. JOHN, and D. WHITAKER, 2006: Construction of resolvable spatial row-column designs. Biometrics, **62**, 103-108.

YATES, F., 1936a: Incomplete Latin squares. Journal of Agricultural Science Cambridge, **26**, 301-315.

YATES, F., 1936b: A new method of arranging variety trials involving a large number of varieties. Journal of Agricultural Science Cambridge, **26**, 424-455.

YU, J., G. PRESSOIR, W.H. BRIGGS, I.V. BI, M. YAMASAKI et al., 2006: A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics, **38**, 203-208.

Comparison of spatial models for sugar beet and barley trials

Bettina U. Müller, Kathrin Kleinknecht, Jens Möhring and Hans-Peter Piepho

Institute for Crop Production and Grassland Science, Universität Hohenheim, 70593 Stuttgart, Germany

Crop Science, **50**, 794-802

The original publication is available at http://crop.scijournals.org/

ABSTRACT

Several spatial methods exist for the adjustment of local trend in one dimension. The aim of this study was to evaluate and to compare the precision of different spatial methods. For this purpose 293 sugar beet and 64 multienvironment barley trials of two German plant breeding companies were analysed using a baseline model, which comprised a block and replicate effect, and different one-dimensional spatial models augmenting the baseline model. Model fit was assessed using the Akaike Information Criterion, the phenotypic correlation of the adjusted genotype means between two environments, and the relative efficiency. For the sugar beet and barley trials the baseline model outperformed the spatial models in the majority of cases, while in some cases the addition of a spatial component proved beneficial. Based on these results we propose a conservative approach to spatial modelling starting with a baseline model and then checks, whether adding a spatial component improves the fit. Among the alternative models studied, the first-order autoregressive and the linear variance models were the most promising candidates.

ABBREVATIONS

AIC: Akaike Information Criterion, AR1: first-order autoregressive model, $AR1_f$: AR(1) model with fixed block effect, $AR1_r$: AR(1) model with random block effect, Base: Baseline model, $Base_f$: Baseline model with fixed block effect, $Base_r$: Baseline model with random block effect, GAU: Gaussian model, GAU_f: Gaussian model with fixed block effect, GAU_r: Gaussian model with random block effect, LV: Linear variance model, LV_{fn}: LV with fixed block effect and with nugget, LV_{rn}: LV with random block effect and with nugget, LV_f: LV with fixed block effect and without nugget, LV_r: LV with random block effect and without nugget, Sph: Spherical model, Sph_f: Spherical model with fixed block effect, Sph_r: Spherical model with random block effect, v.d.: variance of differences.

Arrangement of check plots in augmented block designs when spatial analysis is used

Bettina U. Müller, André Schützenmeister and Hans-Peter Piepho
Institute for Crop Science, Universität Hohenheim, 70593 Stuttgart, Germany

Plant Breeding, **129**, 581 - 589

The original publication is available at http://www.wiley.com/bw/journal.asp?ref=0179-9541

ABSTRACT
Early generation plant breeding trials are often laid out in unreplicated designs with replicated checks. This paper compares the systematic arrangement of check plots to an allocation, which has two or more different arrangements of check plots within blocks. Four uniformity trials were overlaid with augmented designs, which differed in block size, number and type of checks. Another aim was to compare different spatial models and a baseline model, which did not consider spatial trend. Model fit was assessed using the average empirical variance (EMP) for each arrangement. For three of the four uniformity trials the lowest EMP was found for the arrangement of check plots with different defined distributions of checks within blocks. The systematic arrangement was superior only in one uniformity trial. We propose therefore to allocate checks with different arrangements within blocks. The comparison of the spatial models showed that the linear variance model, the AR(1) model, and the spherical model, all assuming a nugget, were the most promising candidates. A further gain in precision could be detected by a two-dimensional analysis.

Keywords: Uniformity trial, spatial analysis, augmented designs, check plots, unreplicated trials, nearest-neighbour methods.

Extension and evaluation of intercropping field trials using spatial models

Heike Knörzer[1], Bettina U. Müller[2], Buqing Guo[3], Simone Graeff-Hönninger[1], Hans-Peter Piepho[2], Pu Wang[3], and Wilhelm Claupein[1]

[1]University of Hohenheim, General Crop Farming, Fruwirthstraße 23, 70593 Stuttgart, Germany
[2]University of Hohenheim, Bioinformatics, Fruwirthstraße 23, 70593 Stuttgart, Germany
[3]Agricultural University, College of Agronomy and Biotechnology, No. 2 West Yuan Ming Yuan Road, Beijing 100094, China

Heike Knörzer and Bettina U. Müller equally contributed to this work.

Agronomy Journal, **102**, 1023-1031.

The original publication is available at http://agron.scijournals.org/

ABSTRACT
Intercropping has often been considered as a secluded cropping system within one field. However, in African and Asian countries, where intercropping is widespread, the system can be looked on at a much larger scale: small fields alternate as strips with different crops grown on them, turning the collection of fields into a kind of unplanned intercropping. The more fragmented the agricultural landscape, the more relevant the borders can become. Traditionally, statistical analysis of intercropping experiments has been done by a simple analysis of variance without taking spatial models into account. But especially strip intercropping experimental arrangements lack in randomization as the cropping system imposes alternating strips with several crop rows. Thus, spatial variability and its effect on yield have to be regarded differently. Two different features of intercropping experiments were studied in the present paper: statistical peculiarities of intercropping designs and the border effect which is a key component of intercropping performance. Field trial results from Germany and China indicated that intercropping showed significant border row effects within the first four rows. For statistical analysis, different spatial models were added to the baseline model to account for the spatial trend and to check whether or not standard models are suitable for analyzing intercropping experiments. The results showed that for the German experiment the baseline model fitted well in the year 2008 and a common analysis of variance seemed to be well suited. However, for the Chinese experiments and the German experiment in the year 2009 the spatial models improved the model fit.

A general method for controlling the genome-wide Type I error rate in linkage and association mapping experiments in plants

Bettina U. Müller[1], Benjamin Stich[2] and Hans-Peter Piepho[1]

[1] Institute for Crop Science, Universität Hohenheim, 70593 Stuttgart, Germany
[2] Max Planck Institute for Plant Breeding Research, 50829 Köln, Germany

Heredity, DOI: 10.1038/hdy.2010.125; in press

The original publication is available at http://www.nature.com/hdy/

ABSTRACT
Control of the genome-wide Type I error rate (GWER) is an important issue in association mapping and linkage mapping experiments. For the latter, different approaches, such as permutation procedures or Bonferroni correction, were proposed. The permutation test, however, cannot account for population structure present in most association mapping populations. This can lead to false positive associations. The Bonferroni correction is applicable, but usually on the conservative side, because correlation of tests cannot be exploited. Therefore, a new approach is proposed which controls the genome-wide error rate, while accounting for population structure. This approach is based on a simulation procedure, which is equally applicable in a linkage and an association mapping context. Using the parameter settings of three real datasets, it is shown that the procedure provides control of the GWER as well as the generalized genome–wide Type I error rate ($GWER_k$).

Keywords: Association mapping, genome-wide Type I error rate, linkage mapping, mixed model, Monte Carlo simulation, parametric bootstrap.

