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Design and Assessment
of Breeding Strategies
for Hybrid Wheat in Europe

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To my father

Contents

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Abbreviations

AEX	Anther extrusion
CHAs	Chemical hybridization agents
CMS	Cytoplasmic male sterility
CNV	Copy number variation
Eps	Earliness *per se*
FHB	Fusarium head blight
fRD	Functional Rogers' distance
GCA	General combining ability
GWA	Genome-wide association
HELP	Hybrid-enabled line profiling
MPH	Midparent heterosis
Ppd	Photoperiod
QTL	Quantitative trait loci
Rht	Reduced height
SCA	Specific combining ability
SNP	Single nucleotide polymorphism
SPS	Spikelets per spike
SSR	Simple sequence repeats
VAEX	Visual anther extrusion
Vrn	Vernalization

1 General Introduction

The long and successful history of allohexaploid bread wheat (*Triticum aestivum* L.; $2n = 6x = 42$; AABBDD), which dominates wheat production today, started about 10,000 years ago when modern agriculture and wheat domestication began in the Fertile Crescent of the Near East (Lev-Yadun et al. 2000; Salamini et al. 2002; Marcussen et al. 2014). The evolution of modern bread wheat was marked by several ploidy changes, events which are quite common among flowering plants (Salamini et al. 2002). Bread wheat possesses three independent subgenomes A, B, and D, while its wild progenitors were diploid ($2n = 2x = 14$) (Marcussen et al. 2014). The two diploid wild grasses *Triticum urartu* (AA) and *Aegilops speltoides* (BB) gave rise to tetraploid wild emmer (*Triticum turgidum*, AABB) by polyploidization (Marcussen et al. 2014). Bread wheat then originated by spontaneous hybridization between *Triticum turgidum* and the D genome donor *Aegilops tauschii* (DD) (Marcussen et al. 2014). The great success of wheat in different geographic regions and under varying climatic conditions is partly attributable to its 17-gigabase complex hexaploid genome (IWGSC 2014).

Wheat is the most important crop in global agricultural production as it possesses the largest global cultivation area (FAOSTAT 2017). In 2014, the world wheat production was around 729 million tons on 220 million hectares, representing the third largest crop production in the world, after maize and rice (FAOSTAT 2017). Wheat is used as animal feed and is the main staple food for about one third of the world's population, providing about 20 %

of the global dietary energy, and is globally the second main food crop for human consumption after rice (FAO 2013; Ray et al. 2013; Shiferaw et al. 2013; IWGSC 2014). Therefore, wheat plays a key role for global food security.

The global population continues to grow, reaching nearly 7.6 billion in the middle of 2017, and is projected to exceed 9 billion by the middle of this century (UN 2017). This ever-growing world population is accompanied by an increasing demand for meat and dairy products due to growing affluence and an expanding biofuel consumption (Evans 2009; Godfray et al. 2010). Simultaneously, global food production is threatened by competition for arable land between food and non-food uses, increasing input costs, and weather extremes as a result of climate change (Godfray et al. 2010; Lobell et al. 2011; OECD/FAO 2013). Thus, agriculture is facing more challenges than ever before, while estimates project a required doubling of the global food demand by 2050 (Godfray et al. 2010; Foley et al. 2011; Tilman et al. 2011; Ray et al. 2012; Ray et al. 2013).

Introduced in the 1960s, the so-called 'Green Revolution' enabled substantial yield increases in wheat for many years, mainly through the introduction of dwarfing genes in combination with large amounts of fertilizer and pesticide applications (Hedden 2003). During the past years, however, about 37 % of the global wheat growing areas are affected by stagnating wheat yields (Ray et al. 2012). Since arable land is finite, there is a strong demand for smart and sustainable agricultural innovations to increase yield and to meet the challenges of future food security. Plant breeders take a key role to feed the world and must therefore adopt innovative breeding technologies to substantially contribute to the required increase in crop production. Here, the exploitation of the hybrid technology also in selfing species, as already successfully done in rice, could significantly boost grain yield (Tester and Langridge 2010; Xu et al. 2014).

In contrast to wheat, recent global yield growth rates were much higher in maize and rice and they also exceeded wheat in their global production

quantity even with a smaller cultivation area (Alston et al. 2009; Ray et al. 2013; FAOSTAT 2017). This difference can, to a large extent, be attributed to the utilization of the hybrid technology, which facilitates increased yields through the phenomenon of heterosis and assures a higher return on investment (Whitford et al. 2013). Especially the latter is also very closely associated with agricultural productivity growth (Alston et al. 2009). Wheat has an autogamous nature and the first scientific wheat breeding in Germany and France was initiated by Wilhelm Rimpau and Henri de Vilmorin, respectively, and started in the late 19th century (Lupton 1987). Traditionally, line breeding is the breeding method of choice for wheat and more than 99 % of the registered wheat varieties in Europe are derived from line breeding programs exploiting self-fertilization of wheat (Longin et al. 2012). However, a main drawback of line breeding is the high level of farm saved seeds (BDP 2010), which leads to a lower turn-over rate of certified seeds each year and therefore to a lower return on investment. The protection of intellectual property is a major concern of breeding companies regarding their high investments and the long breeding cycles of about 10 years prior to the release of new varieties. Consequently, the plant breeding industry puts lower investment in line breeding compared to hybrid crops.

The phenomenon of heterosis

The term heterosis, in its systematic and scientific form, was coined by G. H. Shull and traces back to the beginning of hybrid maize breeding in the US in 1908 (Shull 1914; Crow 1998). Heterosis describes the increased vigor and fitness of a crossbred heterozygous genotype compared to corresponding inbreds (Shull 1952; Lamkey and Edwards 1999). In the literature, heterosis is often expressed as midparent heterosis, which is the deviation of the hybrid performance from the mean of the parents. In contrast, better-parent heterosis describes the deviation of the hybrid performance from the performance of the better parent. However, these definitions of heterosis are more of a

scientific nature and are only of secondary importance for the plant breeder and particularly for the farmer. From an economic point of view, the most interesting comparison is that between the hybrid performance and the performance of the best commercial non-hybrid variety available on the market. This comparison is agronomically relevant and is known as commercial heterosis, and is used as a measure for the usefulness and long-term success of any hybrid breeding program.

The genetic basis and biology of heterosis is still not fully understood although heterosis has now been fascinating scientists for more than 100 years (Shull 1908; East 1908). There are three possible quantitative genetic explanations for the causes of heterotic effects: dominance, overdominance, and epistasis. The dominance hypothesis assumes that deleterious recessive alleles at multiple loci contributed from the one parent are suppressed or masked by dominant alleles from the other parent (Davenport 1908; Bruce 1910; Keeble and Pellew 1910; Jones 1917; Jiang et al. 2017). Therefore, the hybrid would carry more favorable dominant alleles than either of its parents. The overdominance hypothesis explains heterosis with the heterozygosity at individual loci leading to superior performance and an advantage of the hybrid (East 1936; Hull 1945; Crow 1948; Jiang et al. 2017). If only overdominance contributed to heterosis, the hybrid would always be the best performing genotype (Jiang et al. 2017). Finally, heterosis can be explained by epistasis, which assumes favorable interaction effects between different loci (Richey 1942; Schnell and Cockerham 1992; Jiang et al. 2017). The types of digenic epistatic effects include additive-by-additive, additive-by-dominance and dominance-by-dominance effects with other loci (Jiang et al. 2017). With the presence of epistasis, heterosis at a specific locus becomes a function of the genetic background (Goodnight 1999). However, none of these three classical theories is mutually exclusive and probably all explanations contribute to heterosis (Yu et al. 1997; Lamkey and Edwards 1999; Hua et al. 2003; Li et al. 2008; Zhou et al. 2012; Jiang et al. 2017).

The contribution of the different theories and the genetic architecture of heterosis greatly varies among species, crosses, and the trait of interest

(Schnable and Springer 2013). In addition, the event of polyploidization displays a special case of capturing heterotic gene combinations leading to fixed heterosis and a higher probability that epistatic interactions are more pronounced (Kaeppler 2012). Molecular approaches aiming to dissect the basis underlying heterosis included genomic, epigenetic, proteomic, metabolic, quantitative trait loci (QTL), association mapping and network studies, but the molecular basis and genetic mechanisms of heterosis remain elusive and there is no unifying explanation (Birchler et al. 2003; Baranwal et al. 2012; Chen 2013; Groszmann et al. 2013; Schnable and Springer 2013; Itabashi et al. 2018). Nonetheless, heterosis remains the basis of hybrid breeding and offers great potential for agriculture to deal with the increasing demands for food and feed of future generations.

The success of hybrid breeding

Maize is one of the best examples for the success of hybrid breeding. US maize productivity did not change significantly between 1866 and the 1930s, when solely open-pollinated maize varieties were available for the market (Hallauer 1999; Tracy 2004). After the discovery of heterosis in maize, at the beginning of the 20th century, it took more than 20 years, until the 1930s, before the economic success of commercial hybrid breeding emerged (Crow 1998). First maize hybrids in the US in the 1920s showed a commercial heterosis of about 15 % compared to the best open-pollinated varieties (Duvick 1999). However, the production of single-cross maize hybrid seed was too expensive at early stages due to the low performance of inbred lines. In retrospect, the invention of double-cross hybrids by Jones (1918) made hybrid maize seed production commercially successful. Four-way hybrids were more vigorous and removed the restriction to use inbred lines, suffering under inbreeding depression, for the production of hybrid seed (Lee and Tracy 2009). Later on, inbred line performance has been improved and single-cross hybrids dominated the market as they possess the highest level of heterosis.

From the late 1930s on, there was a strong linear increase in US maize yields for more than 70 years (Hallauer 1999; Duvick et al. 2004; Tracy 2004). Clearly, this increased productivity cannot be solely attributed to genetic improvement and includes several other reasons like increased inputs in plant nutrition and plant protection or changes in management practices. Nevertheless, probably more than 50 % of this increase can be attributed to genetic improvement (Tracy 2004), which was facilitated by the successful introduction of the hybrid technology. Thus, the maximum exploitation of heterosis through hybrid breeding was an essential factor for yield increase in maize and nowadays hybrid varieties have replaced open-pollinated varieties (Duvick 2005; Troyer 2006).

Based on the experiences in hybrid maize breeding, it became evident early on that higher heterosis and hybrid performance could be achieved when the parental lines are derived from two genetically divergent germplasm pools, referred to as heterotic groups (Hallauer 1999). Following the definition of Melchinger and Gumber (1998), a heterotic group "denotes a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups", while a heterotic pattern defines a specific pair of heterotic groups showing an optimum exploitation of heterosis. Heterotic groups promote the genetic divergence between parents in a systematic way, mainly as a result of genetic drift and the reciprocal recurrent selection for general combining ability (Melchinger 1999; Reif et al. 2007; Gerke et al. 2015). Since heterosis can be considered as a function of heterozygosity, heterotic groups optimize the exploitation of heterosis and hybrid performance (Martin et al. 1995; Falconer and Mackay 1996; Melchinger 1999; Fu et al. 2014). Consequently, considering a dominance model, fully contrasting allele frequencies would assure maximum heterosis (Miranda Filho 1999). Furthermore, heterotic groups lead to a lower ratio of specific (σ^2_{SCA}) compared to general combining ability (σ^2_{GCA}), and simplify hybrid breeding as early testing, hybrid prediction based on general combining ability (GCA) effects as well as genomic prediction become more

efficient (Melchinger 1999; Reif et al. 2007; Technow et al. 2014). Moreover, a clear two-pool concept also facilitates the stacking of dominant major QTL and the handling of the desired hybrid mechanism (Longin et al. 2012). Therefore, heterotic groups are often designated as the backbone of hybrid breeding and are of major importance for the long-term success of a hybrid breeding program (Melchinger and Gumber 1998). However, heterotic groups are not established overnight and the choice of germplasm to start heterotic groups should be taken carefully since it forms the basis for generations of breeders.

Heterotic groups must usually be established through long-term breeding strategies. For example, heterotic groups in the US Corn Belt evolved from long-term reciprocal recurrent selection (Duvick et al. 2004). Moreover, heterotic groups can be explained by the Wahlund effect (Wahlund 1928), which describes the effect of reduced heterozygosity in subdivided and diverged populations. By contrast, crosses between diverged populations possess an increased level of heterozygosity and thus heterosis. For instance, Central European heterotic groups Dent and Flint were geographically separated and have been established based on geographic origin (Schnell 1992).

The great success of hybrid maize breeding in the US Corn Belt has motivated breeders and scientists alike to transfer the hybrid technology to other outcrossing crops. However, the introduction of successful hybrid breeding requires some main prerequisites: (i) a sufficiently high degree of heterosis, (ii) a reliable and cost-efficient hybrid mechanism which prevents self-pollination, and (iii) a reliable system for the identification and prediction of superior hybrids. All these prerequisites are easily met for maize, where manual emasculation is readily achieved due to the separation of the male and female flowers. By contrast, the flowering biology of other crops requires more advanced hybrid mechanisms, like cytoplasmic male sterility (CMS) or chemical hybridization agents (CHAs) (Fu et al. 2014). For instance, hybrid rye breeding in Central Europe has started in the 1970s and the discovery of the 'Pampa' (P) type CMS cytoplasm and the identification of

the Petkus and Carsten heterotic pools enabled the success story of hybrid rye breeding (Geiger and Miedaner 1999). Nowadays, also hybrid rye varieties have replaced open-pollinated varieties in many countries due to their superior grain yield performance (Geiger and Miedaner 2009). Moreover, hybrid varieties have successfully been established in many other crops such as sunflower, sugar beet, and also in many vegetable production systems, underscoring the hybrid breeding potential (Pickett 1993; Wehner 1999; Colombo and Galmarini 2017). In small grain cereals, particularly in wheat, hybrid breeding is not established yet, but is feasible and promises several advantages to deal with the projected worldwide food demand and is therefore again attracting strong attention in the public and private wheat breeding community (Würschum et al. 2018).

Hybrid wheat breeding

In contrast to allogamous crops, hybrid breeding in autogamous crops has long been considered as unattractive mainly due to several additional layers of complexity (Figure 1). However, also autogamous crops can greatly benefit from the hybrid technology to achieve increased yield through the exploitation of heterosis, higher yield stability and a higher return on investment (Hallauer et al. 1988; Longin et al. 2012). For instance, autogamous crops like rice, sorghum and more recently rapeseed were successfully shifted from line to hybrid breeding. During the first wave of hybrid wheat breeding with its peak in the 1980s, studies reported a midparent heterosis for grain yield of a about 10 % (Merfert et al. 1987; Pickett 1993; Longin et al. 2012). Indeed, recent research based on an extensive number of hybrids tested in multi-environmental field trials confirmed that hybrid wheat holds great potential to increase yield gain, yield stability and global productivity of wheat (Longin et al. 2013; Longin et al. 2014; Whitford et al. 2013; Mühleisen et al. 2014). In hybrid crosses within elite germplasm, a maximum commercial heterosis of 1 Mg ha^{-1} seems feasible, which roughly reflects breeding
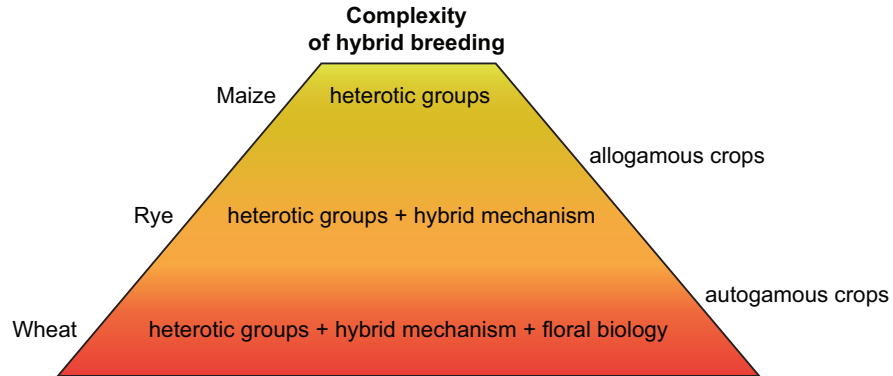
Figure 1: Components contributing to increasing complexity of hybrid breeding in different crops.

progress of 15 years within line breeding (Laidig et al. 2014; Zhao et al. 2015). Particularly in the context of climate change, the higher yield stability of hybrid wheat and its generally higher tolerance to biotic and abiotic stress is of great relevance and makes the hybrid technology very attractive (Jordaan 1999; Longin et al. 2013; Mühleisen et al. 2014). A recent study showed that hybrid wheat is able to combine its higher yield potential with lower susceptibility to Fusarium head blight when compared to line varieties (Miedaner et al. 2017). Moreover, hybrid wheat appears to have a lower susceptibility to frost, leaf rust and Septoria tritici blotch compared to line varieties (Longin et al. 2013). These examples clearly illustrate the potential of hybrid wheat, particularly in the context of climate change and future food security, but history showed that hybrid wheat breeding is not trivial but a challenging venture.

