

Research in the German priority program: "Heterosis in plants"

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In 2003 the German Research Foundation (DFG) decided to support a supraregional priority program entitled "Heterosis in plants: genomic research for causal analysis of a biological key phenomenon and fundamentals of its optimum exploitation in plant breeding". This interdisciplinary framework program brought together scientists from ten different German universities and research organizations representing different disciplines including molecular biology, plant breeding, bioinformatics, genetics, and plant physiology. The goals of this program were: (1) to better understand the genetic, molecular, and physiological basis of heterosis, and (2) to improve the theoretical and practical tools for a better exploitation of heterosis in plant breeding. These aims were addressed by (1) analyzing heterosis at the molecular level for relevant traits in model species (*Arabidopsis* and maize); by testing genetic hypotheses for heterosis of yield in related and unrelated crops; and by analyzing specific physiological processes relevant for the understanding of the molecular basis of heterosis. Moreover, these studies aimed on (2) the long term goal of predicting heterosis with molecular and bioinformatic tools; the evaluation of the concept of heterotic groups and patterns in hybrid breeding; and the development of new approaches to identify heterotic groups and broadening their genetic basis.

The end of this six year research effort will be marked by an international conference in September 2009 that will bring together world leaders in plant heterosis research at the University of Hohenheim.

For details on this conference please refer to:

<https://www.uni-hohenheim.de/heterosis/home.htm>

More details on this framework program can be found at:

<https://www.uni-hohenheim.de/plantbreeding/350a/dfg/indexd.html>

In the following section brief summaries of goals, results, and publications of the different projects involved in this framework program are provided in alphabetical order:

Molecular identification and characterization of loci causing heterosis in *Arabidopsis thaliana*

Thomas Altmann/Rhonda C. Meyer, Leibniz Institute of Plant Genetics and Crop Plant Research

Heterosis for biomass has been shown to be a widespread occurring phenomenon in *Arabidopsis thaliana* that is manifested as early as 6 days after sowing and enhanced by higher light intensities (Meyer *et al.*, 2004). A high predictive power of metabolic composition for biomass features could be demonstrated (Meyer *et al.*, 2007). Using large RIL and IL populations, several

QTL for heterosis for biomass were identified, involving a combination of dominant, overdominant and epistatic effects (Meyer *et al.*, 2009).

Analysis of the genetic basis of heterosis in oilseed rape (*Brassica napus*) via comparative QTL mapping and expression QTL analysis

Rod Snowdon and Wolfgang Friedt, University of Giessen; Wolfgang Ecke and Heiko Becker, University of Goettingen

The overall aim of this project is a comparative analysis of the genetic control of heterosis in oilseed rape (*Brassica napus* L.) by QTL mapping of heterosis-relevant loci in different *B. napus* mapping populations and alignment to eQTL for heterotically expressed genes. Field and greenhouse evaluations were used for identification of QTLs involved in early biomass and seed yield heterosis, respectively (Radoev *et al.*, 2008). In ongoing work, hybrids that show particularly high or low heterosis for seedling biomass traits are being used for expression QTL analysis using next generation sequencing-based EST tag profiling. Through comparison of eQTL positions to heterosis QTL hotspots, we hope to identify genes that are potentially involved in regulation of heterosis in oilseed rape. Additionally, the contribution of “fixed heterosis” due to polyploidy is being analysed using a set of synthetic allopolyploids.

Molecular studies on marginal overdominance in maize (*Zea mays* L.)

Hartwig H. Geiger, Vera Kuehr, University of Hohenheim

A genetic hypothesis for explaining heterosis called ‘marginal overdominance’ (Wallace, 1968) was to be verified by gene expression and enzyme activity studies in unrelated inter-pool hybrids compared with their parent lines. Transcript abundance of the target genes and enzyme activity of the target enzymes showed no consistent pattern of expression across several differing conditions like stage of development, type of tissue, and environment; across these conditions, overdominance was observed for several gene (or enzyme)/hybrid combinations. In some of these cases differences in gene action between P1 and P2 were observed under different conditions and these findings partly verified the hypothesis of Wallace (1968) and yet it could be shown that in all cases of overdominance across conditions at least one single condition displayed overdominance. Thus, the hypothesis of ‘marginal’ overdominance could not fully be verified in this project.

Metabolic phenotype of heterosis in *Zea mays*

Alfons Gierl, Lilla Roemisch-Margl, Technische Universitaet Muenchen

Based on methods for stable isotope labeling of developing maize kernels we have characterized carbohydrate metabolic fluxes in maize kernels (Ettenhuber *et al.*, 2005), in the B73 and Mo17 inbred lines, their F1-hybrids, and F2-progeny (Spielbauer *et al.*, 2006). The hybrid state revealed no significant reprogramming of relative carbohydrate fluxes in heterotic hybrids compared with inbreds. Similarly, among several kernel mutants with strongly reduced yield and low

starch content, only the *bt2* and *sh2* mutants, which lack AGPase, had a significantly altered flux pattern that was characterized by increased hexose cycling. Our results indicate that the carbohydrate flux pattern in maize kernels is very robust to genetic, developmental, and environmental perturbations.

Molecular dissection of heterosis manifestation during early maize root development

Frank Hochholdinger, University of Tuebingen

Heterosis is already manifested very early during maize (*Zea mays* L.) root development and can be detected in terms of primary root elongation, cortical cell size, and lateral root density (Hoecker *et al.*, 2006; Hochholdinger and Hoecker, 2007). Proteome and transcriptome wide analyses of gene expression in young maize seedling roots (Hoecker *et al.*, 2008a; 2008b) suggest organ specific patterns of non-additive gene expression and imply that global expression trends but also the consistent non-additive expression of specific genes might contribute to the manifestation of heterosis.