6. General Discussion

To assess the performance of a cultivar or line it is important for the breeder and for the crop producer to get an unbiased estimate of its genotypic value. For this purpose it is important to differentiate between the genotypic and non-genotypic sources of variation, which affect the phenotypic value. A good statistical analysis of phenotypic data is therefore a prerequisite in order to make the best possible use of non-genotypic and genotypic information. Mixed model approaches can be extended by incorporation of spatial coordinates of field plots or marker information to optimize the estimates of non-genotypic and genotypic information. The objective of this thesis was therefore to optimize the estimation of the genotypic value by using different mixed model approaches extended by spatial analysis and association mapping for plant breeding and plant production trials.

6.1 Modeling of genotypic values as fixed or random effects in the mixed model

Experimental data, like plant breeding and plant production data, can be analysed by a mixed model to estimate the genotypic values and treatment effects. The response is modelled by fixed and random effects plus a residual error that represents all the variability, which cannot be accounted for by the random and fixed effects of the model. A long-standing and essentially unresolved question is, if the genotype effect should be taken as random or fixed. Searle (1992) recommended considering the effect as random if the number of genotypes is large, which might be the case for early generation trials of plant breeding experiments. Smith et al. (2005) suggests to first formulate the specific aim of the study, and upon this, to make the decision whether the genotype

should be random or fixed. From the point of view of Smith et al. (2005), a fixed genotype effect is appropriate, if differences between specific pairs of genotypes should be detected. Specific differences estimated as random genotypic effect would be biased. A random genotype effect should be chosen if the aim of the analysis is ranking and selection of new genotypes, since it maximises the probability of a correct ranking (Searle et al., 1992; Smith et al., 2005). Another approach is to regard the genotype effects in the early generation trials as random, because the genotypes themselves can be regarded as a random sample from a population of genotypes that could have been generated from the various crosses (Piepho et al., 2008), and to use a fixed genotype effect after some number of selection stages.

The focus in this thesis was mostly on the analysis of a single trial, where the aim of analysis was to determine the differences between specific pairs of varieties so the genotype effect was taken as fixed (Chapters 2, 3, and 4). In Chapter 5 the genotype effect was taken as fixed in the first stage of the analysis to compute the adjusted entry means of the several locations, and in the second stage of the analysis the QTL effect was taken as fixed and the unexplained variation of the genotypes were modelled as random (Stich et al., 2008).

Further effects, which can be modelled as random for increasing the efficiency, are effects representing the structure of field designs, such as block effect (Chapters 2, 3, and 4). By modeling the block effect as random the inter-block information can be used, which usually leads to an increased precision (John and Williams, 1995). The plot errors have to be modelled as random, regardless of whether they are modelled as independent or spatially correlated.

In this thesis mostly the plot errors were modelled spatially correlated by different covariance structures, which were compared in this thesis. This will be discussed in Sections 6.2 and 6.3.

6.2 Tools for model selection

Model selection can be based on the principle that there is no simple true model and the model should be found, which best approximates the complex underlying true model (Burnham and Anderson, 2002). Model comparison tools for finding the best model among several candidate models are the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and the corrected Akaike Information Criterion. All these criteria consist of two terms: the first term is two times the negative log-likelihood; the second term is the penalty, which increases with the number of model parameters and is a characteristic for the different model selection criteria. All these selection criteria search for an approximate model that shows the smallest discrepancy to the true model and they all try to strike the balance between model realism on the one hand and the principle of parsimony on the other hand. There is no consensus as to which model selection criterion is the best. Lee and Gosh (2009) and Spilke et al. (2010) compared the performance of three different model selection criteria for spatial analyses and could not find one single optimal selection criterion. The performance of the criteria to select the true model improves with increasing sample size and model complexity, but not with the variance parameter values. In Chapter 2 also different criteria were used to compare the different models for spatial analysis. In this study the AIC was taken to compare all models with the same fixed effect. Model selection by AIC is restricted to models with the same fixed effects for Restricted Maximum Likelihood (REML).

Provided that the models have different fixed effects, these models can be compared by AIC based on Maximum Likelihood (ML) estimation (Wolfinger, 1996).

In Chapter 2 the phenotypic correlation was chosen as another way for comparing the models with the same fixed effects. The use of this method is restricted to series of experiments. The precision of the spatial methods in Chapter 2 can be compared by the Pearson correlation of adjusted means from different environments and therefore the adjusted genotype means were calculated from each series of trials in the different environments for the different used spatial models and baseline model. The adjusted genotype means for each of the models were correlated between pairs of environments. The phenotypic correlation is influenced by the genotype-environment interaction and therefore the phenotypic correlation was low. However, the phenotypic correlation can be used as a relative measure for the model fit to compare the models to each other because all spatial models and baseline model are influenced by the same genotype-environment interaction effects and therefore relative differences in correlations reflect differences in the precision of the spatial models.

Two other criteria were used in Chapter 2 and 3, i.e. the average predicted variance (PRE) and the average empirical variance (EMP) of pairwise genotype contrasts, which were proposed by Baird and Mead (1991) and by Wu and Dutilleul (1999). PRE is defined as the sum of the variances of pairwise genotype contrasts whereas EMP is defined as the sum of the squared pairwise genotype contrasts.

If the PRE of the spatial model was higher than the PRE of the baseline model, the estimated efficiency of the spatial model was higher than for the baseline model. However, this criterion could not be used as a method to evaluate the model comparisons. The estimate of variance of each pairwise genotype contrast strictly

depends on the model and therefore the estimates of the efficiency cannot be used directly as a criterion for preferring a model over another.

For uniformity trials, which were considered in Chapter 3, the EMP could be used as a criterion to compare different spatial models and the baseline model, because all plots had the same genotypic effect and therefore the true differences between the genotypes were zero. The differences between the adjusted genotype means can therefore be used to assess the precision via the EMP criterion in Chapter 3.

In addition to the comparison of different spatial models, in Chapter 3 also different augmented designs were compared by the EMP criterion. To make correct selections with high probability, one must have precise pairwise comparisons among all treatments. Classical measures of design efficiency are A- and D-optimality, which are applicable for designs with a large number of entries, where selection is the prime objective (Cochran and Cox, 1957; John and Williams, 1995). All of these design efficiency measures are based on the average design efficiency factor *E,* which is given by the harmonic mean of the average efficiency factors of pairwise treatment factors. The geometric mean efficiency factor is used for the D-optimality. If the geometric mean efficiency factor of a design is larger than for another design, the design is D-optimal. An A-optimal design is a design which maximizes the average design efficiency factor *E* for a given dataset. These classical measures have, however, the disadvantage that an extra error from estimating variance components is ignored. EMP does not have this problem.

In the study of Martin et al. (2006) different criteria for A-optimality are suggested for unreplicated trials: the A_{ns}-criterion, which minimizes the average pairwise variance between test and control varieties, the A_{nn}-criterion for the pairwise variance between

test varieties, and the A_{ss}-criterion for the pairwise variance between control varieties. Martin et al. (2006) compared the different A-criteria and concluded that the A_{ns}- and A_{nn}-criterion are similar if a large number of test varieties are used. The A-criteria, especially the A_{ns}-criterion, are related to the EMP criterion used in Chapter 3. The calculation of the eigenvalues needed to compute the efficiency factors can be computationally expensive, therefore another useful criterion is the (M,S)-optimality criterion, which is a screening mechanism in computer algorithms to generate effect designs (John and Williams, 1995; John and Whitaker, 2000). For the (M,S)-criterion, first a class of designs is build which maximizes the mean of the efficiency factor (M-optimality) and then minimizes within this class the spread of the efficiency factor (S-optimality).