Interest in research and development of hybrid wheat started in the 1960s. Until now, however, the introduction of wheat hybrids in the global wheat market was not crowned with lasting success and hybrid wheat is still a niche product due to several reasons. First, early hybrid wheat breeding in the 1960s and 1970s coincided with the great success of high-yielding semidwarf wheat varieties introduced during the Green Revolution (Wilson and Driscoll 1983; Hedden 2003). The additional benefit of heterosis, therefore, could not compete with the fast improvements of standard line cultivars (Duvick 1999).

Second, the flowering and floral biology of wheat presents an additional layer of complexity compared to allogamous crops like maize or rye and a cost-effective and completely satisfactory hybrid mechanism was missing (Pickett 1993, Figure 1). The ensuing high costs of hybrid wheat seed production were a major reason for its almost non-existent market. Moreover, wheat breeding is not solely focused on yield but combines a large body of breeding goals including disease resistance and bread-making quality. The final favorable combination of these many breeding goals in the hybrid background presents an additional challenge specific for wheat. Hence, a number of issues remain to be solved for a sustainable success of hybrid wheat.

Challenges for hybrid wheat breeding

Heterotic groups are of major importance for the success of a hybrid breeding program in the long run, but are not yet available in wheat. Reciprocal recurrent selection has been already proven as an efficient tool for the establishment of heterotic groups in maize (Hagdorn et al. 2003; Duvick et al. 2004; Gerke et al. 2015) and the additional use of genome-wide marker profiles leading to reciprocal recurrent genomic selection could even accelerate this breeding strategy (Cros et al. 2015). Before turning on the machinery of reciprocal recurrent selection or reciprocal recurrent genomic selection, a beneficial starting point considering several parameters could greatly accelerate the establishment of heterotic groups. Melchinger and Gumber (1998) proposed a multi-stage procedure to identify heterotic groups starting with the grouping of germplasm based on genetic distance. However, a comprehensive study discovering how global wheat genetic diversity could support and accelerate hybrid wheat breeding and the establishment of heterotic groups is still lacking and was subject of this thesis research.

It has been speculated that genetic diversity and heterosis for hybrid wheat breeding could be increased by using spring and winter types as possible heterotic groups (Koekemoer et al. 2011). A similar approach could be

the exploitation of exotic germplasm for hybrid breeding (Goodman 1999). However, genetic divergence between parental lines and resulting heterosis is always linked to the adaptation of germplasm to the target region. Therefore, at least as demonstrated in maize, excessive use of genetic distance between parental components could also lead to negative effects and to a decrease in heterosis when exceeding a certain optimum (Moll et al. 1965; Melchinger and Gumber 1998). Knowledge about the association of heterosis and genetic distance in a broad diversity space to optimally exploit heterosis in wheat is missing. This information is of fundamental importance for hybrid wheat breeding in general and the establishment of heterotic groups in the long-run.

Irrespective of the chosen strategy to establish heterotic groups in wheat, male and female parents must be compatible to each other. The crucial traits for male-female compatibility are plant height and flowering time. Males should be taller than females to facilitate pollen flow and to optimize female seed set. Females should flower about two days earlier than males to allow the florets to gape (Pickett 1993). The synchronization in flowering time is often referred to as nicking. These phenotypic requirements are greatly restricting theoretical cross combinations.

Reduced height (*Rht*) genes were introduced during the Green Revolution and led to an improved harvest index and increased grain yield (Hedden 2003). The two homoeoloci *Rht-B1* and *Rht-D1*, located on group 4 chromosomes, originated from the Japanese cultivar *Norin10* and encode DELLA proteins which are components of the gibberellic acid signal transduction pathway (Börner et al. 1996; Peng et al. 1999; Pearce et al. 2011; Wilhelm et al. 2013b). The *Rht-B1b* and *Rht-D1b* alleles play a major role in controlling plant stature, but additional minor genes fine-tune plant height in wheat (Worland and Snape 2001; Wilhelm et al. 2013a; Wilhelm et al. 2013b; Würschum et al. 2015). Likewise, flowering time in wheat is controlled by different key gene loci affecting photoperiod response (*Ppd*), vernalization requirement (*Vrn*), or earliness *per se* (*Eps*) (Worland and Snape 2001; Snape et al. 2001; Distelfeld et al. 2009; Laurie and Turner 2011). *Ppd* genes divide wheat genotypes into photoperiod-sensitive and photoperiod-insensitive

classes (Distelfeld et al. 2009). Wheat is photoperiod sensitive and the start of flowering requires a critical day length (Langer et al. 2014a). The *Ppd-D1* allele has a major effect on flowering time in wheat and its mutant type promotes early flowering irrespective of day length (Langer et al. 2014a). *Vrn* genes generally divide wheat into spring and winter types (Distelfeld et al. 2009). Winter wheat has vernalization requirement and is the predominant type grown in Europe. Major genes such as *Vrn*, *Ppd* and *Rht* are nowadays routinely used for marker-assisted selection in wheat line breeding programs. In hybrid wheat breeding, phenotypic selection assisted by molecular markers for these candidate genes could be effectively used to fine-tune flowering time and plant height in the male and female parents. However, the frequency of DNA polymorphisms at these important loci as well as their effects under German growth conditions were unknown in large worldwide wheat collections. This knowledge is of relevance for efficient hybrid wheat breeding and for the establishment of heterotic groups.

The self-pollinating nature and cleistogamy of wheat resulted from a long domestication process (Zohary 1967; D'Souza 1970). Additionally, variety registration and seed production within line breeding programs favor closed flowering to ensure high homogeneity. This leads to a lower frequency of genotypes with favorable floral characteristics and good cross-pollination ability required for hybrid breeding. The identification of such lines is challenging and time-consuming because pollination capability in wheat depends on many different traits such as plant height, flowering, opening of the glumes, awness of the lemma, size of stigma, duration of stigma receptivity, stigma exsertion, lodicule size, number of pollen grains per anther, anther dimensions, elongation of the anther filament, extrusion of anthers, dehiscence of anthers, pollen grain, proportional extent of pollen grains released within the floret, longevity of the pollen grain, and the duration of flowering (*cf.* De Vries 1971 or Pickett 1993). Besides, outcrossing in wheat is generally dependent on environmental conditions like temperature, relative humidity or day time (Fruwirth 1905; De Vries 1972; Pickett 1993).

Previous research suggested that the amount of pollen production in wheat is rather low with about 2000–3000 pollen grains (De Vries 1971). This is roughly only one tenth of the amount of pollen grains produced by the allogamous rye (De Vries 1971; Wilson and Driscoll 1983). Wheat pollen is relatively heavy, which has negative impacts on its aerodynamics, suggesting that male and female plants should be planted close to each other for hybrid seed production (Lelley 1966; D'Souza 1970; De Vries 1971; Waines and Hegde 2003). Moreover, wheat pollen has a rather low viability and longevity is in the range of minutes (D'Souza 1970; Pickett 1993). A number of studies suggested that anther extrusion, length of anthers and their filaments are promising indirect indicators for pollen release and hence, for cross-pollination ability (Joppa et al. 1968; Beri and Anand 1971; De Vries, 1974). Langer et al. (2014b) compiled a number of promising flowering and floral traits with relevance for hybrid breeding programs and evaluated these traits in field trials. They observed large genetic variations and high heritabilities for most of the evaluated traits suggesting that improvement by selection seems feasible. Unfortunately, most of the evaluated traits like pollen mass or anther extrusion are difficult and time-consuming to score. Therefore, employing molecular markers tightly linked with major QTL for the trait of interest (Würschum 2012) or genomic prediction based on genome-wide marker profiles (Meuwissen et al. 2001; Heffner et al. 2009) could greatly accelerate and simplify the redesign of the wheat flower. However, little is known about the genetic architecture and inheritance of the traits of interest and further research is required.

Finally, the seed set on female plants in hybrid seed production is the crucial parameter for the success of a hybrid wheat breeding program as it determines seed costs and the competitiveness with line breeding. However, little is known about the trait seed set itself or trait correlations between indirect male floral traits and seed set. The redesign of the wheat floral architecture requires breeding strategies for the long run and their design and assessment was a major aspect of this thesis work.

Objectives

The goal of this thesis was to contribute to breeding strategies for hybrid wheat in Europe. The main objectives were to assess the usefulness of global wheat diversity for hybrid breeding and to dissect the genetic architecture of male floral traits required for the redesign of the wheat flower for an efficient hybrid seed production.

 In particular, the objectives were to:

1. Evaluate global wheat genetic diversity and how it could be used to support the development of heterotic groups in wheat;

2. Assess the usefulness of exotic germplasm for hybrid wheat breeding and for the establishment of heterotic groups;

3. Evaluate the relationship between heterosis and genetic distance in a broad diversity space;

4. Evaluate the importance of male floral traits for hybrid wheat seed production and dissect the genetic architecture underlying male floral traits required for hybrid wheat breeding.

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2 A unified framework for hybrid breeding and the establishment of heterotic groups in wheat

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Abstract Hybrid wheat breeding has great potential to increase the global wheat grain yield level particularly in view of the increasing abiotic and biotic stress challenges as well as variable climatic conditions. For the long-term success of hybrid wheat breeding and the maximum exploitation of heterosis, high-yielding heterotic patterns must be established. Here, we propose a unified framework for hybrid breeding and the establishment of heterotic groups in autogamous crops and exemplify it for hybrid wheat breeding in

Germany. A key component is the establishment of genetic distance between heterotic groups and in this context, we assessed genetic diversity in a global collection of 1110 winter wheat varieties released during the past decades in 35 countries but with a focus on European origin. Our analyses revealed the absence of major population structure but nevertheless suggest genetically distinct subgroups with potential for hybrid wheat breeding. Taking our molecular results and additional phenotypic data together, we propose how global genetic diversity can be used to accelerate and support reciprocal recurrent selection for the development of genetically distinct heterotic groups in hybrid wheat breeding.

3 Multiply to conquer: Copy number variations at *Ppd-B1* and *Vrn-A1* facilitate global adaptation in wheat

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Abstract Copy number variation was found to be a frequent type of DNA polymorphism in the human genome often associated with diseases but its

importance in crops and the effects on agronomic traits are still largely unknown. Here, we employed a large worldwide panel of 1110 winter wheat varieties to assess the frequency and the geographic distribution of copy number variants at the *Photoperiod-B1* (*Ppd-B1*) and the *Vernalization-A1* (*Vrn-A1*) loci as well as their effects on flowering time under field conditions. We identified a novel four copy variant of *Vrn-A1* and based on the phylogenetic relationships among the lines show that the higher copy variants at both loci are likely to have arisen independently multiple times. In addition, we found that the frequency of the different copy number variants at both loci reflects the environmental conditions in the varieties' region of origin and based on multi-location field trials show that *Ppd-B1* copy number has a substantial effect on the fine-tuning of flowering time. In conclusion, our results show the importance of copy number variation at *Ppd-B1* and *Vrn-A1* for the global adaptation of wheat making it a key factor for wheat success in a broad range of environments and in a wider context substantiate the significant role of copy number variation in crops.

4 Genome-based reevaluation of heterosis in genetically diverse hybrid wheat

Philipp H. G. Boeven[1], Yusheng Zhao[2], Patrick Thorwarth[1], Hans Peter Maurer[1], Mario Gils[3], Ralf Schachschneider[3], Johannes Schacht[4], Erhard Ebmeyer[5], Ebrahim Kazman[6], Vilson Mirdita[7], Jost Dörnte[8], Stefan Kontowski[9], Ralf Horbach[10], Hilmar Cöster[11], Josef Holzapfel[12], Andreas Jacobi[13], Ludwig Ramgraber[14], Carsten Reinbrecht[15], Norbert Starck[16], Pierrick Varenne[17], Anne Starke[4], Friederike Schürmann[9], Jens Möhring[18], C. Friedrich H. Longin[1], Jochen C. Reif[2], Yong Jiang[2], Tobias Würschum[1]

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Abstract Heterosis refers to the improved trait values of hybrids compared to their parental lines. However, the usefulness of exotic, genetically distant germplasm for hybrid breeding remained elusive. Here, we employed a unique data set comprising 2,046 wheat hybrids and found about 10 % average midparent heterosis in crosses between elite lines as well as in crosses between elite and exotic lines. We show that heterosis for grain yield is not decreasing in crosses exploiting a maximum level of available global wheat diversity. Moreover, implementing a novel distance measure giving weight to heterosis loci revealed a strong positive association between heterosis and genetic distance, for which the analysis of the genetic architecture of heterosis provided a mechanistic understanding. As the absolute hybrid response was mainly driven by parental *per se* performance, elite lines are favorable for hybrid wheat breeding. Collectively this work expands the genome-based understanding of heterosis in crops, an important pillar towards global food security.

Genome-based reevaluation of heterosis in genetically diverse hybrid wheat

Philipp H. G. Boeven[1], Yusheng Zhao[2], Patrick Thorwarth[1], Hans Peter Maurer[1], Mario Gils[3], Ralf Schachschneider[3], Johannes Schacht[4], Erhard Ebmeyer[5], Ebrahim Kazman[6], Vilson Mirdita[7], Jost Dörnte[8], Stefan Kontowski[9], Ralf Horbach[10], Hilmar Cöster[11], Josef Holzalpfel[12], Andreas Jacobi[13], Ludwig Ramgraber[14], Carsten Reinbrecht[15], Norbert Starck[16], Pierrick Varenne[17], Anne Starke[4], Friederike Schürmann[8], Jens Möhring[18], Sang He[2], C. Friedrich H. Longin[1], Jochen C. Reif[2✉], Yong Jiang[2], and Tobias Würschum[1]

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1 Abstract

2 Heterosis refers to the improved trait values of hybrids compared to their parental lines.

3 However, the usefulness of exotic, genetically distant germplasm for hybrid breeding remained

4 elusive. Here, we employed a unique data set comprising 2,046 wheat hybrids and found ~10%

5 average midparent heterosis in crosses between elite lines as well as in crosses between elite

6 and exotic lines. We show that heterosis for grain yield is not decreasing in crosses exploiting

7 a maximum level of available global wheat diversity. Moreover, implementing a novel distance

8 measure giving weight to heterosis loci revealed a strong positive association between heterosis

9 and genetic distance, for which the analysis of the genetic architecture of heterosis provided a

10 mechanistic understanding. As the absolute hybrid response was mainly driven by parental *per*

11 *se* performance, elite lines are favorable for hybrid wheat breeding. Collectively this work

12 expands the genome-based understanding of heterosis in crops, an important pillar towards

13 global food security.

14

1 **INTRODUCTION**

2 The targeted exploitation of heterosis has revolutionized plant breeding in the past century and is an

3 ongoing success story in many crops[1,2]. Wheat is a key global staple crop providing about 20% of the

4 global dietary energy, but its yield trends are insufficient to ensure the World's future food security[3,4].

5 Recent studies have shown that hybrid wheat holds great potential to increase yield gain, yield stability

6 and thus global productivity of wheat[5–8].

7 Three mutually nonexclusive theories have been proposed to explain heterosis: (i) dominance[9–

8 12], (ii) over-dominance[13–15], and (iii) epistasis[16,17]. Although the genetic mechanism(s) underlying

9 heterosis remained elusive[18], for successful hybrid breeding the rule of Saint Benedict could be

10 customized as follows: *Rationali cum intervallo labora, ut maxime augeatur heterosis – work with*

11 *(genetic) distance to maximize heterosis*. This well accepted principle is applied in many hybrid breeding

12 programs and is based on quantitative genetic theory, as heterosis depends on directional dominance

13 and the difference in gene frequencies between particular lines or populations[19]. Since the difference in

14 gene frequencies corresponds to the Rogers' distance in case of homozygous parental lines, genetic

15 distance has been proposed as a predictor for hybrid performance and as a proxy for grouping of

16 germplasm into heterotic groups[20,21]. Thus, the idea of introgressing exotic germplasm to support the

17 divergence in allele frequencies between opposite heterotic pools and to increase heterosis appears

18 obvious and has been discussed in several studies[22–27]. The crucial question is, however, if there is a

19 steady increase of heterosis with genetic distance between the hybrid parents or an optimum that

20 maximizes heterosis.

21 A prominent study in maize by Moll *et al.*[28] suggested that genetic distance cannot be taken to

22 extremes to increase heterosis. These authors used eight parental maize populations from four different

23 geographical regions to produce 28 hybrid crosses, and found that heterosis for grain yield increased

24 with increasing geographic distance between the parental populations, but then decreased again in the

25 crosses considered genetically most diverse. Despite the limitations of this work, it since then became

26 well accepted in the community of quantitative geneticists and hybrid breeders, that there is a certain

27 but unknown optimum level of genetic distance and its exceedance is followed by a decrease in

28 heterosis, presumably due to adaptation issues of the parents or negative epistatic interactions between

29 unadapted genes[19,21].