Analysis of the relationship between heterosis and the chromatin modifications of DNA and histones in *Arabidopsis thaliana*

Ali Banaei¹, Florian Mette¹, Michael Seifert¹, Marc Strickert¹, Francois Roudier², Vincent Colot², Andreas Houben¹; ¹Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, ²CNRS, Ecole Normale Supérieure, Paris, France

To test whether an interrelationship between heterosis and histone and DNA modifications exist the chromatin structure of *Arabidopsis thaliana* accessions and their intra-species hybrids were comparably analysed. No large scale differences in the pattern of tested chromatin modifications at microscopic level were found after intra-species hybridization. Genome-wide ChIP on chip analysis (Seifert *et al.* 2009). revealed naive variations of histone H3K4 and H3K27 methylation among Col-0, C24 and Cvi. Parental histone H3K4 methylation patterns were additively inherited after intra-species hybridization in the offspring of Col-0/C24 and Col-0/Cvi. Histone H3K27 methylation showed some dynamics in response to intra-species hybridization. The results are being scanned for chromatin modifications that potentially influence the expression of heterosis, which will enable us to draw conclusions on the importance of histone/DNA modifications on gene regulation in general and intra-species formation.

Quantitative and molecular genetic analysis of embryo heterosis in faba bean (*Vicia faba* L.).

W. Link and S. Dieckmann, University of Goettingen.

Based on a specific diallel mating scheme, heterosis was assessed extremely early, when the faba bean individuals existed as embryo (as mature seed). Mid-parent heterosis for mature seed weight (10.6%) and for juvenile plant biomass (14.5%) was proven to be significant. In both traits, the embryos contributed markedly and significantly *via* their own genes to the genetic variation. For

mature embryo weight heterosis, apparently the parental difference in seed weight was decisive, whereas for juvenile biomass heterosis, it was rather genetic un-relatedness of parents (Voges and Link, 2004; Voges and Link, 2005; Voges and Link, 2006a/b, Dieckmann and Link, 2009). Meanwhile, differential gene expression of reciprocally crossed vs. selfed embryos using microarrays is under way (Weber *et al.*, unpublished). A list of up- and downregulated genes was identified in both reciprocal hybrids when compared to selfed seed. Several storage-associated genes are upregulated which are potentially controlled by abscisic acid, a major phytohormone of seed maturation. Additional gene expression experiments are in progress.

QTL analyses of a testcross design for revealing the genetic and molecular basis of heterosis for biomass-related traits in Arabidopsis

Albrecht E. Melchinger, University of Hohenheim

We developed a theoretical framework for determining the contributions of different types of genetic effects to mid-parent heterosis (MPH) considering the epistatic interactions of individual QTLs with the entire genetic background (Melchinger *et al.*, 2007a) and extended this approach as a multiple-interval mapping (MIM) model (Garcia *et al.*, 2008) and triple testcross (TTC) design (Melchinger *et al.*, 2008). Moreover, we investigated the genetic basis of MPH for growth-related traits in Arabidopsis (Kusterer *et al.*, 2007a, b; Melchinger *et al.*, 2007) and concluded that overdominance, dominance as well as epistatic effects play an important role in heterosis.

Optimum exploitation of heterosis utilizing the concept of heterotic groups and patterns in hybrid breeding

Jochen C. Reif, University of Hohenheim

Heterotic groups and pattern are the backbone of hybrid breeding programs. In theoretical investigations, we present a theoretical proof that the ratio of variance due to specific versus general combining ability effects decreases with increasing genetic divergence between two populations (Reif *et al.*, 2007). This could be confirmed using experimental data in maize (Fischer *et al.* 2008) and triticale (Fischer *et al.*, 2009). Therefore, we conclude that interpopulation improvement becomes more efficient with divergent than with genetically similar heterotic groups, because performance of superior hybrids can be predicted based on general combining ability effects.

Heterosis and associated gene expression pattern in early embryo and endosperm development

Stefan Scholten, University of Hamburg

Hybrid maize embryos exhibit a strong crossbreeding advantage as early as six days after fertilization. Associated gene expression pattern indicate gene regulatory interactions among the parental alleles and imply genes related to

regulatory processes to be involved to specify heterosis in the plant life cycle (Meyer *et al.*, 2007). Allele-specific expression analyses confirm that maize, in contrast to the situation in other plants and in animals, has evolved a strategy to activate the paternal genome immediately following fertilization in zygotes before the first cell division providing an explanation for the heterotic proliferation rates of early hybrid embryo development (Meyer and Scholten, 2007).

Network and integrative data analysis of gene expression and metabolite data from *Arabidopsis thaliana* heterosis experiments

Joachim Selbig and Matthias Steinfath, University of Potsdam; Dirk Repsilber, Research Institute for the Biology of Farm Animals, Dummerstorf

A network structure-based model for heterosis in form of a feed-forward two-layer network was analyzed using metabolite profiles from *Arabidopsis*. It allows for directly relating structural network properties with biological function. The biological networks involved show increasing connectivity of regulatory interactions for the heterozygote crossings (Andorf *et al.*, 2009). The prediction of biomass heterosis in resulting offspring was improved by a combination of parental genetic and metabolic markers, identified via feature selection and minimum-description-length based regression methods (Gaertner *et al.*, 2009). For the integrated analysis of data from several different multi-factorial experiments a general processing schema was developed (Steinfath *et al.*, 2007).

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