In Chapter 3 the A- and D-optimality criteria as well as the (M,S)-criterion were not used. Instead, the average empirical variance (EMP) was used as criterion, which is related to the other criteria and was also applied as a measure in the study of Wu and Dutilleul (1999). The major advantage of EMP over A- or D-optimality is that extra error arising from the estimation of variance components is taken into account (Baird and Mead, 1991). In Chapter 2 EMP was therefore used as measure because the main interest was to compare different designs and models simultaneously.

6.3 Spatial models for optimising the field trial data

A crop producer and especially a plant breeder is confronted with one central problem when comparing the treatments of a field trial: the performance of the treatments is notably affected by the environmental condition, like fertility of the plots, in which the treatments are tested. The correlation of plots decreases with increasing distance of the plots. One useful statistical approach for examining the within-trial heterogeneity is to

use spatial models. The correlation between the plots is modelled by a spatial covariance structure. There are different spatial models which have different covariance structures to model the heterogeneity. A primary concern of many German plant breeding companies is to find one robust preferable spatial model (see Chapter 1). Therefore, different spatial models were compared based on different criteria (Chapter 6.2) in the Chapters 2 and 3. No clear-cut winning model could be found in both studies, which was preferable over all spatial models. In a lot of cases it was noticeable that the baseline model, which does not consider the spatial trend, showed the best model fit. Studies of Piepho and Williams (2010) as well as Chapter 2 and 3 recommend therefore a conservative approach to spatial modelling. This approach starts with a baseline model, which reflects the randomization of the field trial and is not neglecting the row, column, or block effects. Subsequently, a spatial trend is then added and it is checked, if the model fit can be improved. Spatial models which are appropriate as an add-on to the baseline model are the AR1 model and the linear variance model (Williams, 1986). A crucial assumption for this conservative approach, however, is that there is a valid randomized field design. This is because the baseline model is based on the randomized field design, which is founded in randomization theory.

Gilmour et al. (1997) propose another approach, which assumes spatial modelling as an alternative to the models based on the randomization theory. In this study the authors suggest to start first with a spatial covariance model, like the two-dimensional separable AR1 model, and to then extend this model by components like splines for rows and columns.

The studies of Piepho and Williams (2010) as well as the studies of Chapter 2 and 3 have shown that an efficient strategy is to regard the randomization of the field trials

and to extend this baseline model by spatial trend. There are two important aspect of implying the randomization of the field. The first aspect is that by randomization a separation of genotypic and environmental influences is possible. If there is no randomization there is no unequivocal separation of genotypic and environmental influences. In Chapter 4 intercropping trials were analysed, which were restricted in randomization or were not randomized at all. The analysis of the intercropping trials by spatial models led to an improvement of model fit. For these intercropping trials with restricted randomization, however, there was no clear separation between the position and the spatial trend.

The second aspect is the difficulty to find the correct spatial model, which represents in the best way the correlation between the plots and leads to valid results. It is important to regard randomization in the baseline model because there is an uncertainty in the model selection regarding the correct spatial model. The application of spatial models assumes that there is no global trend, which corresponds to the assumption of stationarity. The extension by polynomials and splines can be used if the assumption of stationarity is violated. In Chapter 3 the stationarity was violated for one uniformity trial and the extension of the models by polynomials and splines led to an improvement of the model fit, if there was a global trend.

Gilmour et al. (1997) propose to start with a spatial model and to extend the model by splines and polynomials. The model selection in this study of Gilmour et al. (1997) is based on the sample variogram. Stefanova et al. (2009) extended the model selection process of Gilmour et al. (1997) by using the 95% coverage interval of 1 000 simulated variogram, which were based on simulated data for the specified random effects. A reason for this extension was that inexperienced users had difficulty to interpret the

sample variograms correctly and therefore to select the correct model. Also the use of splines and polynomials can lead to misspecification of the model (Lee et al., 2010). The mentioned aspects show that there is more certainty to find a good model, if there is a field design based on randomization theory. Starting with a baseline model, then adding on spatial models components and further extensions, leads to a higher chance to model the data appropriately. Moreover, the conservative approach is applicable on a routine basis in plant breeding and the risk of the misspecification of the spatial model is minimized by adherence to randomization principles.

Further extension of the model is possible by a two-dimensional analysis of the field trial data. Possible spatial models are the two-dimensional separable AR1 model, which was used in many studies (e.g. Gilmour et al., 1997; Smith et al., 2005), and the two-dimensional linear variance model (Piepho and Williams, 2010). A greater potential efficiency than for a one-dimensional spatial analysis is achieved by these two-dimensional spatial analysis models, if there is a sufficient number of rows and columns (Cullis and Gleeson, 1991). In Chapter 3 it was demonstrated, that for three analysed uniformity trials a two-dimensional analysis led to a better model fit. For one of the analysed uniformity trials the two-dimensional analysis could not lead to an improvement of the model fit, because this trial was characterized by a much higher number of rows than columns. No two-dimensional analysis was done in Chapter 2, because the analysed field trials also had a much higher number of rows than columns.

An aspect of this thesis was to find a spatial method, which is suitable as a routine method. No clear-cut winning model could be found. Therefore, the suggestion is to start first with a baseline model which includes row or column effects, and to extend this model by one-dimensional spatial models, if the model fit can be improved. For the

one-dimensional spatial models the most appropriate models were the linear variance model and the AR1 model. Also more efficient but also more complex are non-stationary trend components like splines and various random effects for “extraneous variation”. These models are, however, too demanding for a routine application. A two-dimensional analysis is appropriate if there are a sufficient number of rows and columns and it may then lead to further improvement.

6.3.1 Optimising the arrangements of check plots within unreplicated plant breeding trials for spatial analysis

Unreplicated trials have an important place in plant breeding trials. Caused by limited seed in the first generations of the plant breeding process, the new test lines are grown in the first stages without replication, together with replicated standard or check varieties. Trials are performed at one or more locations. The basic idea of this method is to adjust the yields of the test lines by the yields of the local check plots. Kempton (1984) and Kempton and Fox (1997) name some important aspects of design and analysis which should be considered, when unreplicated trials are laid out: (1) plot size, (2) choice of check varieties, (3) frequency of checks, (4) arrangement of checks and adjustment of test plots, and (5) the validity of adjustment by check plots.

The application of spatial analyses is also possible for unreplicated trials (Gilmour et al., 1997; Stringer and Cullis, 2002). For a one-dimensional analysis, where correlation is assumed to be in one dimension, the plots should be long and narrow to maximize the correlation between check plots and test lines. In Chapter 2 it was therefore assumed that higher spatial correlation is found along the dimension where plot margins are shorter. A two-dimensional analysis is most effective, if the plots are square, but can

also lead to further improvement if the plots are long and narrow (Piepho and Williams, 2010; Chapter 3).

The most important aspects for the design of the unreplicated trials are the choice, frequency, and the arrangement of the check plots for adjusting the test lines. More than two check genotypes should be chosen for adjusting the test lines, which have a similar genetic background as the test material, and which show the same response to the soil fertility as the test material. Also, the more plots are used as check plot the stronger the adjustment of the test line yields.