1   Jiang *et al.*[29] recently elaborated a quantitative genetic framework to study the genetic

2 architecture of heterosis in hybrid populations. They found that epistatic effects play the most prominent

3 role in the genetic architecture of grain yield heterosis in elite European hybrid wheat. However, such

4 favorable co-adapted gene complexes can be disrupted in genetically extremely diverse crosses and

5 can cause outbreeding depression and finally a decrease in heterosis[30–32]. To date, little is known about

6 the relationship between heterosis and genetic distance outside maize. Moreover, previous studies

7 estimated genetic distances based on geographic origin of the parents or with genome-wide neutral

8 markers, thereby not taking the genetic architecture of heterosis into account. To bridge this gap, we

9 utilized the growing toolbox of genomic approaches and applied it to analyze an extensive hybrid wheat

10 panel comprising 1,750 elite wheat hybrids and 296 hybrids established from crosses between elite

11 wheat lines and exotic germplasm. We show, that midparent heterosis for grain yield in hybrid wheat is

12 constantly increasing with genetic distance, even in genetically distant crosses that exploit the maximum

13 level of genetic diversity available in global winter wheat. Furthermore, we established a new genetic

14 distance measure that gives special weight to dominance effects estimated in a Bayesian framework

15 and elucidated the genetic basis of grain yield heterosis in the different hybrids.

16

1 **RESULTS**

2 **Genetic diversity and divergence are maximized in the hybrid parents**

3 To evaluate the relationship between heterosis and genetic distance, we produced a total of 2,046 wheat

4 hybrids, thereby employing three sets of parental germplasm groups that cover different levels of global

5 wheat diversity (**Supplementary Figs. 1 and 2**). Within the "Elite" set, we sampled 189 and 41 wheat

6 lines as female and male parents, respectively, representing the current Western European elite wheat

7 breeding germplasm. The "Historic" set consisted of 96 cultivars from the past five decades, mainly with

8 Western European background, which represents adapted material that is already a step away from the

9 elite wheat breeding pool. We completed our global wheat diversity collection by tapping into the winter

10 wheat diversity held at the IPK gene bank in Gatersleben and further sampled 101 wheat accessions as

11 the "Exotic" set. This represents a massive step into global wheat diversity as these accessions cover

12 the most diverse material available for winter wheat breeding without leaving the primary gene pool[33],

13 All lines were fingerprinted with a 15 k Infinium SNP array, that performed without bias in all

14 three parental groups (**Supplementary Fig. 3**). The mean polymorphic information content (PIC) of the

15 "Elite" and "Historic" sets was almost identical with 0.315 and 0.314, respectively, while the mean PIC

16 of the "Exotic" set was highest with 0.365, indicating its higher allelic diversity. This corresponded well

17 with the gene diversity H_S, which was 0.33, 0.32, and 0.35 for the "Elite", "Historic" and "Exotic" sets,

18 respectively. The mean pairwise F_{ST} values indicated significant differentiation between all three sets

19 while being highest between the "Elite" and "Exotic" and somewhat lower between the "Elite" and

20 "Historic" sets (**Fig. 1c**). Indeed, the observed F_{ST} of 0.15 between "Elite" and "Exotic" reflects a similar

21 level of genetic differentiation as the Dent and Flint heterotic pools in maize[34]. This trend was further

22 substantiated by distance- and character-based phylogenetic methods, clearly separating the "Elite",

23 "Historic" and "Exotic" sets (**Fig. 1a,b,d**). The curves of linkage disequilibrium (LD) decay as a measure

24 for the haplotype diversity were almost identical for the "Elite" and "Historic" sets, but decayed faster for

25 the "Exotic" set, thus lending further support to the conclusion of the higher genetic divergence of this

26 set (**Fig. 1e**). This was further corroborated by the difference in persistence of LD phase of the "Exotic"

27 set (**Fig. 1f**). The higher degree of long-ranging LD observed in the "Elite" and "Historic" sets likely

28 results from selection and fixation of favorable alleles, a typical characteristic normally found in elite

29 breeding populations[35,36]. Taken together, all analyses underscore the successful sampling of a

30 maximum genetic diversity in the hybrid wheat parents.

1 **Heritability estimates in the hybrid wheat panel**

2 We evaluated the "Elite" set at six and the "Historic" and "Exotic" sets at five agro-ecologically diverse

3 locations and observed high heritability estimates between 0.81 and 0.83 for grain yield

4 (**Supplementary Table 2**). In total, 431 hybrids outperformed the highest yielding released line variety

5 *KWS Smart*, with the best performing hybrid (10.89 Mg ha^{-1}) outyielding it by 0.73 Mg ha^{-1}

6 (**Supplementary Fig. 4**). This yield advantage of 7.2% relative to the best commercial variety

7 corresponds to several years of selection gain in breeding programs and underscores previous results

8 on the potential of hybrid wheat to increase yield[5,37]. Heritability estimates of grain yield heterosis ranged

9 between 0.66 and 0.78 (**Supplementary Fig. 5**). This reflects the high quality of the phenotypic data

10 underlying this study, which consequently provides an excellent foundation to reevaluate heterosis in

11 genetically diverse hybrid wheat.

12

13 **No breakdown of heterosis with maximized diversity**

14 Interestingly, the "Elite", "Historic" as well as "Exotic" hybrids all showed a similar amount of mean

15 relative midparent heterosis with 9.24, 9.48, and 9.17%, respectively (**Fig. 2, Supplementary Fig. 6,**

16 **Supplementary Table 2**). Nonetheless, it is important to note that there was a significant difference (*P*

17 < 0.01) in the mean absolute heterosis between the "Elite" (0.83 Mg ha^{-1}) and "Exotic" (0.72 Mg ha^{-1})

18 hybrids owing to the generally lower yield level of the exotic material. We observed a weak positive

19 correlation between the relative midparent heterosis and genetic distance (*r* = 0.18***), as well as

20 between the absolute midparent heterosis and genetic distance (*r* = 0.13***) (**Fig. 3a**). This observation

21 also held true within each of the three sets of hybrids.

22 The finding that the absolute midparent heterosis was lower in the "Exotic" hybrids than in the

23 "Elite" hybrids should not be interpreted as a decrease of absolute midparent heterosis under maximized

24 genetic distance. First, this difference was rather small and for a similar relative midparent heterosis the

25 absolute heterosis values must become smaller for lower midparent values caused by the lower *per se*

26 performance of the "Exotic" lines (**Supplementary Fig. 6**). Second, the absolute midparent heterosis of

27 "Exotic" hybrids was generally lower, even for those hybrids with a genetic distance between their

28 parental lines similar to that of some of the "Elite" hybrids and third, the trend of a constant increase of

29 absolute midparent heterosis with genetic distance was also clearly discernible in the "Exotic" hybrids.

30 Taken together, our results revealed a slightly lower absolute heterosis in hybrids involving "Exotic"

1   lines, but an increase of absolute and relative midparent heterosis with genetic distance without an

2   apparent breakdown under maximized genetic distance.

3

4   **Accounting for dominance effects revealed a more accurate picture between heterosis and**

5   **genetic distance**

6   Assuming that all marker loci are in LD with QTL involved in heterosis and that there is a substantial

7   degree of dominance at these loci, then genetic distance would indeed be a perfect tool for the prediction

8   of heterosis[38]. In case of neutral markers, however, genetic distance appears to be a weak estimator for

9   heterosis, at least in unrelated material[39]. We aimed to reduce the degree of noise caused by neutral

10   markers by developing and employing a functional Rogers' distance (fRD) that gives weight to markers

11   contributing to heterosis through dominance. These dominance effects were estimated by genomic

12   prediction based on a BayesCπ approach to account for their genetic architecture[40,41]. Taking the whole

13   dataset into account, we observed a strong increase in the correlation from $r = 0.13^{***}$ to $r = 0.64^{***}$ for

14   absolute midparent heterosis and from $r = 0.18^{***}$ to $r = 0.66^{***}$ for relative midparent heterosis when

15   considering the dominance effects to compute the genetic distances (**Fig. 3b**). These trends were

16   confirmed within all three different sets of hybrids, and were further validated by fivefold cross-validation

17   which resulted in only slightly lower values of $r = 0.56^{***}$ and 0.57^{***} for absolute and relative midparent

18   heterosis, respectively (**Supplementary Fig. 7**). Consistently, the mean cross-validated correlations

19   between heterosis and the fRD were similar to the genome-wide prediction accuracies for midparent

20   heterosis when only considering dominance effects (**Supplementary Table 3**).

21   In summary, giving weight to dominance effects when estimating the genetic distance between

22   parental lines revealed a much stronger correlation between heterosis and genetic divergence. This

23   suggests that genome-wide neutral markers have so far obscured the true genetic association between

24   heterosis and genetic distance.

25

26   **Different genetic architecture of grain yield heterosis in "Elite" and "Exotic" hybrids**

27   We next applied the quantitative genetic framework recently elaborated by Jiang *et al.*[29] to elucidate

28   the genetic basis of grain yield heterosis in our two genetically most distinct sets of hybrids, "Elite" and

29   "Exotic". Partitioning the total genetic variance of heterosis into its components revealed the importance

30   of additive-by-additive epistasis, which explained 62% of the genetic variance of grain yield heterosis in

1   the "Elite" set, whereas dominance effects contributed only to 10% of the genetic variance (**Fig. 4g**).

2   This observed pattern is in close agreement with results by Jiang *et al.*[29] who exploited a similar set of

3   elite wheat hybrids. We identified a total of 221 significant effects in the "Elite" set, of which the majority

4   was involved in several types of interactions (**Fig. 4a-c, Supplementary Table 6**). Out of the seven

5   heterotic QTL effects that significantly contribute to heterosis, five were overlapping with those reported

6   by Jiang *et al.*[29], which is quite considerable for a complex trait like grain yield heterosis (**Fig. 4i**).

7   Interestingly, the "Exotic" set showed a different genetic architecture for grain yield heterosis. Here, the

8   contribution of the different genetic components of variance to heterosis was much more balanced.

9   Dominance had a more pronounced role and explained 24% of the genetic variance of grain yield

10 heterosis. In line with this, we identified two dominance effects out of the total 101 significant effects,

11 while for the "Elite" set only one was identified and none by Jiang *et al.*[29]. This is likely due to the "Exotic"

12 parental lines being less subjected to intensive breeding and selection, which was corroborated by the

13 results from the LD analyses and their on average much taller height (**Fig. 1e,f, Supplementary Table**

14 **2**). We assume that favorable additive-by-additive epistatic interactions between loci on homoeologous

15 subgenomes of the allohexaploid wheat genome became fixed during line breeding, an effect termed

16 fixed heterosis, thus explaining the more prominent role of dominance in exotic wheat germplasm.

17 The comparatively low contribution of dominance effects to the total genetic variance of

18 heterosis and the observed strong increase in the correlation between fRD and heterosis are not

19 mutually contradictory. Rather, the loci given weight for the fRD based on their dominance effects also

20 capture different types of epistatic interactions, as revealed by our mapping approach and the

21 correlations between the kinship matrices for dominance and the three types of digenic epistatic

22 interactions (**Supplementary Tables 3-5**). Thus, the genome-based analysis of the genetic architecture

23 of grain yield heterosis provides the mechanistic understanding for the observations regarding the

24 correlation between heterosis and genetic distance.

25

1 **DISCUSSION**

2 The association between heterosis and genetic distance is one of the central elements in hybrid

3 breeding theory and has intrigued and inspired many scientists as well as plant breeders during the past

4 decades[21,26,27,38,39,42–47]. The long history of hybrid breeding in maize has led to a substantial increase in

5 genetic differentiation between the established heterotic pools[48]. For hybrid crops in their infancy, such

6 as wheat, breeders need to decide which germplasm to use for an optimal exploitation of heterosis and

7 for the establishment of heterotic groups. Here, we particularly revisited the concept based on the study

8 by Moll *et al.*[28] that suggests a certain optimum genetic distance for maximum heterosis. Despite its

9 importance for hybrid breeding this concept has since neither been unambiguously confirmed nor

10 disproved, even in maize.

11 Interestingly, we observed no evidence for a breakdown of heterosis under maximized genetic

12 distance. The average relative midparent heterosis was about 10% in all three sets of hybrids, even in

13 the genetically most distant crosses between elite and exotic lines. However, in line with quantitative

14 genetic theory and the assumption of a certain contribution of dominance to heterosis in wheat, we did

15 observe an increase of heterosis with increasing genetic distance estimated by genome-wide markers.

16 For the first time, however, the growing genomics toolbox allowed us to take this kind of analysis a step

17 further by implementing a distance measure that gives special weight to markers involved in the

18 expression of heterosis. The strongly increased correlation between heterosis and this fRD illustrates

19 that genome-wide neutral markers obscure the true genetic association between heterosis and genetic

20 distance (**Fig. 5a**). Thus, only a subset of the genome contributes to grain yield heterosis. We

21 hypothesize that this finding challenges the paradigm of hybrid breeding to keep established heterotic

22 groups strictly separated, as maintaining a high genetic distance based on markers that do not contribute

23 to heterosis appears obsolete and may even hinder the introgression of favorable alleles and breaking

24 of haploblocks, thereby limiting *per se* performance.

25 Consequently, breeders could tap into wheat diversity for hybrid breeding without passing into

26 a sub-optimum and losing relative grain yield heterosis. The absolute heterosis, however, was on

27 average lower for the "Exotic" hybrids compared to the "Elite" hybrids (**Fig. 5b**). This can be attributed

28 to the lower *per se* performance of these exotic lines, due to their less intensive selection for yield and

29 potentially being less adapted to the growing conditions of the field trials (**Supplementary Figs. 8 and**

30 **9**). Notably, genetic distance will inevitably be confounded with *per se* performance and adaptation. Moll

31 *et al.*[28] observed a decrease in heterosis in the most divergent crosses, but these were also the ones

1   that included the two Mexican populations that were low in yield even under conditions where they were

2   commonly grown. This illustrates, that also the study underlying the "Moll concept" faced the issue that

3   the levels of genetic divergence were confounded with adaptation and *per se* performance and even

4   growing the hybrids in the different environments, as done by Moll *et al.*[28], does not allow to disentangle

5   these confounding effects. Thus, in order to maximize hybrid performance, crosses among high-yielding

6   elite lines appear more promising for hybrid breeding in wheat than the direct utilization of exotic

7   germplasm, as the lower parental *per se* performance of exotic lines cannot be compensated by

8   heterotic response. In general, the observed hybrid performances are encouraging and underscore the

9   potential of hybrid wheat breeding to increase grain yield levels globally. Collectively our results illustrate

10   the power of genome-based approaches to dissect the complex phenomenon heterosis.

11

1 **METHODS**

2 Methods, including statements of data availability and any associated accession codes and references,

3 are available in the online version of the paper.

4

5

8

9 **AUTHOR CONTRIBUTIONS**

10 J.C.R., C.F.H.L., P.H.G.B. and T.W. conceived and designed the study. P.H.G.B., C.F.H.L., J.C.R., M.G., R.S., J.S., E.E., E.K.,

11 V.M., J.D., S.K., R.H., H.C., J.H., A.J., L.R., C.R., N.S., P.V., A.S. and F.S. acquired and contributed data. P.H.G.B., Y.J. and Y.Z.

12 processed the data, performed the analyses and analyzed the results. S.H. imputed the SNP data. H.P.M. did SSR analyses. J.M.

13 and P.T. supported in data analyses. P.H.G.B., Y.J, Y.Z., J.C.R. and T.W. interpreted the results and wrote the manuscript.