Different designs for unreplicated trials were proposed, like augmented designs (Federer, 1961), α-α designs (Williams and John, 2003), partially replicated (p-rep) designs (Cullis et al., 2006), or augmented p-rep designs (Williams et al., 2010), which include replicated check plots or entries and are applicable for spatial analysis. Federer (1961) proposed the augmented designs where a number of replicated check plots are laid out in a block design and each block is augmented with a number of unreplicated test varieties. Cullis et al. (2006) and Smith et al. (2006) introduced the p-rep design, which replaces checks by some of the entries, leading to partially replicated (p-rep) designs. These designs are useful when trials are repeated across *l* locations. At each location 1/*l*-th of entries can be replicated, such that each entry is tested with two replicates in one of the *l* locations. These authors allocate the replicated entries in such a way that efficiency is optimized for analysis by a particular spatial model. Williams et al. (2010) proposed a design, which combines the approaches of augmented design and p-rep design, the so called augmented p-rep design. An augmented p-rep design is based on an augmented design where the controls are replaced with partially replicated entries.

Kempton (1984) as well as Kempton and Fox (1997) pointed out that a disadvantage of the augmented designs is that the positions of the check plots are randomized and produce an irregular arrangement of check plots. Thereby the first visual assessment of the performance of the new lines is complicated. However, if there are different distances among the check plots, the spatial covariance within the plots can be estimated more accurately as shown in Chapter 3. Although randomization of augmented designs was suggested (Federer and Raghavarao, 1975; Martin et al., 2006), the arrangement of the check plots is often systematic in practice (Kempton, 1984; Besag and Kempton, 1986). Mostly, breeders arrange the check plots systematically every 5^{th} or 6^{th} plot. In Chapter 3 therefore different augmented designs were compared, which differed in their frequency and arrangement of check plots. Augmented designs with a non-systematic arrangement of check plots and a systematic arrangement of check plots were compared, based on four dataset of uniformity trials. A main result of Chapter 3 was that for most of the different arrangements, a non-systematic arrangement of check plots had a lower EMP than the systematic arrangement. For more accurate estimation of the genotypic values, it is therefore better to have a non-systematic arrangement, especially to estimate the spatial models. The non-systematic arrangement is characterized by different distances within the check plots. Because of this, the covariance within smaller distances can be estimated more accurately.

6.3.2 Optimising the analysis of plant production trials by spatial models

One point, which was not considered in the studies of spatial analysis presented in this thesis, is the possible presence of competition effects between neighboured plots. These effects were not considered because the breeders who conducted the analysed trials reported no evidence of competition effects. However; if there would be competition

between the neighboured plots, a further improvement can be obtained by regarding the information of plant height. The methods appropriate for this purpose will be briefly discussed in this section.

Competition among genotypes cannot be removed by randomization and replication. There are studies, which show a high competition effect between maize and grain plots. (Lorgeou, 1986, cited by David et al., 2001, Van Waes, 1997, cited by David et al., 2001). Furthermore, in Chapter 4 a competition effect between maize and wheat was observed for German trials. However a higher competition effect was visible in Chapter 4 between maize and pea than between maize and wheat. The competition effects may be influenced by the differences of plant height, maturity, leaf area, density, or planting date (David et al., 2001). The competition effect is a source of bias for species comparison. Methods to do this are either to increase the plot size, which increases the land area needed and the amount of seed required, or to modify the experimental design by reducing the interference by an optimal allocating of the species to the plots. David and Kempton (1996), David et al. (1996), and Büchse (2003) proposed designs based on block designs, α-designs, and split-plot designs which group cultivars with similar heights as neighboured plots. These designs are applicable if there is prior information on the cultivar heights or maturity. Büchse (2003) proposed the designs of David and Kempton (1996) as a method when cultivars of different groups should be compared, because all differences have the same standard error. If the differences between the groups are not of interest and there is a high competition effect, Büchse (2003) proposed to use a split-plot design. A further design, which was proposed by Azais et al. (1993), is the neighbour-balanced design, which is applicable if there is no prior information on cultivar heights. For these designs all experimental treatments have once each other

treatment as right and once as left neighbour. These designs imply that the number of replications is divisible by the number of treatments minus one. A limitation of these designs is therefore the high number of replications necessary for a high number of tested treatments. Wilkinson et al. (1983) proposed partially neighbour balanced designs, where each treatment has once each other treatment on the right side or on the left side.

An additional method is to use the information about the height of the entries in a covariance analysis (David et al., 2000; David et al., 2001). The information of the heights can be included on the one hand via a covariate computed as the differences in height between the plot to the neighboured plot and on the other hand via a competition effect for each cultivar. In the study of David et al. (2001) both methods were compared for maize trials in which the two inner rows of four rows or all rows were harvested separately. The results showed that the most efficient way to control interference is to harvest only the two inner rows, while not harvesting the border rows. If all rows were harvested, the extension by a covariate led to an improvement. Besag and Kempton (1986) proposed a way to integrate the information of neighbour competition effects by modelling a neighbour incidence matrix using a vector of centred treatment effects. All the proposed methods are a further extension to the spatial models considered in the present thesis, which can lead in some cases to further improvements of the model fit. In Chapter 2 and 3 no correction for a competition effect between neighboured plots was performed because there was no information for plant height and because no competition was reported by the breeder for the analysed datasets in Chapter 2 and 3. It can be conjectured that a further improvement of the model fit could maybe obtained by correction for the competition effect. In some cases the autoregressive correlation was

negative, which could be an indication that there was a competition effect between the neighboured plots.

Inter-specific as well as intra-specific competition was studied in Chapter 4 for intercropping trials. A mixed model analysis was done for each species separately, which were affected by the inter-specific as well as intra-specific competition. No correction of the yield by cultivar heights was done in the study, because interest was in significant differences between the intercropped and the monocropped plots, and such differences are caused by competition. Soil fertility and other environmental effects which influenced the species yields were modelled by a spatial analysis. Therefore, this analysis did correct for competition.

In some circumstances, like for intercropping trials, randomization is not possible, and a spatial analysis is the only way ahead. The extension of intercropping trials by spatial analysis led to an improvement of the model fits. It is, however, important to note, that spatial analysis is not an alternative for randomization. Whenever possible the principle of randomization should be maintained, because it is the best way to avoid biases (Cochran and Cox, 1957; John and Williams, 1995; Cox, 2009). Two important aspects why spatial analysis is not an alternative to randomization were mentioned in Section 6.3. Spatial analysis should be regarded as an add-on to an analysis which is based on randomization theory, which may lead to further improvement of precision, however, not as an alternative to randomization.

6.4 Genome-wide error rate in association mapping experiments

The availability of genetic variation is required for progress in plant breeding. One main aspect of plant breeding is to exploit this genetic variability and to use methods, which improve the prediction of the genotypic values, e.g. spatial models (Chapter 2 and 3) or

marker-based approaches (Chapter 5). In conventional breeding the genotypic variation is estimated by measuring the phenotypic performance only. An increased efficiency of the breeding efforts was observed by using modern plant breeding approaches, which estimate the genotypic value directly at the genomic level, like genomic selection (Meuwissen et al., 2001; Meuwissen, 2009; Goddard and Hayes, 2009; Heffner et al., 2009; Piepho, 2009) or detection of QTL which affect a specific trait, using linkage mapping (Lander and Botstein, 1989; Stuber et al., 1999) or association mapping (Bodmer, 1986; Thornsberry et al., 2001; Yu et al., 2006). The difference between the latter two QTL mapping methods is that in linkage mapping, there are only a few opportunities for recombination to occur within families and pedigrees with known ancestry. Association mapping is a statistical approach which detects and localizes association between phenotypic trait variation and a polymorphic gene locus in a germplasm collection with different origins and morphological properties (Zhu et al., 2008). The detection of QTL which influence the trait is based on the assumption that historical recombination exists among loci which are closely linked (Sørensen et al., 2007; Ersoz et al., 2008). Linkage disequilibrium caused by the presence of the population structure affects significantly the results of the association mapping approach and leads to an increasing rate of false positives, if not controlled correctly.

Different methods were proposed to control false positive marker-phenotype associations in linkage mapping, like the Bonferroni correction and the permutation test. Both methods have their disadvantages; the Bonferroni correction is conservative, and the permutation test does not regard the population structure. If applied to association mapping, any correlations between trait and population structure are destroyed by permutation. Chapter 5 therefore proposes a new approach for controlling the rate of

false-positive associations between marker and phenotype. The advantages of this new approach are that it is less conservative than the Bonferroni correction and that in difference to the permutation procedure of Churchill and Doerge (1994) the information of the population structure is accounted for in the computation of the threshold, i.e. the associations between trait and population structure are not destroyed.

False-positive associations may occur if the population structure is not accounted for the threshold computation. However, there are two aspects, related to population structure, in which the association mapping method can have little power to detect an association correctly and lead to false-negative associations. One situation occurs, if the considered trait is strongly associated with the population structure. Flowering time in maize is one example (Thornsberry et al., 2001; Yu and Buckler, 2006). For this trait the distribution of functional alleles is highly correlated with the population structure, which can be caused by local adaption and therefore lead to false-negative associations when taking into account population structure in the analyses (Stich et al., 2008; Myles et al., 2009; Stich and Melchinger, 2010).

Another aspect, which influences the power to detect an association, is the distribution of allele frequencies at the functional polymorphism. Results of empirical studies suggest that most alleles are rare. These rare alleles have mostly little influence on the population as whole, even if the allele has a strong effect on the phenotype and, thus, are difficult to detect by association mapping (Myles et al., 2009; Stich and Melchinger, 2010).

A way to circumvent both problems, which lead to false-negative association in association mapping experiments, if the population structure is regarded, is to create segregating populations. First the correlation between population structure and

phenotypic traits can be broken (Manenti et al., 2009) and secondly the allele frequencies in the progenies can be increased to enhance the power of mapping by establishing segregating populations (Myles et al., 2009; Stich and Melchinger, 2010). The new approach of Chapter 5 is also applicable for segregating populations derived from multiple crosses to control the genome-wide Type I error rate.

6.5 Conclusion

The prediction of the genotypic estimates can be improved by optimizing the field design or by extension of mixed models by spatial trend and marker information. The results of the studies showed, that the integration of spatial trend can lead to an improvement of the model fit. However the baseline model, which does not regard spatial trend, was in a lot of cases the best model compared to the spatial models. The studies showed that the best way is to start with the baseline model and to extend this model by a spatial component, if the model fit can be improved. No spatial model could be found which was preferable over all the other spatial models.

The result of a second study was that optimizing the field designs for unreplicated trials leads to a better prediction of the genotypic estimates. In this study the systematic allocation of check plots was compared to a non-systematic allocation of check plots. Three of the four analysed datasets showed that the non-systematic allocation of check plots leads to a better prediction. A valid randomized design is a good basis for getting a good prediction of genotypic estimates or treatments. In a further study a spatial analysis was done for intercropping trials, which were non-randomized or had restricted randomization. Also in this study the extension of the baseline model by spatial components led in some cases to an improvement. However, no clear separation of

spatial trend and the tested position effect was possible because of the restricted randomization or non-randomization of the field trials.

The extension of the mixed models by marker information can improve the prediction of the genotypic estimates further and QTLs can be detected by the association mapping approach, which are associated with a specific trait. Different methods were proposed which controls the genome-wide error for detecting specific traits. These methods are either too conservative or not accounting for the population structure. In this thesis, therefore, a new approach was proposed which calculates a threshold for the genome-wide error rate for association mapping experiments, which is not too conservative and is regarding the population structure.

In summary, the thesis has proposed and evaluated different approaches for improving the prediction of the genotypic values and treatment effect estimates by integration of spatial trend, marker information, and further extensions, like splines and polynomial regression, as well as by using an optimized field design.

References

AZAIS, J.M., R.A. BAILEY, and H. MONOD, 1993: A catalogue of efficient neighbour-designs with border plots. Biometrics, **49**, 1252-1261.

BAIRD, D., and R. MEAD, 1991: The empirical efficiency and validity of two neighbour models. Biometrics, **47**, 1473-1487.

BESAG, J., and R. KEMPTON, 1986: Statistical analysis of field experiments using neighbouring plots. Biometrics, **42**, 231-251.

BODMER, W.F., 1986: Human genetics: the molecular challenge. Cold spring harbour symp. Quant. Biol., **51**, 1-3.

BÜCHSE, A., 2003: Reduction of interplot interference in variety trials by grouping similar genotypes. Vortrag 49th Biometric Conference of the German Region of International Biometric Society, Wuppertal, March.

BURNHAM, K.P., and D.R. ANDERSON, 2002: Model Selection and Multimodel Inference. 2nd edn. Springer, New York.

CHURCHILL, G.A., and R.W. DOERGE, 1994: Empirical threshold values for quantitative trait mapping. Genetics, **138**, 963-971.

COCHRAN, W.G., and D.R. COX, 1957: Experimental designs. 2nd ed. Wiley, New York.

COX, D.R., 2009: Randomization in design of experiments. International Statistical Review, **77**, 415-429.

CULLIS, B.R., and A.C. GLEESON, 1991: Spatial analysis of field experiments - An extension to two dimensions. Biometrics, **47**, 1449-1460.

CULLIS, B.R., A.B. SMITH, and N.E. COOMBES, 2006: On the design of early generation variety trials with correlated data. Journal of Agricultural, Biological and Environmental Statistics, **11**, 1-13.

DAVID, O., and R.A. KEMPTON, 1996: Designs for interference. Biometrics, **52**, 597-606.

DAVID, O., R.A. KEMPTON, and I.M. NEVISON, 1996: Designs for controlling interplot competition in variety trials. Journal of Agricultural Science, **127**, 285-288.

DAVID, O., H. MONOD, and J. AMOUSSOU, 2000: Optimal complete block designs to adjust for interplot competition with a covariance analysis. Biometrics, **56**, 389-393.

DAVID, O., H. MONOD, J. LORGEOU, and G. PHILIPPEAU, 2001: Control of interplot interference in grain maize: A multi site comparison. Crop Science, **41**, 406-414.

ERSOZ, E.S., J. YU, and E.S. BUCKLER, 2008: Applications of linkage disequilibrium and association mapping in maize. In: KRIZ A., B. LARKINS (eds) Molecular Genetic Approaches to Maize Improvement. Springer, Berlin.

FEDERER, W.T., 1961: Augmented designs with one-way elimination of heterogeneity. Biometrics, **17**, 447-473.

FEDERER, W.T., and D. RAGHAVARAO, 1975: On augmented designs. Biometrics, **31**, 29-35.

GILMOUR, A.R., B.R. CULLIS, and A.P. VERBYLA, 1997: Accounting for natural and extraneous variation in the analysis of field experiments. Journal of Agricultural, Biological, and Environmental Statistics, **2**, 269-293.

GODDARD, M.E., and B.J. HAYES, 2009: Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics, **10**, 381-391.

HEFFNER, E.L., M.E. SORRELLS, and I.-L. JANNINK, 2009: Genomic selection for crop improvement. Crop Science, **49**, 1-12.