14

15 **COMPETING FINANCIAL INTERESTS**

16 The authors declare no competing financial interests.

17

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1 **ONLINE METHODS**

2 **Plant materials and field trials.** This study was based on three different sets of wheat hybrids denoted

3 in the following as "Elite", "Historic" and "Exotic". The "Elite" set initially comprised 434 potential female

4 lines provided by the 13 following wheat breeding companies: Bayer CropScience AG, Deutsche

5 Saatveredelung AG, KWS LOCHOW GmbH, Limagrain GmbH, Pflanzenzucht Oberlimpurg, RAGT-

6 Saaten GmbH, Saatzucht Bauer GmbH, Saatzucht Josef Breun GmbH & Co. KG, Saatzucht Streng-

7 Engelen GmbH & Co. KG, Secobra Saatzucht GmbH, Strube Research GmbH & Co. KG, Syngenta

8 Seeds GmbH, and W. von Borries-Eckendorf GmbH & Co. KG (**Supplementary Fig. 1a**). We genotyped

9 those lines with 22 SSR markers and based on genetic distances among them (Rogers' distance > 0.2)

10 and by maximizing the allelic diversity (> 97 % of alleles maintained after selection), selected 189 out of

11 the 434 as female parents for the hybrids. Forty-one male lines were selected based on suitable floral

12 characteristics[49] and were provided by the two wheat breeding companies Limagrain GmbH and

13 Nordsaat Saatzucht GmbH (**Supplementary Fig. 1b**). Within the "Elite" set, we were able to produce

14 enough seeds for 1,750 elite wheat hybrids by crossing the 189 elite female lines and the 41 elite male

15 lines in an incomplete factorial mating design using chemical hybridization agents (**Supplementary Fig.**

16 **2a**). The sterility of the female parents after chemical hybridization agent application was checked by

17 bagging 1-3 plants. The 1,750 hybrids, their 189 female and 41 male parental lines, as well as 11

18 commercial varieties as checks, i.e. 8 line varieties (quality class A: *JB Asano*, *Julius*, *RGT Reform*;

19 quality class B: *Colonia*, *KWS Loft*, *Rumor*, *Tobak*; quality class C: *Elixer*), and 3 hybrids (quality class

20 B: *Hybred*, *Hystar*; quality class C: *LG Alpha*), were evaluated for grain yield (Mg ha^{-1}), heading date

21 (days from January 1st) and plant height (cm) at six agro-ecologically diverse locations in Germany in

22 the growing season 2015/16. The locations were Asendorf, Biendorf, Gatersleben, Hadmersleben,

23 Rosenthal, and Seligenstadt (**Supplementary Table 1**). The experimental design for each group of 506

24 entries, that can be harvested on a single day, was an α-design with size of the incomplete blocks of

25 11. Sowing density was 200 seeds per m^{-2} for all entries and plot size at the different locations ranged

26 from 7.2 to 11 m². Plots were treated with fertilizers, fungicides and herbicides according to best

27 agronomic practices.

28 The "Historic" set consisted of 96 wheat lines selected based on a temporal and spatial selection

29 strategy (**Supplementary Fig. 1c,d**). About half of these wheat varieties were released between the

30 1960's and 1980's, but also some more recently released varieties were included (**Supplementary Fig.**

1   **1c**). The spatial, i.e. geographical, component mainly focused on Western European origins. More than

2   80 % of the varieties were released in Germany, France or Great Britain, but also cultivars from regions

3   with a more continental climate were included. The "Historic" material is part of the winter wheat panel

4   described recently by Boeven *et al.*[27]. All of these lines were used as female components with an elite

5   male tester mix in a topcross mating design using chemical hybridization agents (**Supplementary Fig.**

6   **2c**). The elite male tester mix was provided by Nordsaat Saatzucht GmbH and comprised two

7   unreleased breeding sibling lines with known good male floral characteristics. The sterility was again

8   checked by bagging 1-3 female plants. We were able to produce enough seeds for 96 hybrids derived

9   from crosses of 96 "Historic" female lines with the male tester mix.

10 For the "Exotic" set, a random sample of 1,500 gene bank accessions obtained from the

11 gene bank IPK Gatersleben were screened for their male floral characteristics in observation plots at

12 the IPK Gatersleben. Finally, 101 accessions were selected and crossed as male components in an

13 incomplete factorial mating design with 9 German elite varieties (*Famulus*, *Franz*, *Glaucus*, *JB Asano*,

14 *Patras*, *RGT Reform*, *Rumor*, *Tabasco*, *Türkis*) used as female testers, that were emasculated by a

15 chemical hybridization agent (**Supplementary Fig. 2b**). The sterility was checked by bagging 1-3 female

16 plants. According to available passport data and information obtained from the genetic resources

17 information and analytical system (GRIS) for wheat and triticale (http://wheatpedigree.net), the

18 acquisition date of more than 50% of these lines pre-dates the year 1970 and more than 20 worldwide

19 origins were represented by this random gene bank sample (**Supplementary Fig. 1e,f**). We were able

20 to produce enough seeds for 200 hybrids.

21 The "Historic" and "Exotic" hybrids, as well as their female and male parental lines were

22 evaluated for grain yield (Mg ha^{-1}), heading date (days from January 1st) and plant height (cm) at five

23 agro-ecologically diverse locations in Germany in the growing season 2015/16. The locations were

24 Stuttgart-Hohenheim, Renningen, Gatersleben, Schackstedt and Böhnshausen (Supplementary Table

25 1). Heading date was only recorded at two locations. The experimental designs were unreplicated α-

26 designs. To avoid neighboring effects of the much taller gene bank accessions, the material was split

27 into two adjacent trials according to plant height. The "tall trial" (243 entries) included all "Exotic" lines >

28 100 cm and their hybrids, while the "short trial" (378 entries) included all "Exotic" lines < 100 cm, their

29 hybrids and all their female testers, all "Historic" hybrids, the male tester mix and also its single

30 components, and the same common elite checks as used in the "Elite" trial, and 95 further genotypes

1 not considered in this study. The two adjacent trials were linked by 10 commercial medium-long check

2 varieties (*Bernstein*, *Capo*, *Discus*, *Hybery* (hybrid), *Hymack* (hybrid), *KWS Milaneco*, *Midas*,

3 *Naturastar*, *Xantippe*) at each location. Sowing density was 220 seeds m^{-2} for all entries and plot size at

4 the different locations ranged from 7.56 to 12 m². Plots were treated according to best agronomic

5 practices, but N-fertilizers were reduced by 25 % and an additional application of growth regulators was

6 applied to prevent lodging.

7 In all trials, data were recorded for heading date as the number of days from January 1st to the

8 day when half of the heads of a plot had emerged from the flag leaves, for plant height in cm from the

9 ground to the tip of the erected ears, excluding awns, at the growth stage of dough development of the

10 kernels, and for grain yield in Mg ha^{-1} with a moisture content of 140 g H_2O kg^{-1}. Homogeneity of hybrids

11 was visually assessed on a scale from 1 (uniform) to 9 (50/50 mix of two genotypes) about two to three

12 weeks after flowering. In addition, hybridity of the "Exotic" hybrids was visually assessed on a scale from

13 1 (more than 90 % selfing) to 9 (only hybrids) in observation plots of two rows and 1.25 m length at the

14 IPK Gatersleben, where each hybrid and its parents were sown side by side. We identified 53 hybrids

15 of the "Elite" set and 48 hybrids of the "Exotic" set which did not meet these criteria and were therefore

16 not considered for the subsequent heterosis analyses

17

18 **Phenotypic data analyses.** Data for both experiments were analyzed by a two-stage approach, where

19 first data of each experiment was analyzed separately, and then means across experiments were

20 calculated. All data were screened for outliers using the method 4 "Bonferroni–Holm with re-scaled

21 median absolute deviation standardized residuals" as suggested by Bernal-Vasquez *et al.*[50]. Our mixed

22 model description follows the syntax suggested by Patterson[51], where crossed effects are denoted with

23 a dot operator, fixed and random effects are separated by a colon, with fixed effects appearing first. The

24 phenotypic data of the "Elite" set were analyzed based on the following linear mixed model:

25 $G : Loc + G \bullet Loc + Loc \bullet Exp + Loc \bullet Exp \bullet Block$ (1)

26 where *G*, *Loc*, *Exp*, and *Block* denote the effects of the genotypes, locations, trials and incomplete

27 blocks, respectively. Error, block and experiment variances were assumed to be heterogeneous among

28 locations. Genotype had 1,991 levels, 1,750 for hybrids, 189 and 41 for female and male parents,

29 respectively, and 11 for checks.

The phenotypic data of the "Historic" and "Exotic" sets were analyzed by a model analogous to model (1). Here, genotype had 621 levels, 200 for "Exotic" hybrids, 101 for "Exotic" male parental lines, nine for "Exotic" female testers, 96 for "Historic" hybrids and female parental lines, respectively, three for "Historic" male tester and its components, 11 for elite checks, 95 for additional genotypes and 20 for the ten medium-long check varieties coded separately for the "tall" and "short" trial. The reason for the latter was a plausibility check before analyses. We tested for identical yield of the ten commercial medium long check varieties in both experiments using the following linear mixed model:

$$ML_{checks} + Trial + ML_{checks} \bullet Trial : Loc + ML_{checks} \bullet Loc \qquad (2)$$

where ML_{checks}, $Trial$, and Loc denote the effects of the medium long checks, trials and locations, respectively. Extending the data and the model by including all data did not change the result that there was a significant and consistent overestimation of medium-tall genotypes in the "short trial" compared to the "tall trial" (on average 0.53 Mg ha^{-1}). As neighboring effects are one plausible explanation, we tried to account for this by treating differences in plant height of the neighboring plots as co-variable. However, this co-variable was not significant leaving a still significant systematic yield effect of the "tall trial". We, therefore, decided not to rely on the checks for connecting the two trials and rather coded the medium-long checks of each trial as different genotypes. Thus, trial adjustment was done across locations but within the same group of genotypes.

In a second step, the "Elite", "Historic" and "Exotic" sets were analyzed in a joint analysis based on the common elite checks. Here, adjusted entry means and corresponding standard errors of genotypes from the first step were used in the following linear mixed model:

$$G + Set : G \bullet Set \qquad (3)$$

where G and Set denote the genotypes and sets, respectively. Owing to the use of one divided by the squared standard errors of means as weighting factor, we set the residual variance to one applying method 3 suggested by Möhring and Piepho[52].

We assumed fixed genotypic effects to obtain best linear unbiased estimators (BLUEs) of the genotypic values of hybrids, females and males. BLUEs were used to calculate midparent heterosis (MPH) for each hybrid as MPH = F_1 − MP, where F_1 refers to the performance of a hybrid and MP refers to the midparent value of the two parental lines P_1 and P_2. Relative midparent heterosis (MPH %) was calculated for each hybrid as MPH (%) = (MPH / MP) × 100. Better-parent heterosis (BPH) was calculated as BPH = F_1 – P_{Better}, where P_{Better} refers to the performance of the better performing parental line. Relative better-parent heterosis was calculated as BPH (%) = (BPH / MP) × 100. Correlations based

1   on BLUEs were tested with Pearson's product moment correlation coefficients. BLUEs of different

2   genotypic groups were compared by Student's t-tests.

3   Variance components were estimated by the restricted maximum likelihood (REML) method

4   treating all effects as random except for the group effect. Binary dummy variables were used to estimate

5   variance components for each group. The "Elite" set was analyzed with the following mixed model:

6    $Group : Loc + Loc \bullet Exp + Loc \bullet Exp \bullet Block + Group \bullet Loc$

7    $+ Checks + Checks \bullet Loc + Females + Males$

8    $+ GCA_{Female} + GCA_{Female} \bullet Loc + GCA_{Male} + GCA_{Male} \bullet Loc + SCA$ (4)

9   where *Group* refers to the genetic group effects (hybrids, females, males, and each check have a

10   separate level), *Checks* to the effect of the checks, *Females* to the effect of the female parents, *Males*

11   to the effect of the male parents, *GCA* denotes general combining ability effects, and *SCA* the specific

12   combining ability effect. For sake of simplicity, dummy variables were suppressed in the model stated

13   above. We assumed group-specific error variances. Similarly, the "Historic" and "Exotic" sets were

14   analyzed with the following linear mixed model:

15   $Group + Male_{Tester} : Loc + Loc \bullet Trial + Loc \bullet Trial \bullet Block + Group \bullet Loc$

16   $+ Female_{Historic} + Elite_{Cultivars} + Male_{Exotic} + Rest$

17   $+ GCA_{Female_{Historic}} + GCA_{Female_{Exotic}} + GCA_{Female_{Exotic}} \bullet Loc$

18   $+ GCA_{Male_{Exotic}} + GCA_{Male_{Exotic}} \bullet Loc + SCA_{Exotic}$ (5)

19   where *Group* refers to the genetic group effects (hybrids "Historic" and "Exotic", female parents

20   "Historic", male parents "Exotic", elite varieties including all checks and "Exotic" female parents, and

21   Rest), and *Elite_Cultivars* to the effects of the elite checks and "Exotic" female parents, Rest to the effect of

22   genotypes not further considered in this study. The male tester within the "Historic" set was considered

23   as fixed effect. We assumed group-specific error variances. Broad sense heritability (*h²*) on an entry-

24   mean basis was estimated as the ratio of genotypic to phenotypic variance, $h^2 = \sigma_G^2 / (\sigma_G^2 + \frac{\sigma_{G \times L}^2}{L} + \frac{\sigma_e^2}{LR})$,

25   where σ_G^2 and $\sigma_{G \times L}^2$ refer to the total genotypic variance of the different genetic groups and their

26   interaction with location, respectively, L to the number of locations, R to the number of replications and

27   σ_e^2 to the residual variance. Except for checks within the "Elite" set and GCA of hybrids produced in an

28   incomplete factorial mating design, $\sigma_{G \times L}^2$ was confounded with σ_e^2. Genotypic variance of hybrids was

29   assumed to be the sum of GCA and SCA variances. Heritability of midparent heterosis was estimated

30   as described by Jiang et al. [29]. Briefly, midparent heterosis was calculated based on block-corrected

1 values of hybrids and their parents at each location. We then used these values and fitted a linear mixed

2 model including random genotype and location effects. Heritability was estimated as $h^2 = 1 - \frac{\overline{\vartheta}_{BLUP}}{2\sigma_G^2}$,

3 where $\overline{\vartheta}_{BLUP}$ is the mean variance of a difference of two BLUPs[53]. All statistical analyses were performed

4 within the R environment [54] and with the software package ASReml-R 3.0[55].

5

6 **Genotypic data and analyses.** All parental lines were fingerprinted using a 15 k Infinium SNP array

7 that contains a subset of markers from the 90 k Illumina Infinium assay[56]. The development of the 15 k

8 SNP-chip and genotyping was performed by TraitGenetics GmbH (www.traitgenetics.com) and resulted

9 in a total of 13,006 polymorphic SNP markers. In the rare event of missing marker data, imputation was

10 performed by Random Forest regression[57]. After quality tests, 10,059 high-quality SNPs with available

11 map position remained that were used for all subsequent analysis. Genotyping of one "Elite" male line

12 and two females lines failed, which were consequently excluded from the subsequent analyses.

13 The polymorphic information content (PIC) as a measure of genetic diversity was calculated for

14 each marker as PIC = 1 − (p^2 + q^2), where p and q denote the frequencies of the two alleles. In addition,

15 we calculated the gene diversity statistic H_S[58] using the R package 'HIERFSTAT'[59]. F_{ST} statistic for each

16 pair of subpopulations among "Elite", "Historic", and "Exotic", was estimated using the method of[60 60] as

17 implemented in the R package 'diveRsity'[61] and visualized by a neighbor-joining tree using the R

18 package 'ape'[62]. The 95% confidence intervals for the F_{ST} statistic were obtained from 1000 bootstrap

19 replicates. Population structure among parental lines was analyzed by principal coordinate analysis

20 (PCoA)[63] and cluster analysis was based on modified Rogers' distances[64] using the software package

21 Plabsoft[65]. The neighbor-joining tree was generated using the R package 'ape'[62]. Bayesian clustering

22 analysis was implemented using the software package ADMIXTURE version 1.23[66]. The most probable

23 number of ancestral populations capturing the major population structure in the data was found by

24 fivefold cross-validation. Genetic distance was measured by Rogers' distance[67]. In addition, a functional

25 Rogers' distance (fRD) was calculated by incorporating estimated dominance effects of the SNPs as:

26 $$f_{RD}(X,Y) = \frac{1}{L}\sum_{u=1}^{L} w_u \sqrt{\frac{\sum_{j=1}^{n_u}(X_{uj}-Y_{uj})^2}{2}} \qquad\qquad (5)$$

27 where X and Y represent two genotypes under consideration, X_{uj} and Y_{uj} are allele frequencies of the

28 j-th allele at the u-th locus, n_u is the number of alleles at the u-th locus, L refers to the number of loci,

and w_u is the dominance weight for the u-th locus. The dominance weight w for the u-th locus was

calculated as the value of w_u divided by the mean of all dominance effects resulting in a scaled weight

with a mean of one. Dominance effects of SNPs were estimated based on the BayesCπ approach

previously outlined in detail by [37][37]. We performed a five-fold cross-validation with 100 runs to estimate

dominance effects and to assess the association between heterosis and fRD. The trends between the

different measures of genetic distance and heterosis were visualized by fitting natural smoothing splines.

The extent of pairwise linkage disequilibrium (LD) was assessed using the squared allele-frequency correlations (r^2) according to[68]. Decay of LD with genetic map distance for the "Elite" and

"Exotic" sets was assessed by fitting natural smoothing splines to the r^2 values. The threshold for LD

due to linkage was calculated based on the method described by Breseghello and Sorrels[69]. The

persistence of linkage phase between the "Elite", "Historic" and "Exotic" sets was inferred by calculating

LD as the correlation coefficient r, where r can take values between -1 and 1. The correlation of r

between the different sets was defined as R and plotted against the genetic map distance[70] and was

again assessed by fitting natural smoothing splines. The LD parameters r and r^2 were calculated using

the software package PLINK[71].

Partitioning of genetic variance components for MPH. Genetic variance components for MPH were

estimated by fitting an extended genomic best linear unbiased prediction model[72,73] including dominance

and digenic epistatic effects. Briefly, the model can be described as follows:

$$y = g_d + g_{aa} + g_{ad} + g_{dd} + e \tag{7}$$

where y is the vector of MPH values for all hybrids, g_d, g_{aa}, g_{ad} and g_{dd} are vectors of genetic values

contributed by dominance, additive-by-additive, additive-by-dominance and dominance-by-dominance

effects, respectively, and e is a residual term. In the model we assume $g_d \sim N(0, K_d \sigma_d^2)$,

$g_{aa} \sim N(0, K_{aa}\sigma_{aa}^2)$, $g_{ad} \sim N(0, K_{ad}\sigma_{ad}^2)$, $g_{dd} \sim N(0, K_{dd}\sigma_{dd}^2)$ and $e \sim N(0, TT'\sigma_e^2)$, where K_d, K_{aa}, K_{ad} and K_{dd}

are marker-derived kinship matrices for the different genetic effects. T is a $r \times (r + s)$ matrix of linear

transformation from the vectors of the original trait (grain yield) to the vectors of MPH, where r is the

number of hybrids and s is the number of parental lines.

Note that in the model the residual term is not assumed to be independently distributed. The

reason is that we usually assume independent residual terms for the original trait grain yield, but the

1   MPH values are derived from the original trait values in the form of the linear transformation T. The

2   marker-derived kinship matrices are also specific to MPH instead of the original trait. We refer to Jiang

3   *et al.*[29] for more details on the implementation of the model. The variance components σ_d^2, σ_{aa}^2, σ_{ad}^2 and

4   σ_{dd}^2 were estimated by the multi-kernel method in the R package BGLR[74].

5

6   **Definition of heterotic effects.** The heterotic effect of a locus is the genetic contribution of the locus to

7   MPH, which is a complex combination of the dominance effect of the locus itself and the epistatic

8   interaction effects with the entire genetic background[29]. The precise definition is described as follows:

9   Let Q be the set of all QTL for the original trait. QTL were coded as 0, 1, or 2, depending on the

10   number of a chosen allele at each locus. Considering one hybrid, we denote by R_{kl} ($k, l = 0$ or 2) the

11   subset of loci where the female parent has genotype k and the male parent has genotype l. For $i, j \in Q$,

12   let d_i be the dominance effect of the i-th QTL, aa_{ij} is the additive-by-additive epistatic effect between

13   the i-th and the j-th QTL, ad_{ij} is the additive-by-dominance epistatic effect between the i-th and the j-

14   th QTL, and dd_{ij} is the dominance-by-dominance epistatic effect between the i-th and the j-th QTL. The

15   heterotic effect of the i-th locus was defined as:

16

$$h_i = \begin{cases} d_i - \dfrac{1}{2}\displaystyle\sum_{j\in R_{20}} aa_{ij} + \dfrac{1}{2}\displaystyle\sum_{j\in R_{02}} aa_{ij} + \dfrac{1}{2}\displaystyle\sum_{j\in R_{22}} ad_{ji} - \dfrac{1}{2}\displaystyle\sum_{j\in R_{00}} ad_{ji} + \dfrac{1}{2}\displaystyle\sum_{j\in R_{20}\cup R_{02}} dd_{ij} & if\ i \in R_{20} \\[2em] d_i - \dfrac{1}{2}\displaystyle\sum_{j\in R_{02}} aa_{ij} + \dfrac{1}{2}\displaystyle\sum_{j\in R_{20}} aa_{ij} + \dfrac{1}{2}\displaystyle\sum_{j\in R_{22}} ad_{ji} - \dfrac{1}{2}\displaystyle\sum_{j\in R_{00}} ad_{ji} + \dfrac{1}{2}\displaystyle\sum_{j\in R_{20}\cup R_{02}} dd_{ij} & if\ i \in R_{02} \\[2em] \dfrac{1}{2}\displaystyle\sum_{j\in R_{20}\cup R_{02}} ad_{ij} & if\ i \in R_{22} \\[2em] -\dfrac{1}{2}\displaystyle\sum_{j\in R_{20}\cup R_{02}} ad_{ij} & if\ i \in R_{00} \end{cases} \tag{8}$$

17   With this definition, the MPH value of each hybrid is the sum of heterotic effects across all loci,

18   i.e., $MPH = \sum_{i\in Q} h_i$.

19

20   **Genome-wide scan for significant heterotic effects.** We applied the following three-step procedure

21   developed by Jiang *et al.*[29] to detect significant heterotic effects: First, genome-wide association

22   mapping was performed to identify significant component effects (i.e. dominance and di-genic epistatic

23   effects). We used a standard linear mixed model with a marker-derived kinship matrix controlling for the

1   structure of multiple levels of relatedness and polygenic background effects[75]. Since presence of

2   epistasis was assumed, it is necessary to apply a model controlling the polygenic background effects

3   consisting of both main and epistatic effects[76]. The model can be described as follows:

4
$$y = m\alpha + g_d + g_{aa} + g_{ad} + g_{dd} + e \qquad (9)$$

5   where y, g_d, g_{aa}, g_{ad}, g_{dd} and e are the same as in Eq. (7). In particular, α is the genetic effect being

6   tested, m is the corresponding coefficient. More precisely, α is the dominance effect of any marker or

7   the epistatic interaction effect for any pair of markers. We assumed that α is an unknown fixed

8   parameter. The other assumptions are the same as for Eq. (7). For computational efficiency, the model

9   was transformed to a standard linear regression model in which only the residual terms are random.

10   The transformed model is equivalent to the original one, provided that the influence of different α on the

11   estimation of variance components is negligible[76,77]. After the transformation, the significance of the

12   effect α can be assessed by an F-test. We refer to Jiang *et al.*[29] for more details on the implementation

13   of the model.

14   In the second step, the significant component effects were integrated into the heterotic effects

15   according to Eq. (8). All non-significant effects were set to zero.

16   Finally, the heterotic effect h_i of each locus was tested by a permutation test. More precisely,

17   for each locus, the MPH values of all hybrids can be predicted using the heterotic effect of this particular

18   locus. Then the Pearson correlation coefficient between the predicted and observed MPH values was

19   calculated and a permutation test for the correlation coefficient was performed.

20   In the first and third step, the genome-wide threshold for *P* values was determined in the

21   following way: For the "Elite" panel, the threshold was *P* < 0.05 after Bonferroni-Holm correction for

22   multiple testing[78]. For the "Exotic" panel, the power of detecting significant epistatic effects was severely

23   hindered by the small population size. We therefore used a modified Bonferroni correction method based

24   on the effective number of independent markers p_e, which was obtained by performing principal

25   component analysis for the marker LD matrix[79]. The threshold for dominance and heterotic effects was

26    $P < 0.05/p_e$ and for epistatic effects $P < 0.05/(p_e(p_e-1)/2)$.

27

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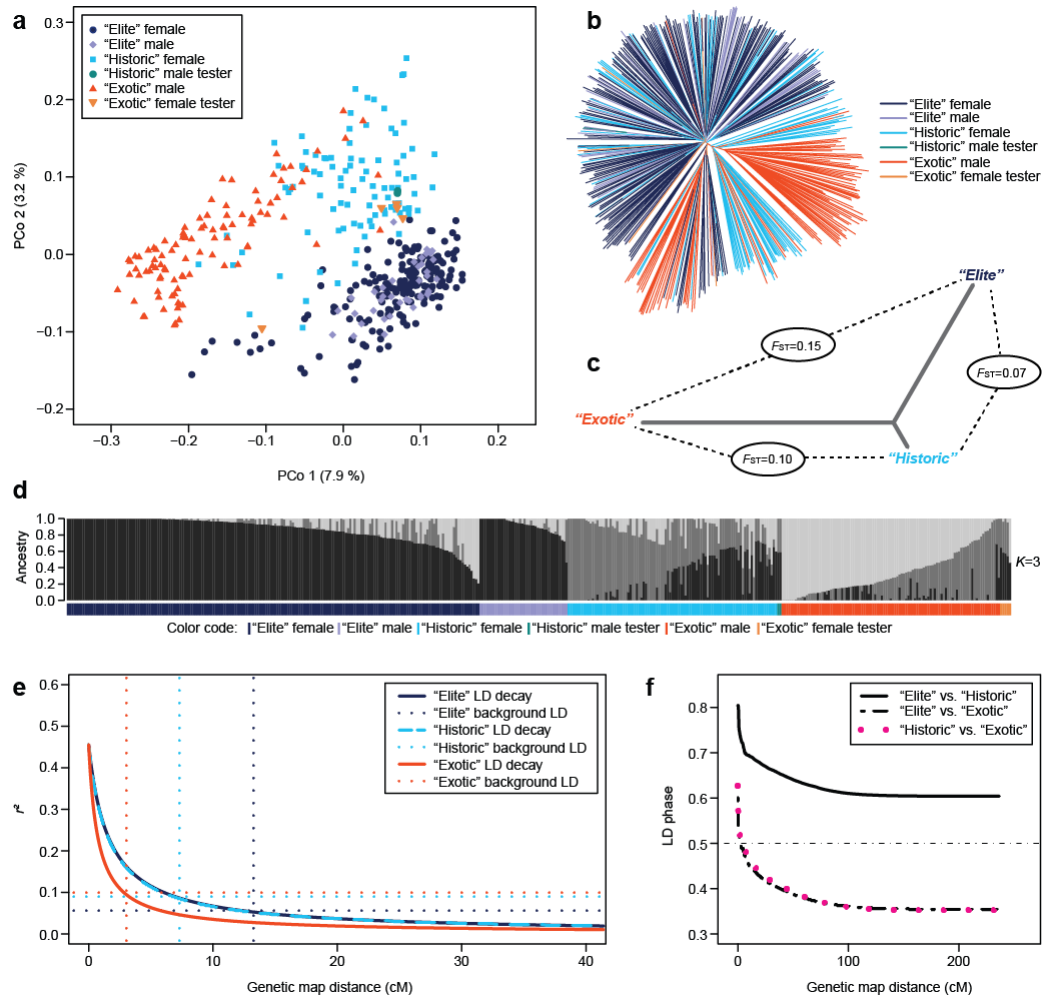
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3 **Fig. 1 | Genetic diversity is maximized in the hybrid wheat parents.** The 227 "Elite" parents, 98

4 "Historic" parents and 106 "Exotic" parents were analyzed for population structure and linkage

5 disequilibrium (LD) parameters. **a,** Principal coordinate analysis based on modified Rogers' distances.

6 Percentages in parentheses refer to the proportion of genotypic variance explained by the first and

7 second principal coordinate. **b,** Neighbor-joining tree based on modified Rogers' distances. **c,** Neighbor-

8 joining tree based on the results of F_{ST} statistics for the three sets. **d**, Bayesian clustering analysis

9 (ADMIXTURE) with K=3. **e,** Smoothing spline of the LD measure r^2 plotted against the genetic map

10 distance within the three sets. Dotted lines show the 95% percentile of LD between pairs of unlinked

11 markers as a population-specific critical value for LD due to genetic linkage. **f,** Smoothing spline of

12 pairwise correlations of LD phase (R) between the three sets plotted against the genetic map distance.

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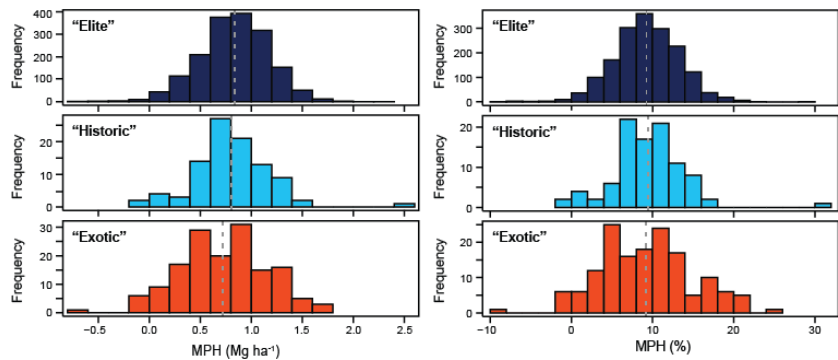
3 **Fig. 2 | Heterosis for grain yield.** Distribution of absolute and relative midparent heterosis (MPH) for

4 grain yield for the "Elite", "Historic" and "Exotic" hybrids. The dashed vertical line indicates the mean.

5
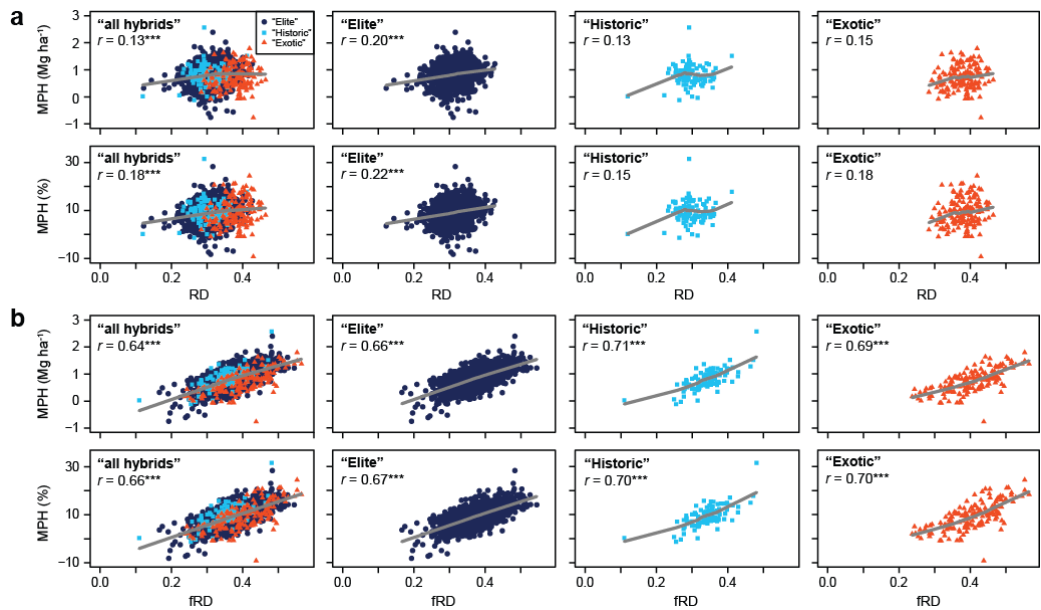
Fig. 3 | Association between grain yield heterosis and genetic distance. Association between absolute or relative midparent heterosis and **a**, Rogers' distance (RD) or **b**, functional Rogers' distance (fRD), shown for the different sets of hybrids. The grey lines are locally weighted regression lines.
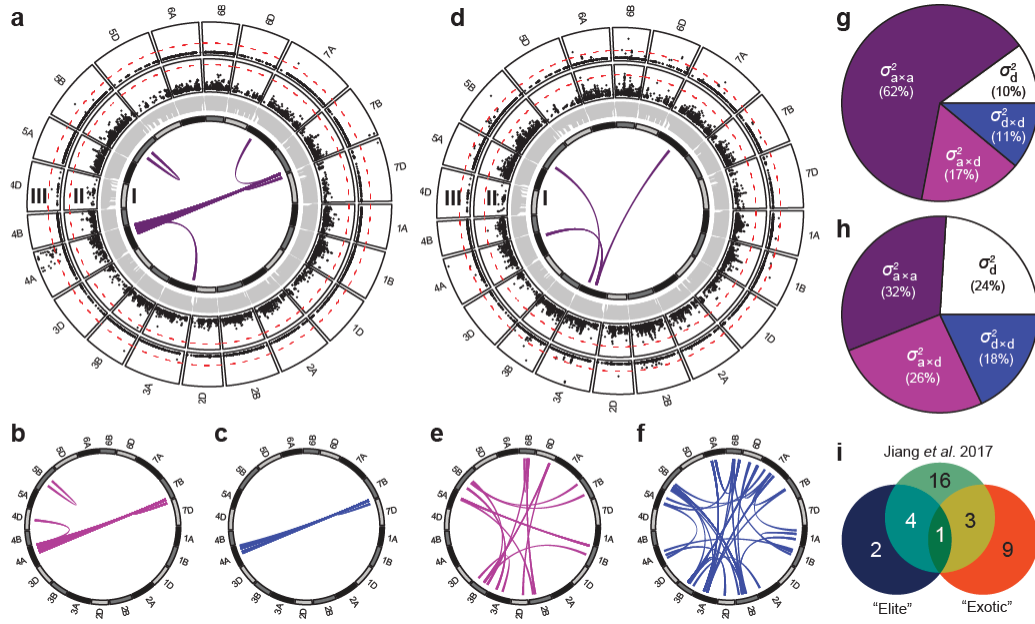
Fig. 4 | Genetic architecture of midparent heterosis for grain yield in wheat in the "Elite" and "Exotic" hybrids. a-f, Wheat chromosomes are indicated as bars in the inner circle; gray shadings differentiate homoeologous chromosomes. Gray connector lines represent the genetic-map positions of SNPs on the chromosomes. Colored links in the centers of the circles represent significant digenic epistatic interactions: additive-by-additive interactions (**aI,dI**), additive-by-dominance interactions (**b,e**), and dominance-by-dominance interactions (**c,f**). Manhattan plots for the dominance effects (**aII,dII**) and the heterotic effects (**aIII,dIII**) from GWAS. Significance thresholds are indicated as red dashed lines. **g,h,** Relative contributions of the genetic components of midparent heterosis for grain yield (σ_d^2, dominance variance; $\sigma_{a\times a}^2$, additive-by-additive variance; $\sigma_{a\times d}^2$, additive-by-dominance variance; $\sigma_{d\times d}^2$, dominance-by-dominance variance) estimated via Bayesian generalized linear regression. **i,** Venn diagram showing the number of overlapping heterotic QTL between the "Elite" and "Exotic" sets and the study by Jiang *et al.*[29].
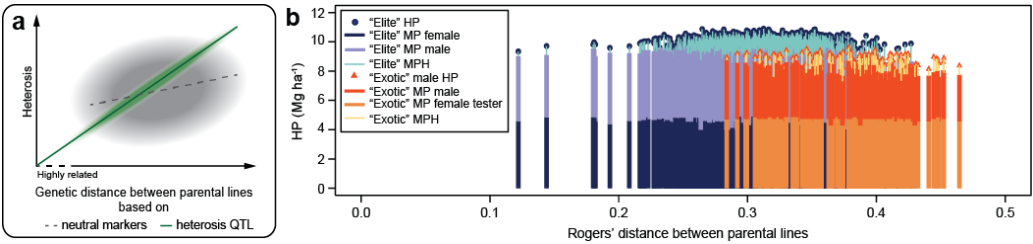
Fig. 5 | Heterosis and genetic distance. a, Schematic representation of the genetic association between heterosis and genetic distance measures. **b,** Components of hybrid performance associated with Rogers' distance between parental lines. *HP*, hybrid performance; *MP*, midparent value; *MPH*, absolute midparent heterosis