JOHN, N.A., and E.R. WILLIAMS, 1995: Cyclic and Computer Generated Designs. 2nd edition. Chapman and Hall, London.

JOHN, N.A., and D. WHITAKER, 2000: Construction of cyclic designs using integer programming. Journal of Statistical Planning and Inference, **36**, 357-366.

KEMPTON, R.A., 1984: The design and analysis of unreplicated trials. Vorträge für Pflanzenzüchtung, **7**, 219-242.

KEMPTON, R.A., and P.N. FOX, 1997: Statistical Methods for Plant Variety Evaluation. Chapman and Hall, London.

LANDER, E.S., and D. BOTSTEIN, 1989: Mapping Mendelian factors underlying quantitative traits using RFLP markers. Genetics, **121**, 185-199.

LEE, H., and S.K. GHOSH, 2009: Performance of information criteria for spatial models. Journal of Statistical Computation and Simulation, **79**, 93-106.

LEE, Y., W. LEE, and H.-P. PIEPHO, 2010: Inferences for random-effect models with singular precision matrix. American Statistician, in review.

LORGEOU, J., 1986: Etude des effets d'allée frontale et de concurrence sur le comportement de quelques variétés de mais dans les essais en petites parcelles. Technical Report, AGPM, Pau, France.

MANENTI, G., A. GALVAN, A. PETTINICCHIO, G. TRINCUCCI, E. SPADA, A. ZOLIN, S. MILANI, A. GONZALEZ-NEIRA, and T.A. DRAGANI, 2009: Mouse genome-wide

association mapping needs linkage analysis to avoid false-positive loci. PLOS Genetics, **5**, 1000331. (doi:10.1371/journal.pgen.1000331)

MARTIN, R.J., J.A. ECCLESTON, N. CHAUHAN, and B.S.P. CHAN, 2006: Some results on the design of field experiments for comparing unreplicated treatments. Journal of Agricultural, Biological and Environmental Statistics, **11**, 1-17.

MEUWISSEN, T.H.E, B.I. HAYES, and M.E. GODDARD, 2001: Prediction of total genetic value using genome-wide dense marker maps. Genetics, **157**, 1819-1829.

MEUWISSEN, T.H.E., 2009: Accuracy of breeding values of 'unrelated' individuals predicted by dense SNP genotyping. Genetics Selection Evolution, **41**, 35.

MYLES, S., J. PEIFFER, P.J. BROWN, E.S. ERSOZ, Z. ZHANG, D.E. COSTICH, and E.S. BUCKLER, 2009: Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell, **21**, 2194-2202.

PIEPHO, H.-P., J. MÖHRING, A.E. MELCHINGER, A. BÜCHSE, 2008: BLUP for phenotypic selection in plant breeding and variety testing. Euphytica, **161**, 209-228.

PIEPHO, H.-P., 2009: Ridge regression and extensions for genomewide selection in maize. Crop Science, **49**, 1165-1176.

PIEPHO, H.-P., and E.R. WILLIAMS, 2010: Two-dimensional linear variance structures for field trials. Plant Breeding, **129**, 1-8.

SEARLE, S.R., G. CASELLA, and C.E. MCCULLOCH, 1992: Variance components. Wiley, New York.

SMITH, A.B., B.R. CULLIS, and R. THOMPSON, 2005: The analysis of crop cultivar breeding and evaluation trials: an overview of current mixed model approaches. Journal of Agricultural Science, **143**, 449-462.

SMITH, A.B., P. LIM, and B.R. CULLIS, 2006: The design and analysis of multi-phase plant breeding experiments. Journal of Agricultural Science, **144**, 393-409.

SØRENSEN, A.P, J. STUURMAN, J. ROUPPE VAN DER VOORT, and J. PELEMAN, 2008: Molecular breeding: maximizing the exploitation of genetic diverstiy. In: R.K. Varshney and R. Tuberosa (eds) Genomics-Assisted Crop Improvement. Springer.

SPILKE, J., C. RICHTER, and H.-P. PIEPHO, 2010: Model selection and its consequences for different split-plot designs with spatial covariance and trend. Plant Breeding, online, DOI: 10.1111/j.1439-0523.2010.01795.x

STEFANOVA, K.T., A.B. SMITH, and B.R. CULLIS, 2009: Enhanced diagnostics for spatial analysis of field trials. Journal of Agricultural, Biological and Environmental Statistics, **14**, 392-410.

STICH, B., J. MÖHRING, H.-P. PIEPHO, M. HECKENBERGER, E.S. BUCKLER et al., 2008: Comparison of mixed-model approaches for association mapping. Genetics, **178**, 1745-1754.

STICH, B., and A.E. MELCHINGER, 2010: An introduction to association mapping in plants. CAB Reviews, in press.

STRINGER, J.K., and B.R. CULLIS, 2002: Application of spatial analysis techniques to adjust for fertility trends and identify interplot competition in early stage sugarcane selection trials. Australian Journal Agricultural Research, **53**, 911-918.

STUBER, C.W., M. POLACCO, and M.L. SENIOR, 1999: Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Science, **39**, 1571-1583.

THORNSBERRY J.M., M.M. GOODMANN, J. DOEBLEY, S. KRESOVICH, D. NIELSEN et al., 2001: Dwarf8 polymorphisms associate with variation in flowering time. Nature Genetics, **28**, 286-289.

VAN WAES, J., 1997: Border effects in variety trials with grain maize in Belgium and the relation with plant height and plant density. Technical Report, Centre for Agricultural Research, Gent, Belgium.

WILKINSON, G.N., S.R. ECKERT, T.W. HANCOCK, and O. MAYO, 1983: Nearest neighbour analysis of field experiments (with discussion). Journal of Royal Statistical Society B, **45**, 151-211.

WILLIAMS, E.R., 1986: A neighbor model for field experiments. Biometrika, **73**, 279-287.

WILLIAMS, E.R., and J.A. JOHN, 2003: A note on the design of unreplicated trials. Biometrical Journal, **45**, 751-757.

WILLIAMS, E.R., H.-P. PIEPHO, and D. WHITAKER, 2010: Augmented p-rep designs. Biometrical Journal, tentatively accepted.

WOLFINGER, R.D., 1996: Heterogeneous variance-covariance structures for repeated measures. Journal of Agricultural, Biological, and Environmental Statistics, **1**, 205-230.

WU, T., and P. DUTILLEUL, 1999: Validity and efficiency of neighbor analyses in comparison with classical complete and incomplete block analyses of field experiments. Agronomy Journal, **91,** 721-731.

YU J., G. PRESSOIR, W.H. BRIGGS, I.V. BI, M. YAMASAKI, et al. 2006: A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics, **38**, 203-208.

ZHU, C., M. GORE, E.S. BUCKLER, and J. YU, 2008: Status and prospects of association mapping in plants. The Plant Genome, **1**, 5-19.

7. Summary

Different effects, like the design of the field trial, agricultural practice, competition between neighboured plots, climate as well as the spatial trend, have an influence on the non-genotypic variation of the genotype. The prediction of the genotypic value of an agronomic trait, like yield, is influenced by the non-genotypic variation. The error, which results from the influence of the non-genotypic variation, can be separated from the phenotypic value by field design and statistical models, like a mixed model. An exact prediction of the genotypic value is important for the selection in plant breeding experiments as well as in the evaluation of genotypes, which have received different treatments in crop experiments. The integration of different types of information, like spatial trend, can lead to an improved prediction of genotypic values. In addition to the information of spatial trend also the prediction of genotypic values can be improved and QTLs can be detected.