Genome-based reevaluation of heterosis in genetically diverse hybrid wheat

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[7] Bayer CropScience AG, Am Schwabeplan 6, 06466 Stadt Seeland, Germany

[8] Deutsche Saatveredelung AG, Leutewitz 26, 01665 Käbschütztal, Germany

[9] W. von Borries-Eckendorf GmbH & Co. KG, Hovedisserstr. 92, 33818 Leopoldshöhe, Germany

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Supplementary information

1 **Table S1 | Location information.** Locations used for the field trials in the growing-season 2015/16

2

Trial	Location	Latitude	Longitude	Altitude	Mean annual precipitation	Mean annual temperature
"Elite"	Asendorf	52°44'17.934" N	9°0'24.105" E	45 m	751 mm	9.3 °C
"Elite"	Biendorf	51°45'0" N	11°50'59" E	79 m	470 mm	8.8 °C
"Elite"	Gatersleben	51°50'35.7" N	11°18'1.512" E	156 m	519 mm	8.7 °C
"Elite"	Hadmersleben	51°58'37.916" N	11°18'10.414" E	91 m	500 mm	8.7 °C
"Elite"	Rosenthal	52°18'18.89" N	10°10'52.88" E	70 m	700 mm	9.8 °C
"Elite"	Seligenstadt	49°51'16.30" N	10°06'2.30" E	280 m	606 mm	9.2 °C
"Exotic/Historic"	Böhnshausen	51°51'31.401" N	10°57'44.669" E	130 m	580 mm	9.2 °C
"Exotic/Historic"	Gatersleben	51°51'39.986" N	11°18'4.471" E	156 m	519 mm	8.7 °C
"Exotic/Historic"	Renningen	48°44'29.583" N	8°55'15.35" E	484 m	690 mm	7.9 °C
"Exotic/Historic"	Schackstedt	51°43'12" N	11°37'11.999" E	134 m	504 mm	8.8 °C
"Exotic/Historic"	Stuttgart-Hohenheim	49°43'2.646" N	9°11'12.699" E	406 m	697 mm	8.8 °C

3

1 **Table S2 | Summary statistics and heterosis**. Shown for grain yield, heading time, and plant height

Source	Grain yield (Mg ha^{-1})	Heading (days)	Plant height (cm)
"Elite" hybrids (*n* = 1697)			
h²	0.81	0.94	0.94
Mean (Min; Max)	9.93 (7.48; 10.89)	152.74 (148.19; 158.31)	90.02 (72.68; 105.18)
Mean MPH (Min, Max)	0.83 (-0.75; 2.39)	-0.76 (-3.62; 2.77)	6.26 (-7.54; 18.83)
Mean MPH% (Min; Max)	9.24 (-8.21; 28.30)	-0.49 (-2.36; 1.82)	7.50 (-8.96; 23.49)
Mean BPH (Min; Max)	0.60 (-1.63; 1.91)	-1.63 (-7.49; 1.35)	2.40 (-11.55; 14.61)
Mean BPH% (Min; Max)	6.47 (-17.41; 21.38)	-1.05 (-4.80; 0.89)	2.84 (-13.71; 18.30)
"Historic" hybrids (*n* = 96)			
h²	0.83	0.79	0.76
Mean (Min; Max)	9.35 (8.30; 10.72)	152.97 (148.71; 157.75)	91.02 (83.00; 98.73)
Mean MPH (Min; Max)	0.81 (-0.12; 2.57)	-1.30 (-4.79; 1.39)	5.96 (-1.64; 13.30)
Mean MPH% (Min; Max)	9.48 (-1.32; 31.51)	-0.84 (-3.06; 0.91)	6.33 (-1.73; 14.03)
Mean BPH (Min; Max)	0.47 (-0.62; 2.03)	-2.42 (-6.51; 0.28)	3.03 (-4.02; 11.46)
Mean BPH% (Min; Max)	5.27 (-6.16; 23.34)	-1.56 (-4.20; 0.18)	3.46 (-4.62; 13.14)
"Exotic" hybrids (*n* = 152)			
h²	0.82	0.64	0.84
Mean (Min; Max)	8.76 (7.59; 9.63)	155.22 (151.93; 158.40)	112.71 (94.07; 124.03)
Mean MPH (Min; Max)	0.72 (-0.77; 1.79)	-1.41 (-5.37; 6.13)	9.02 (-1.22; 32.81)
Mean MPH% (Min; Max)	9.17 (-9.18; 24.44)	-0.88 (-3.23; 4.09)	9.15 (-1.03; 37.59)
Mean BPH (Min; Max)	-0.66 (-1.49; 0.14)	-3.30 (-10.09; 1.36)	-13.33 (-38.11; 31.05)
Mean BPH% (Min; Max)	-7.00 (-16.29; 1.46)	-2.07 (-6.12; 0.88)	-9.19 (-25.00; 34.88)

2 *MPH*, absolute midparent heterosis; *MPH%*, relative midparent heterosis; *BPH*, absolute better parent

3 heterosis; *BPH%*, relative better parent heterosis

4

1   **Table S3 |** Genome-wide prediction accuracies for mid-parent heterosis in the "Elite" and "Exotic" sets

2   considering different genetic effects

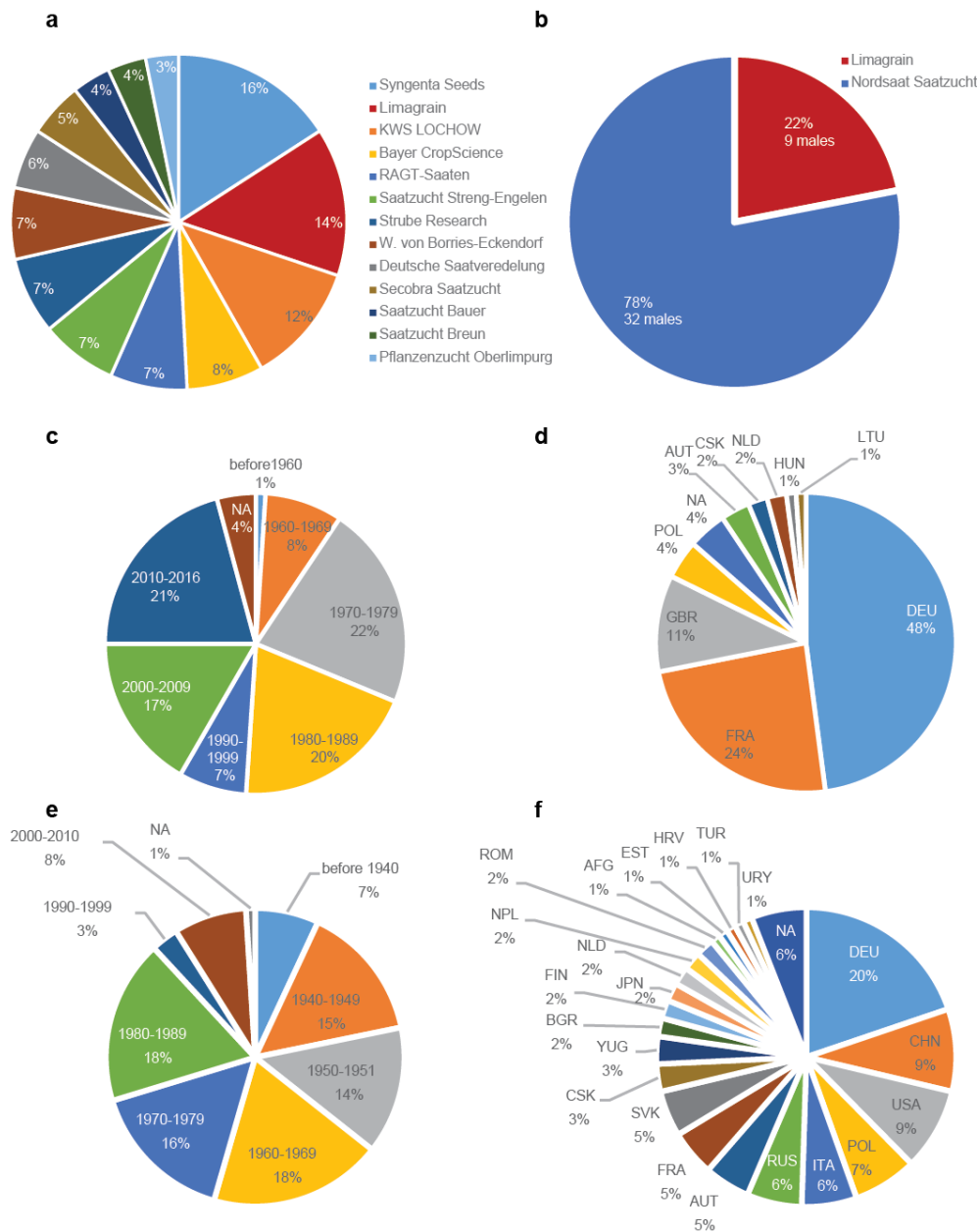| Model	"Elite"	"Exotic"
D	0.54	0.64
AA	0.70	0.68
D+A	0.68	0.68
D+AA+AD	0.69	0.69
D+AA+AD+DD	0.68	0.68

3

4

5   **Table S4 |** Correlations among kinship matrices of dominance (D), and respective digenic epistatic (AA,

6   AD, DD) variance components (VC) within the "Elite" set

VC	D	AA	AD	DD
D	1.00	0.47	0.76	0.99
AA		1.00	0.75	0.53
AD			1.00	0.78
DD				1.00

7

8

9   **Table S5 |** Correlations among kinship matrices of dominance (D), and respective digenic epistatic (AA,

10   AD, DD) variance components (VC) within the "Exotic" set

VC	D	AA	AD	DD
D	1.00	0.83	0.81	0.99
AA		1.00	0.96	0.85
AD			1.00	0.82
DD				1.00

11

12

13   **Table S6 |** Number of significant genetic effects and heterotic effects detected in the "Elite" and "Exotic"

14   sets

Genetic effect	"Elite" [a]	"Exotic" [b]
D	1	2
AA	106	5
AD	81	19
DD	33	75
Heterotic effect	46	18

15   [a] "Elite" set: Treshold $P < 0.05$ after Bonferroni correction, [b] "Exotic" set: Treshold $P < 0.05$ after modified Bonferroni correction

31

a

- Syngenta Seeds
- Limagrain
- KWS LOCHOW
- Bayer CropScience
- RAGT-Saaten
- Saatzucht Streng-Engelen
- Strube Research
- W. von Borries-Eckendorf
- Deutsche Saatveredelung
- Secobra Saatzucht
- Saatzucht Bauer
- Saatzucht Breun
- Pflanzenzucht Oberlimpurg

b

- Limagrain
- Nordsaat Saatzucht

Fig. S1 | Plant material information of parental lines. Breeders' affiliation of **a**, 189 female lines and **b**, 41 male lines and used within the "Elite" set. **c,** Decade of release and **d,** origin of 96 female lines within the "Historic" set. **e,** Decade of release and **f,** origin of 101 male lines within the "Exotic" set. *AFG*, Afghanistan; *AUT*, Austria; *BGR*, Bulgaria; *CHN*, China, *CSK*, former Czechoslovakia; *DEU*, Germany; *EST*, Estonia; *FIN*, Finland; *FRA*, France; *GBR*; Great Britain; *HRV*, Croatia; *HUN*, Hungary; *ITA*, Italy; *JPN*, Japan; *LTU*, Lithuania; *NA*, no data available; *NLD*, Netherlands; *NPL*, Nepal; *POL*, Poland; *ROM*, Romania; *RUS*, Russia; *SVK*, Slovak Republic; *TUR*, Turkey; *URY*, Uruguay; *USA*, United States of America; *YUG*, former Yugoslavia

a 189 Females

41 Males

b 101 Males

9 Female testers

c Male tester × 96 Females

1

2

3 **Fig. S2 | Schematic illustration of the mating designs.** Produced and tested hybrids are indicated by

4 green boxes. **a,** "Elite"; incomplete factorial mating design. **b,** "Exotic"; incomplete factorial mating

5 design. **c,** "Historic"; topcross mating design.
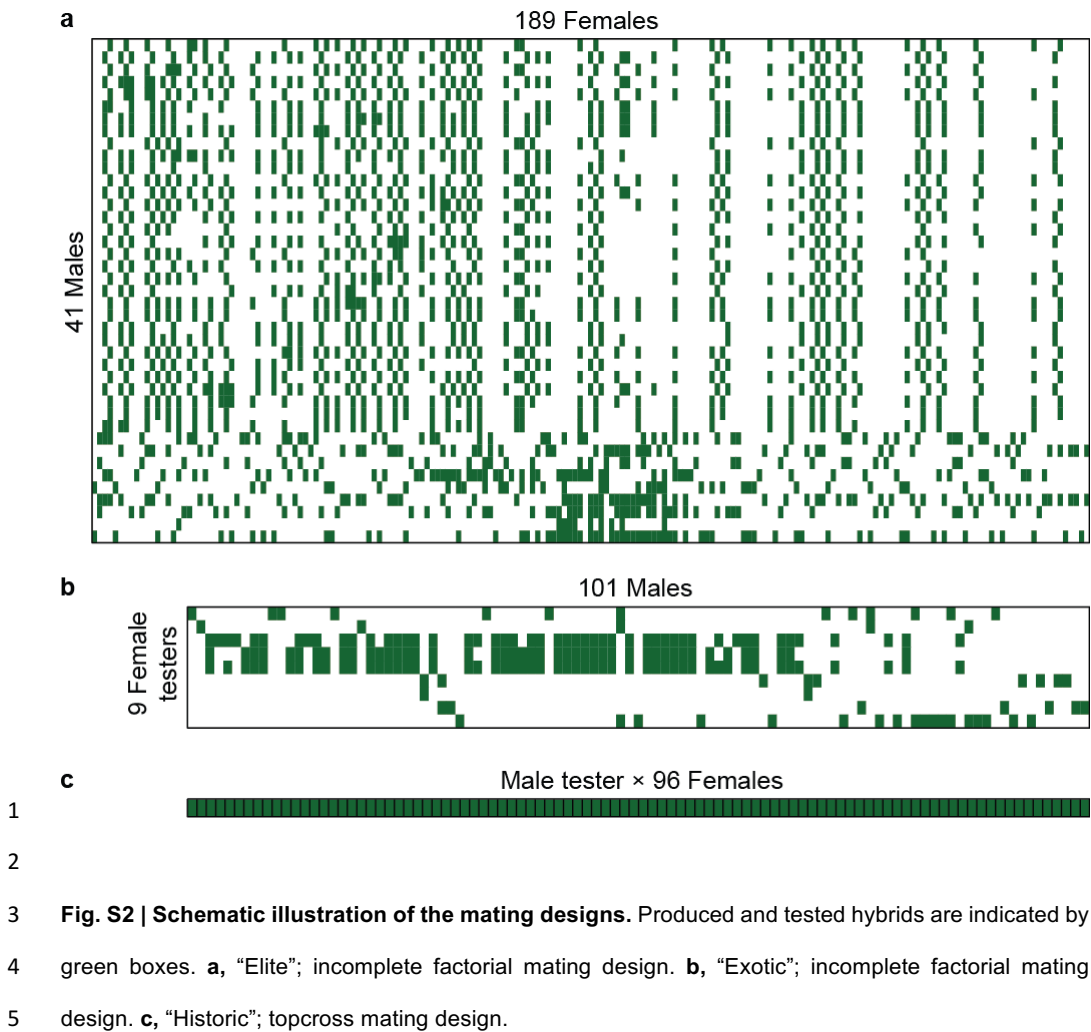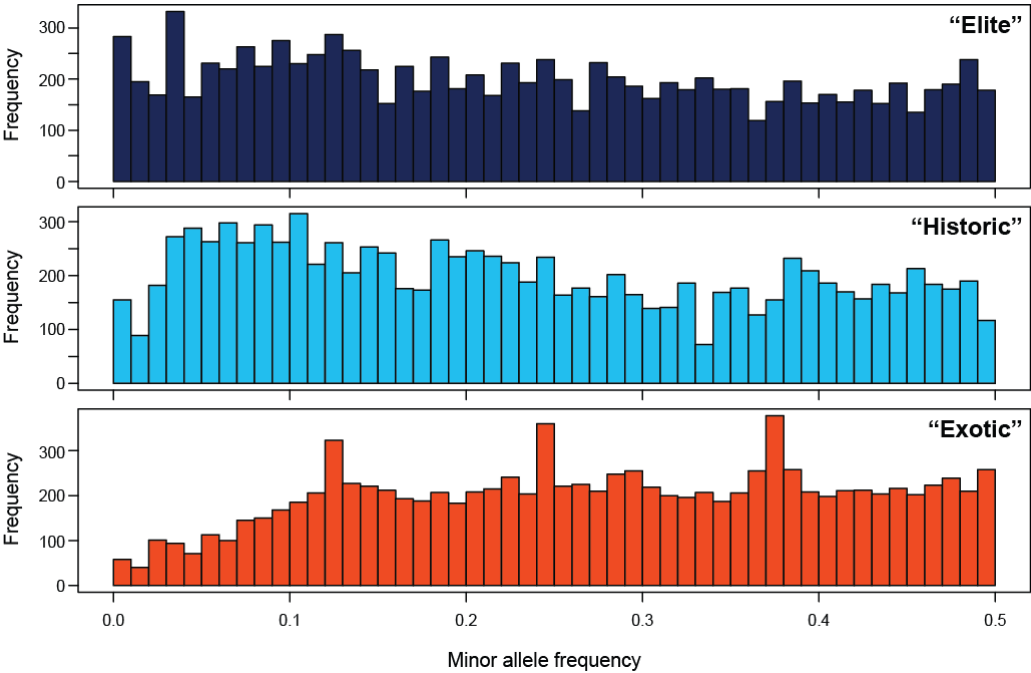
1

2

3 **Fig. S3 | Assessment of SNP array.** Distribution of the minor allele frequency (MAF) in the three groups

4 "Elite", "Historic", and "Exotic", showing that the SNP array is equally informative in all three groups of

5 parental lines. Ascertainment bias in the "Exotic" set would result in a high proportion of markers with

6 low MAF, which is not the case here.

7
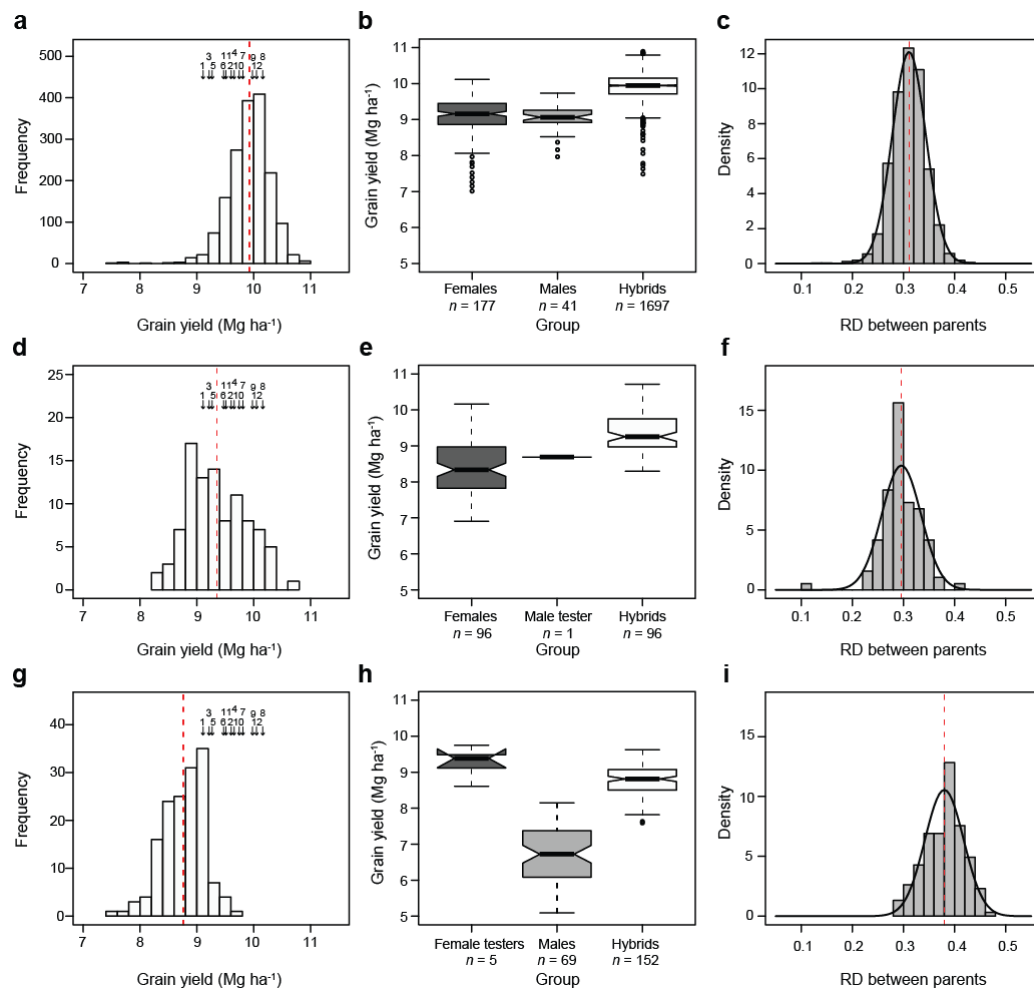
Fig. S4 | Distributions of grain yield and Rogers' distance between parents. **a,d,g,** Distributions of grain yield for 1,697 "Elite" hybrids, 96 "Historic" hybrids and 152 "Exotic" hybrids. **b,e,h,** Boxplots showing grain yield of female parents, male parents, and hybrids for "Elite", "Historic" and "Exotic" sets. **c,f,i,** Distributions of Rogers' distances between 217 "Elite" parents, between 98 "Historic" parents, and between 74 "Exotic" parents. 1=*Colonia*, 2=*Elixer*, 3=*Hybred*, 4=*Hystar*, 5=*JB Asano*, 6=*Julius*, 7=*KWS Loft*, 8=*KWS Smart*, 9=*LG Alpha*, 10=*RGT Reform*, 11=*Rumor* and 12=*Tobak*.
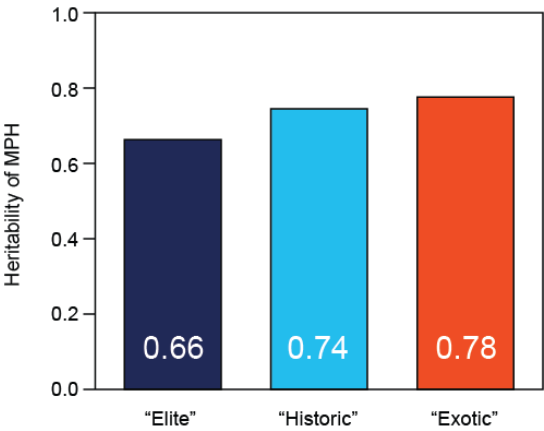
Fig. S5 | Heritability estimates for midparent heterosis of grain yield.
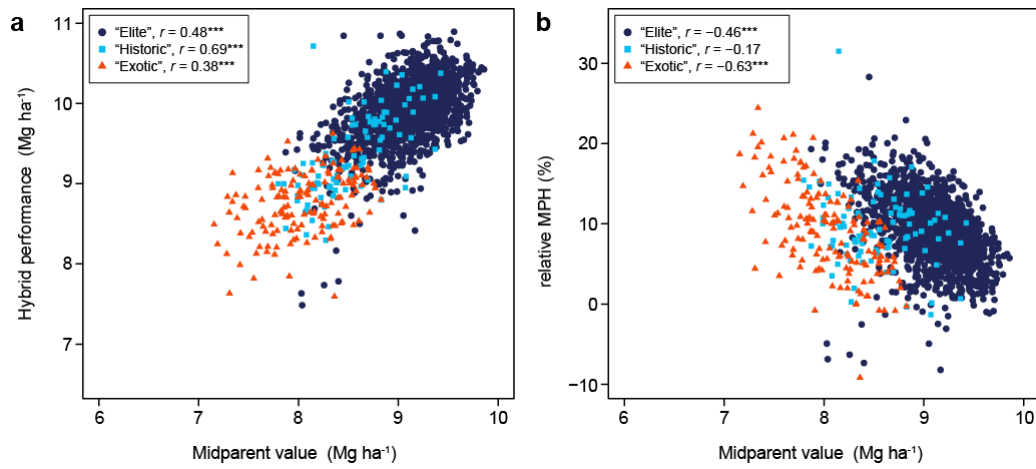
Fig. S6 | Hybrid response, heterosis and *per se* performance. The positive association between (**a**) midparent value and hybrid performance as well as (**b**) the negative association between midparent value and heterosis was observed within each group, accompanied with a shift from "Elite" to "Exotic".
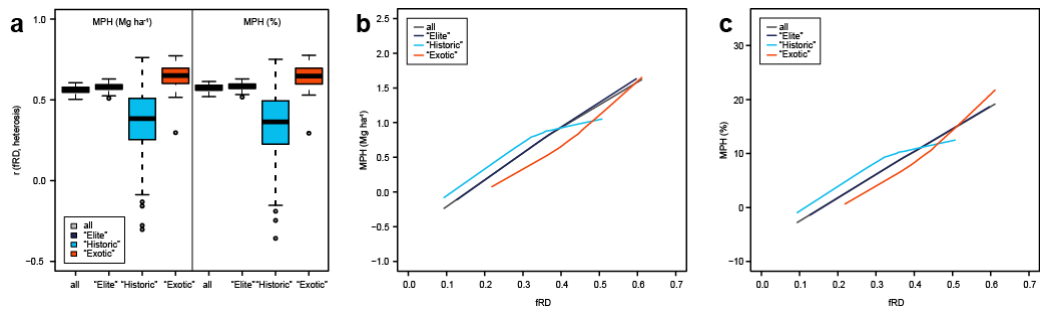
Fig. S7 | Cross-validated correlation between heterosis and the functional Rogers' distance (fRD). a, Boxplots showing correlations between heterosis and the functional Rogers' distance for dominance effects estimated in 80% of the hybrids and the correlation assessed in the remaining 20%. The greater variation of values within the "Historic" set can be explained as an effect of the topcross mating design. **b,c,** Natural smoothing splines to the cross-validated values between fRD and **(b)** absolute and **(c)** relative midparent heterosis.
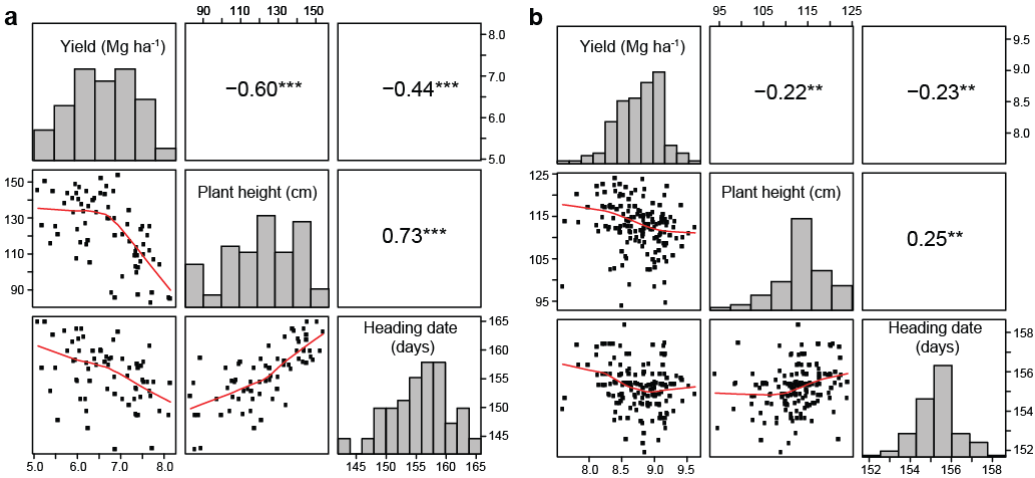
Fig. S8 | Phenotypic trait correlations. a, "Exotic" lines and **b,** "Exotic" hybrids. Plant height and yield were found to be strongly negatively correlated within the "Exotic" lines, but much less so in their hybrids from crosses with adapted elite material.
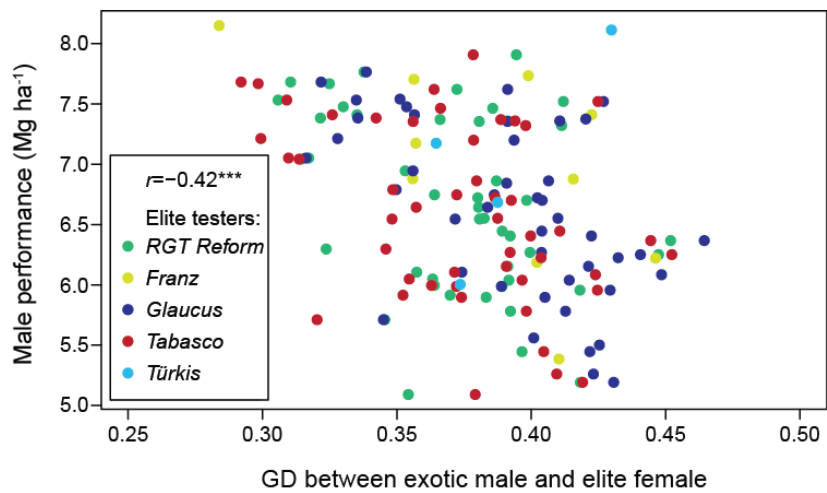
Fig. S9 | Adaptation of "Exotic" males. Association between "Exotic" line *per se* performance and genetic distance (GD) to the respective elite female tester.

5 Genetic architecture of male floral traits required for hybrid wheat breeding

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[2] KWS LOCHOW GMBH, 29303 Bergen, Germany

Abstract Hybrid wheat breeding is a promising approach to increase grain yield and yield stability. However, the identification of lines with favorable male floral characteristics required for hybrid seed production currently poses a severe bottleneck for hybrid wheat breeding. This study therefore aimed to unravel the genetic architecture of floral traits and to assess the potential of genomic approaches to accelerate their improvement. To this end,

we employed a panel of 209 diverse winter wheat lines assessed for male floral traits and genotyped with genome-wide markers as well as for *Rht-B1* and *Rht-D1*. We found the highest proportion of explained genotypic variance for the *Rht-D1* locus (11–24 %), for which the dwarfing allele *Rht-D1b* had a negative effect on anther extrusion, visual anther extrusion and pollen mass. The genome-wide scan detected only few QTL with small or medium effects, indicating a complex genetic architecture. Consequently, marker-assisted selection yielded only moderate prediction abilities (0.44–0.63), mainly relying on *Rht-D1*. Genomic selection based on weighted ridge-regression best linear unbiased prediction achieved higher prediction abilities of up to 0.70 for anther extrusion. In conclusion, recurrent phenotypic selection appears most cost-effective for the initial improvement of floral traits in wheat, while genome-wide prediction approaches may be worthwhile when complete marker profiles are already available in a hybrid wheat breeding program.