The present work consists of four studies from the area of plant breeding and crop science, in which the prediction of the genotypic values was optimized with inclusion of the above mentioned aspects. Goals of the work were

(1) to compare the different spatial models and to find one model, which is applicable as routine in plant breeding analysis,

(2) to optimize the analysis of unreplicated trials of plant breeding experiments by improving the allocation of replicated check genotypes,

(3) to improve the analysis of intercropping experiments by using spatial models and to detect the neighbour effect between the different cultivars, and

(4) to optimize the calculation of the genome-wide error in association mapping experiments by using an approach which regards the population structure.

Different spatial models and a baseline model, which reflects the randomization of the field trial, were compared in two of the studies. In one study the models were compared on basis of different efficiency criteria with the goal to find a model, which is applicable as routine in plant breeding experiments. In another study the different spatial models and the baseline model were compared on unreplicated trials, which are used in the early generations of the plant breeding process. Adjacent to the comparison of the models in this study different designs were compared with the goal to see if a non-systematic allocation of check genotypes is more preferable than a systematic allocation of check genotypes.

The results of both studies are that no spatial model could be found, which is preferable over all other spatial models. In a lot of cases the baseline model, which regards only the randomization but no spatial trend, was better than the spatial models. Different spatial models, like the autoregressive model and the linear variance model of Williams, led to an improvement of the model fit. In both studies the basic principle was followed to start first with the baseline model, which is based on the randomization theory, and then to extend it by spatial trend, if the model fit can be improved.

In the second study the systematic and non-systematic allocation of check plots in unreplicated trials were compared to solve the question if a non-systematic allocation leads to more efficient estimates of genotypes as the systematic allocation. The non-systematic allocation of check plots led to an improved estimation with lower averaged standard errors in three of four uniformity trials. The systematic allocation of check plots was preferable in one of the four analysed uniformity trials. A characteristic of this uniformity trial to the other uniformity trials was that there was a global trend within the field. An extension of the model by polynomial regression and spline approaches to model

the global trend led to a model improvement, however not to a change in the optimal allocation for this uniformity trial.

Also different spatial models and a baseline model were tested and compared on intercropping experiments adjacent to the plant breeding experiments. A characteristic for intercropping experiments is that different cultivars could be laid out simultaneously or successively on the same area. In this study it was tested, if an improvement is expectable for these non randomized or restricted randomized trials by using a spatial analysis. As well as an analysis was done in this study, if the border plot of the different cultivars - wheat, maize, and legume - are influenced by the neighboured cultivar and if there are significant differences to the inner plots. The extension of the baseline model by spatial trend has shown that in some cases the spatial models led to a model improvement. It is important to note for these trials, that there was no complete randomization of the position effect and hence for these trials no clear separation was possible for position effect and spatial trend. The position of the cultivars, border plot or inner plot, had a significant influence on yield. If maize was cultivated adjacent to pea, the yield of the border plot of maize was much higher than the inner plot of maize. When wheat was cultivated behind maize, there were no significant differences in the yield, if the plot was a border plot or inner plot.

In addition to optimizing the field design for unreplicated trials and the extension of the models by spatial trend the marker information was integrated in a further study. An approach was proposed in this study, which calculates the genome wide error for association mapping experiments and accounts for the population structure. Advantages of this approach in contrast to previously published approaches (Bonferroni correction and permutation test) are that the approach on the one hand is not too conservative and on the

other hand accounts the population structure. The adherence of the genome wide error was tested on three datasets, which were provided by different plant breeding companies.

The results of these studies show that by the different extensions, like integration of spatial trend and marker information, and modifications of the field design, an improved prediction of the genotypic values can be achieved.

8. Zusammenfassung

Unterschiedliche Einflüsse, wie Versuchsdesign, landwirtschaftliche Versuchstechnik, Konkurrenz zwischen benachbarten Parzellen, Klima sowie räumlicher Trend wirken sich auf die nicht-genotypische Variation eines Genotyps aus. Die Schätzung des genotypischen Wertes für ein agronomisches Merkmal, wie zum Beispiel Ertrag, wird beeinflusst durch den nicht-genotypischen Anteil an der Variation des Genotyps. Über Versuchsdesign und statistische Modelle, beispielsweise ein gemischtes Modell, kann ein sich durch den Einfluss der nicht-genotypischen Variation ergebender Fehler vom phänotypischen Wert getrennt werden. Eine genaue Schätzung der genotypischen Werte ist sowohl für den Selektionsprozess der Pflanzenzüchtung wichtig, als auch in der Beurteilung von Genotypen, mit unterschiedlicher Behandlung in pflanzenbaulichen Experimenten. Die Integration von Informationen, wie räumlicher Trend, kann zu einer verbesserten Schätzung des genotypischen Wertes führen. Neben der räumlichen Information kann über die Nutzung von Markerinformation auch die Schätzung des genotypischen Wertes verbessert werden und QTL detektiert werden.

Die vorliegende Arbeit besteht aus drei pflanzenzüchterischen und einer pflanzenbaulichen Studie, in denen die Schätzung der genotypischen Werte unter Einbeziehung der oben genannten Aspekte optimiert wurde. Zielstellung der Arbeit war:

(1) Die unterschiedlichsten geostatistischen Verfahren zu vergleichen und ein Verfahren heraus zu filtern, das routinemäßig in der pflanzenzüchterischen Auswertung zu verwenden ist.

(2) Die Analyse von unwiederholten pflanzenzüchterischen Versuchen durch eine verbesserte Allokation wiederholter Standardgenotypen zu optimieren.

(3) Die Analyse von pflanzenbaulichen Intercropping Versuchen durch eine geostatistische Auswertung zu verbessern und den Nachbarschaftseffekt zwischen den unterschiedlichen Kulturarten zu erfassen.

(4) Die Berechung des genomweiten Fehlers in Assoziationsstudien durch ein Verfahren unter Berücksichtigung der Population zu optimieren.

Die unterschiedlichsten räumlichen Modelle und ein Grundmodell, welches nur die Randomisation des Feldversuches widerspiegelte, wurden in zwei Studien miteinander verglichen. In der ersten Studie wurden die Modelle anhand unterschiedlicher Effizienzkriterien verglichen mit dem Ziel ein Modell zu finden, das in der Pflanzenzüchtung als Routineanalyse einsetzbar ist. In der zweiten Studie wurden die unterschiedlichen räumlichen Modelle und das Grundmodell an unwiederholten Versuchen, die in den ersten Generationen des Pflanzenzüchtungsprozess angewendet werden, gegenübergestellt. In dieser Studie wurde auf Basis von Blindversuchsdaten neben den räumlichen Modellen auch abgewogen, ob eine nicht-systematische Anordnung von Standardgenotypen einer systematischen Anordnung von Standardgenotypen vorzuziehen ist.

Die Ergebnisse beider Studien zeigten, dass kein räumliches Modell gefunden werden konnte, das den anderen räumlichen Modellen vorzuziehen ist. Es war ersichtlich, dass in vielen Fällen das Grundmodell mit Randomisation des Feldversuches und ohne Berücksichtigung räumlichen Trends Vorteile gegenüber den räumlichen Modellen offenbarte. Unterschiedlichste räumliche Modelle, wie zum Beispiel das autoregressive Modell und das lineare Varianzmodell nach Williams, konnten zu einer Verbesserung der Modellanpassung führen. In beiden Studien wurde der Grundsatz verfolgt, zuerst mit dem auf der Randomisationstheorie basierenden Grundmodell zu beginnen und dieses in der

Folge, sofern eine offensichtliche Verbesserung zu erwarten war, durch den räumlichen Trend zu erweitern.

In der zweiten Studie wurde die systematische und nicht-systematische Verteilung der Standards in unwiederholten Versuchen gegenübergestellt mit der Frage, ob eine nicht-systematische Anordnung von Standards zu einer verbesserten Schätzung der Genotypen führt als eine systematische Anordnung der Standards. Eine nicht-systematische Anordnung der Standards führte in drei von vier Blindversuchen zu einer verbesserten Schätzung mit geringeren mittleren Standardfehlern. Die systematische Anordnung war in einem der vier untersuchten Blindversuche vorzuziehen. Dieser Blindversuch zeichnete sich gegenüber den anderen Blindversuchen durch einen globalen Trend aus, der im Feld vorherrschte. Eine Erweiterung dieses Models um Polynomregression und Splineansätze, um den globalen Trend zu modellieren, führte zu einer Modellverbesserung, jedoch zu keiner allokativen Veränderung der Standardverteilung.

Die unterschiedlichen räumlichen Modelle und das entsprechende Grundmodell wurden neben den pflanzenzüchterischen Versuchen auch an pflanzenbaulichen Intercropping - Experimenten untersucht und verglichen. Für Intercropping - Experimente ist charakteristisch, dass mehrere Kulturarten simultan oder nacheinander auf der gleichen Fläche angebaut werden. In dieser Studie sollte überprüft werden, ob zum einen eine Verbesserung durch eine geostatistische Auswertung unvollständig bzw. nicht randomisierter Versuche zu erwarten ist. Zum anderen fand eine Untersuchung der Randparzellen verschiedener Kulturarten - Weizen, Mais und Gemüse - auf Beeinflussung durch benachbarte Kulturarten statt, die Unterschiede im untersuchten Merkmal zur Mittelparzelle betrachtete. Die Erweiterung des Grundmodells um den räumlichen Trend zeigte in Einzelfällen eine Verbesserung durch die räumlichen Modelle. Wiederkehrend

wurden in vielen Fällen jedoch Vorteile des Grundmodells bezüglich der Modellanpassung festgestellt. Dabei gilt für diese Versuche zu beachten, dass keine vollständige Randomisation vom Positionseffekt und folglich keine saubere Trennung von Positionseffekt und räumlichen Trend möglich war. Sowohl die Randposition als auch die mittlere Parzelle hatte eine Auswirkung auf den Ertrag der Kulturart. Sofern Mais neben Erbse angebaut wurde, war der Ertrag der Randparzelle bei Mais höher. Für die Kombination Weizen neben Mais konnte kein signifikanter Unterschied im Ertrag festgestellt werden.

Neben der Optimierung des Versuchsdesigns für unwiederholte Versuche und der Erweiterung der Modelle um den räumlichen Trend wurde in einer weiteren Studie die Markerinformation eingebunden. Ein Ansatz wurde in dieser Studie vorgestellt, der den genomweiten Fehler unter Berücksichtigung der Populationsstruktur einhält. Die Vorteile dieses Ansatzes gegenüber den bisher publizierten Verfahren (Bonferroni-Korrektur und Permutationstest) sind der nur moderat konservative Charakter und die Berücksichtigung der Populationsstruktur. Die Einhaltung der genomweiten Fehlerrate wurde anhand von drei Datensätzen verschiedener Pflanzenzüchtungsunternehmen überprüft.

Die Ergebnisse der Studien zeigen, dass über unterschiedliche Erweiterungen und Verbesserungen, wie zum Beispiel die Integration von räumlicher Information, und Markerinformation sowie eine Optimierung des Feldversuchdesigns eine verbesserte Schätzung des genotypischen Wertes erzielt werden kann.

ACKNOWLEDGMENTS

My appreciation is extended to all the people who helped me to conduct the investigations of this research. First I am very grateful to Prof. Dr. Hans-Peter Piepho for his advice, continuous scientific support, and creative suggestions during this thesis work.

Thanks to Prof. Dr. A.E. Melchinger for serving on my graduate committee.

Many thanks to all the member of the GABI GAIN project for their support and helpfulness.

I also would like to thank Prof. Dr. Simone Graeff-Hönninger, Kathrin Kleinknecht, Dr. Heike Knörzer, Jens Möhring, Andre Schützenmeister, PD Dr. Benjamin Stich, and Prof. Dr. W. Claupein for being co-authors of the publications. Thanks to Helmut Kärcher, Herbert Grözinger, Andreas Lohrer, and Thomas Truckses of the experimental station Ihinger Hof for conduction the uniformity trials. My thanks to Dr. Karin Hartung and Andrea Richter for their support in conducting the trials. I gratefully thank Anita Rapp, Zeynep Akyildiz, and Gerdi Frankenberger for their great help in organizational matters.

I thank all the present and alumni members of the Crop Science institute for creating a pleasant working environment. Especially, I wish to thank Dr. Irina Kuzaykova, Dr. Karin Hartung, Jens Möhring, Ulrike Semmler-Busch, Torben Schulz-Streeck, and Andrea Richter.

As well as I want to thank Dr. Sandra Fischer, Dr. Christin Falke, Maren Grossmann, Kathrin Kleinknecht, Dr. Heike Knörzer, Vanessa Prigge, Dr. Elena Orsini, Dr. Christiane von der Ohe, Delphine van Ingelhandt, Dr. Katinka Wilde, Sankalp Bhosale, Johannes Empl, Dr. Peter Risser, PD Dr. Benjamin Stich, Andre Schützenmeister, Dr. Hans-Henning Voss, and Thilo Wegenast for creating a pleasant time in Hohenheim.

Finally I want to thank my family for their great support during the last three years of this thesis.

Curriculum Vitae

Name	Bettina Ulrike Müller	
Date and place of Birth	06.04.1980 in Ostfildern/Ruit	
School education		
	1986-1991	Elementary school (Grundschule Ochsenfurt) Ochsenfurt
	1991-1993	Highschool (Mozart-Gymnasium**)** Würzburg
	1993-2000	Highschool (Egbert-Gymnasium**)** Münsterschwarzach
University education	10/2000-10/2004, Bioinformatics, University of Applied Science Weihenstephan, Freising Diplom (FH) October 2004	
	10/2004-10/2006, Agricultural Science, University of Göttingen, Göttingen Master of Science October 2006	
	07/2007 – 03/2011 Doctoral candidate and scientific assistant at the Institute for Crop Science, Bioinformatics Unit (Prof. Dr. H.-P. Piepho) at the University of Hohenheim, Stuttgart.	
Professional experience	03/2002 - 08/2002	Lynkeus Biotech GmbH Würzburg, Germany
	09/2003 - 10/2004	Institute of Bioinformatics, University of Leipzig Leipzig, Germany
	04/2005 - 11/2006	Institute for Sugar beet research, University of Göttingen Göttingen, Germany
	02/2007 - 06/2007	Syngenta Seeds, Landskrona, Sweden
	04/2011	Strube Research, Söllingen, Germany

Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass ich nicht früher oder gleichzeitig einen Antrag auf Eröffnung eines Promotionsvorfahrens unter Vorlage der hier eingereichten Dissertation gestellt habe.

Stuttgart - Hohenheim, 2010

Bettina Ulrike Müller