6 Hybrid seed set in wheat is a complex trait but can be improved indirectly by selection for male floral traits

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Abstract Efficient hybrid wheat breeding requires the redesign of the wheat floral architecure to enhance cross-pollination. Several studies evaluated the phenotypic variation and the genetic architecture of male floral traits, but their contribution to the most important trait, hybrid seed set on the female

parent, has not yet been considered. To bridge this gap, we employed 31 male lines and evaluated the hybrid seed set on two female tester lines in crossing blocks. Hybrid seed set showed large genetic variance and high heritability, which demonstrates the potential for the improvement of this trait. However, the assessment of hybrid seed set is difficult as secondary traits like plant height and especially flowering time, as well as the environment largely influence the hybrid seed set. Nevertheless, a moderately high correlation between visual anther extrusion and hybrid seed set opens up the possibility to use visual anther extrusion as an indirect trait for preliminary male screenings. Further research evaluating traits influencing female receptivity coupled with genomics-assisted approaches are highly recommended to develop an improved selection portfolio for maximizing hybrid seed set.

7 General Discussion

Hybrid breeding is a worldwide success story. First and foremost, hybrid breeding has been applied extensively to the agricultural production of the allogamous crop maize (Duvick 1999, 2005; Troyer 2006), but has since also been established in sunflower, sugar beet, rye and many other crops (Longin et al. 2012). This inspired scientists and breeders alike to evaluate the potential of hybrid breeding in wheat.

Wheat has a rather long history of hybrid breeding efforts tracing back to the 1960s and was explored by a number of research groups and companies across many countries including Germany, the former German Democratic Republic, France, Great Britain, the US, China, Australia, India, and South Africa (Merfert et al. 1988; Lucken and Johnson 1989; Pickett 1993; Singh et al. 2010; Koekemoer et al. 2011; Longin et al. 2012). In the 1980s, hybrid wheat breeding and research peaked and the first hybrid cultivars entered the wheat market (Pickett 1993). However, they did not find lasting success. Hybrid wheat was mainly hampered by its high seed production costs due to the self-pollinating flowering biology, the lack of a completely satisfactory hybrid mechanism, and the strong competition of high-yielding line varieties, eventually leading to a decreasing interest in hybrid wheat in the beginning of the 1990s (Pickett 1993). Nevertheless, the stagnation of wheat yields during the past years, ongoing climate change, the need to feed an ever-growing world population, accompanied by a growing toolbox of genomic approaches again led to increasing interest in hybrid wheat. Consequently, solutions to

the issues hampering hybrid wheat breeding need to be developed. These issues can be roughly divided into two thematic components: (i) quantitative genetics dealing with heterosis as well as with the establishment of heterotic groups, and (ii) applied breeding mainly focusing on the production of hybrid seed and the required redesign of the wheat flower. Both thematic components were part of this thesis research and will be discussed in the following paragraphs.

Performance of European hybrid wheat: Substantial heterosis is available

The crucial decision to launch a hybrid wheat breeding program mainly relies on the realized amount of heterosis and the resulting hybrid performance. Longin et al. (2012) reported an average grain yield heterosis of about 10 % in wheat, based on a review of studies performing multi-environmental field trials. This amount of midparent heterosis for grain yield seems realistic and was confirmed in recent studies analyzing grain yield of more than 3,500 wheat hybrids and their parental lines (Longin et al. 2013; Boeven et al. to be submitted). In the wheat market, however, hybrid wheat varieties need to compete with high performing line varieties. Hence, from an economic point of view, the most interesting parameter is the commercial heterosis comparing the performance of hybrids with the best performing released line variety. We tested 1,750 wheat hybrids for grain yield and found that 106 hybrids significantly ($P < 0.05$) outperformed the highest performing released line variety with a maximum yield benefit of 1 Mg ha^{-1} (Figure 2, unpublished data). This yield advantage corresponds to several years of selection gain in line breeding programs and highlights the potential of hybrid wheat (Longin et al. 2013; Zhao et al. 2015; Boeven et al. to be submitted). Interestingly, we did not observe a significant difference in grain yield between lines employed as female or male parents, illustrating that the rather stringent selection for floral characteristics required for the
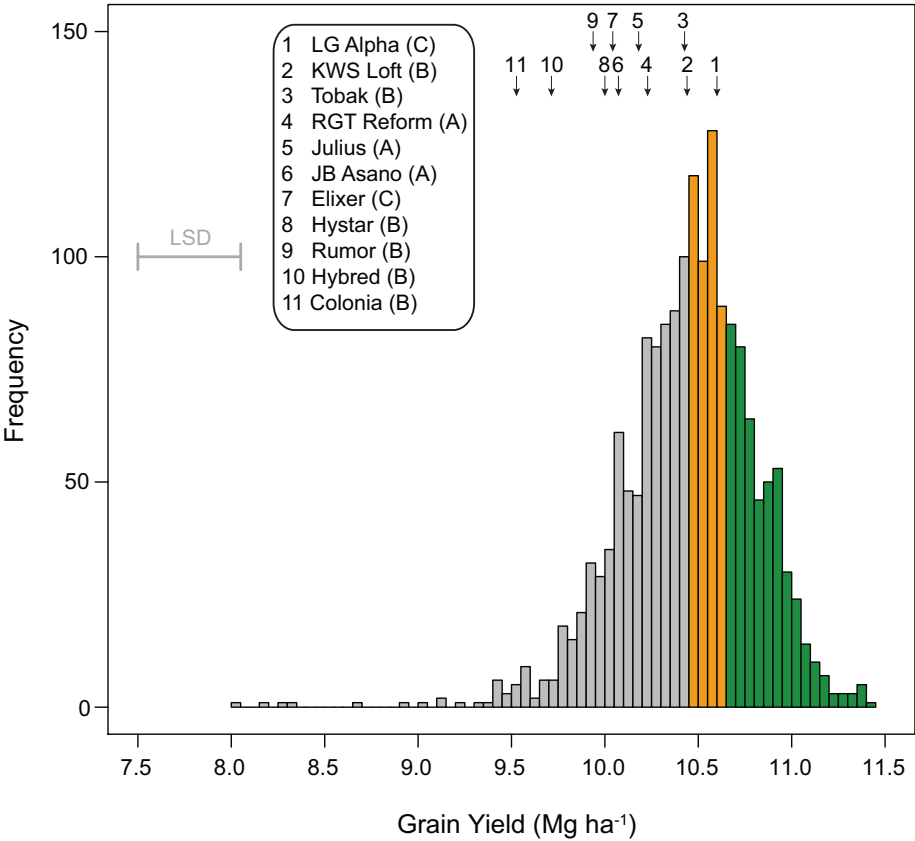
Figure 2: Grain yield performance of hybrid wheat. Distribution of grain yield of 1,750 wheat hybrids tested in six environments (unpublished data, ZUCHTWERT project). Arrows indicate performance of the 11 check varieties, respective quality classes are given in parentheses. Green bars indicate hybrids performing better than the best-unreleased wheat line. Both orange and green bars indicate all hybrids performing better than the best released line variety.

male parents to produce hybrid seed, appears to have no negative trade-off regarding their yield potential.

Undoubtedly, commercial heterosis is always a question of definition. Comparing the best performing unreleased hybrid combination with the best performing released line variety might be an unfair comparison as a released line variety is not fully representing the available maximum potential of line breeding including new lines in pre-registration stages. Taking this scenario into account, only 15 hybrids significantly ($P < 0.05$) outperformed the high-

est performing unreleased line variety but still with a maximum yield benefit of 0.78 Mg ha^{-1} (Figure 2, unpublished data). On the other hand, it must be considered that up to now fewer monetary resources were invested in hybrid compared to line breeding and the used hybrid parents were not pre-selected for their combining ability but represent a more or less random sample of current German line breeding programs.

Fusarium head blight (FHB) is a serious fungal disease in wheat and infections with FHB lead to yield losses and to a contamination of the grain by mycotoxins harmful for livestock as well as for humans (Osborne and Stein 2007). The registration of new wheat varieties in Germany requires at least a moderate resistance to FHB. Interestingly, Miedaner et al. (2017) found a negative average midparent heterosis for FHB and hence, wheat hybrids combined their higher yield potential with a lower susceptibility to FHB. We analyzed grain yield and FHB ratings of 1,750 wheat hybrids and their 189 parental lines. Here, hybrids and lines showed a similar level of FHB disease severity, but the hybrids were still able to combine it with higher yield (Figure 3a, unpublished data). The slightly different result compared to the study by Miedaner et al. (2017) might be explained by the broader sampling strategy and genetic background of the parental lines. Additionally, Longin et al. (2013) observed that hybrid wheat possesses a lower susceptibility to leaf rust and Septoria tritici blotch compared to their parental lines and the general trend that hybrid wheat can combine higher yield with a similar or even lower level of disease susceptibility was also confirmed by our data (Figure 3, unpublished data). These examples underscore the potential of hybrid breeding, facilitating high-yielding and disease-resistant genotypes to meet the challenges of climate change and future food security.

Finally, bread-making quality traits are of essential importance in wheat breeding and processing (Shewry et al. 1995). Ideally, genotypes should combine high grain yield with high bread-making quality. This, however, is difficult to achieve, as these traits are negatively associated. In Germany, new cultivars need to meet minimum standards to enter different quality
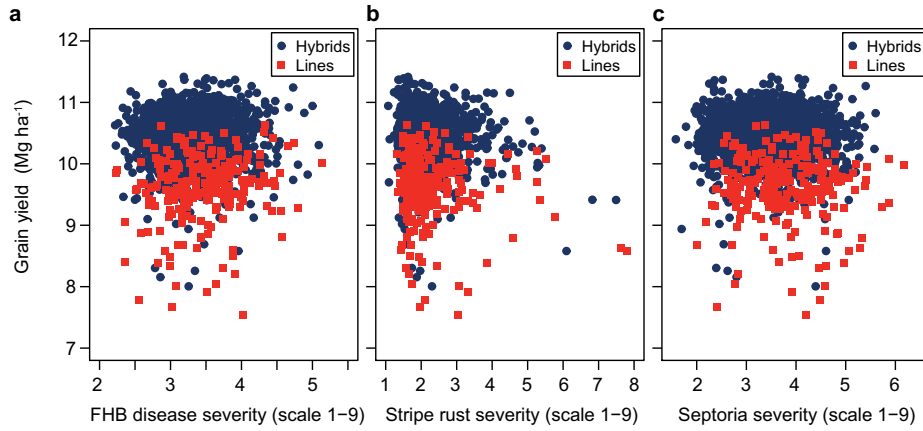
Figure 3: Association of grain yield and disease severity in the groups of hybrids and lines (unpublished data, ZUCHTWERT project). **a** Grain yield and Fusarium head blight severity (FHB). **b** Grain yield and stripe rust severity. **c** Grain yield and Septoria tritici blotch severity.

classes. This classification has a huge impact on the success of new cultivars in the wheat market. For example, two important indirect parameters for bread-making quality are protein content and sedimentation volume. Both traits were reported to show a negative midparent heterosis of on average about 2 to 3 %, respectively (Liu et al. 2016; Thorwarth et al. 2018). In contrast, we found that wheat hybrids showed a positive midparent heterosis for protein yield of about 8 %. In addition, Thorwarth et al. (2018) recently reported for grain protein deviation, based on a bivariate model, a heterosis of about 35 %. This study compared hybrids and lines within different quality classes and stressed that hybrids had a higher grain yield at a given sedimentation volume or protein content. These initial findings are promising towards a more sustainable wheat production but further research is required to determine the bread-making quality of hybrid wheat in more detail.

Establishment of heterotic groups in wheat: A long way to go

Genetic diversity caused by spatial or temporal isolation contributed to the establishment of heterotic groups in different crops (Melchinger and Gumber 1998). For instance in rye, the well-known heterotic groups Carsten and Petkus were identified in a 7×7 complete diallel among open pollinated populations and these identified pools originally trace back to two germplasm groups separated through breeding history (Hepting 1978; Melchinger and Gumber 1998; Geiger and Miedaner 1999). In contrast, the establishment of heterotic groups in autogamous crops is generally more challenging because the amount of heterosis is lower, and the floral biology and pollination control often limit theoretical testcrosses. Moreover, line breeding allows a widespread exchange of germplasm between breeding companies leading to a mix of germplasm impeding the identification of divergent groups as it is the case in current German elite winter wheat (Figure 4).

For these reasons, a more unified approach for the establishment of heterotic groups in wheat is required. In Chapter 2, Boeven et al. (2016b) proposed the *HyBFrame* approach tackling how global wheat genetic diversity could be used to support and accelerate hybrid breeding and the establishment of heterotic groups by reciprocal recurrent selection. Briefly, the *HyBFrame* approach includes eight steps:

(1) adapted germplasm under maximized diversity serves as starting material due to the moderate to high correlations between parental *per se* and hybrid performance (Longin et al. 2013; Figure 5);

(2) identification and consideration of suitable male and female floral traits;

(3) factorial testcrosses to assess GCA;

(4) selection of promising lines as starting material for male and female pools;
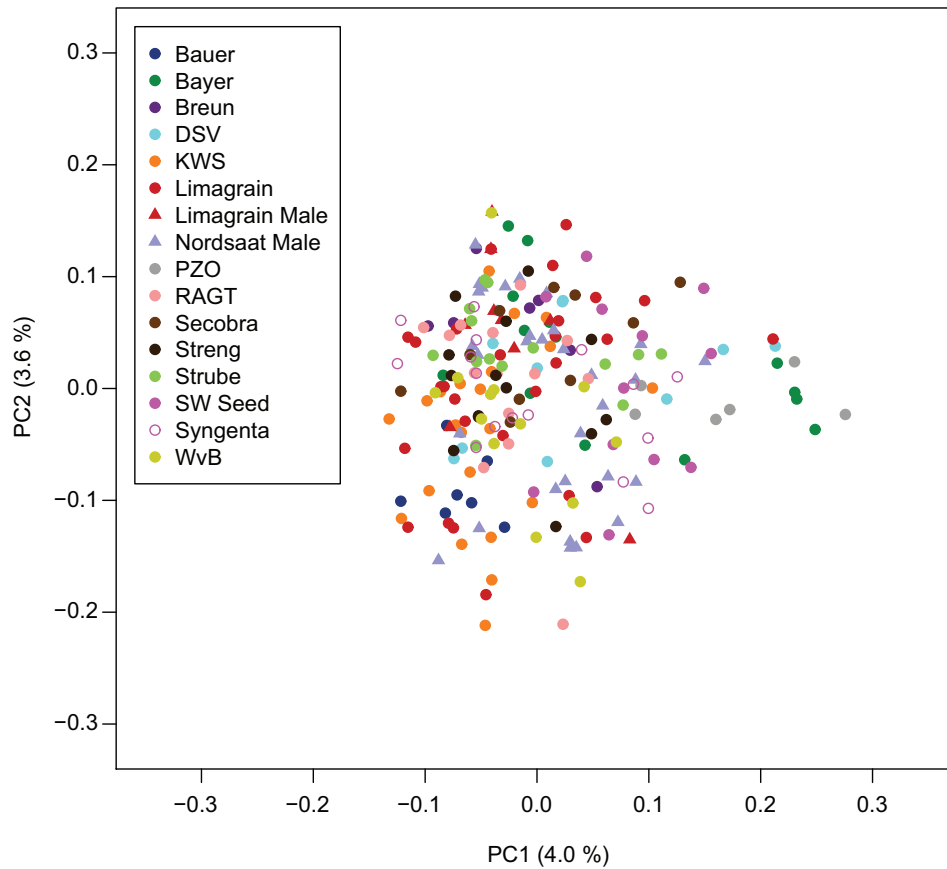
Figure 4: Population structure of German elite winter wheat with regard to breeding companies. The principal coordinate analysis is based on modified Rogers' distances between the 230 "Elite" parental lines used in the study by Boeven et al. (to be submitted). Percentages in parentheses refer to the proportion of genotypic variance explained by the first and second principal coordinate.

(5) consideration of the desired hybrid mechanism;

(6) start of reciprocal recurrent (genomic) selection to improve combining ability and genetic divergence between the pools (Hagdorn et al. 2003; Gerke et al. 2015);

(7) continuous support of reciprocal recurrent (genomic) selection by the knowledge-based introgression of new germplasm not disrupting the established patterns. Breeders should consider predicted GCA, genetic similarity, the necessity of previous backcrossing to improve adaption,

and final testcross performance with proven testers from the opposite pool;

(8) new genotypes fulfilling the before-mentioned requirements and possessing a superior observed GCA enter the respective group. If necessary, further selection of progenies for floral traits, hybrid mechanism related traits, or adaptation traits.

Most of the illustrated *HyBFrame* steps have already been made or are currently ongoing in applied hybrid wheat breeding programs. Nevertheless, the implementation of *HyBFrame* takes many years and needs long-term commitment.

Moreover, Zhao et al. (2015) recently proposed a three-step approach for the genome-based establishment of a heterotic pattern in wheat. This approach is based on the initial production and field evaluation of a preferably large number of wheat hybrids derived from an incomplete factorial mating design using chemical hybridization agents (CHAs). In a first step, the performance of all untested hybrids is estimated by genomic prediction. A
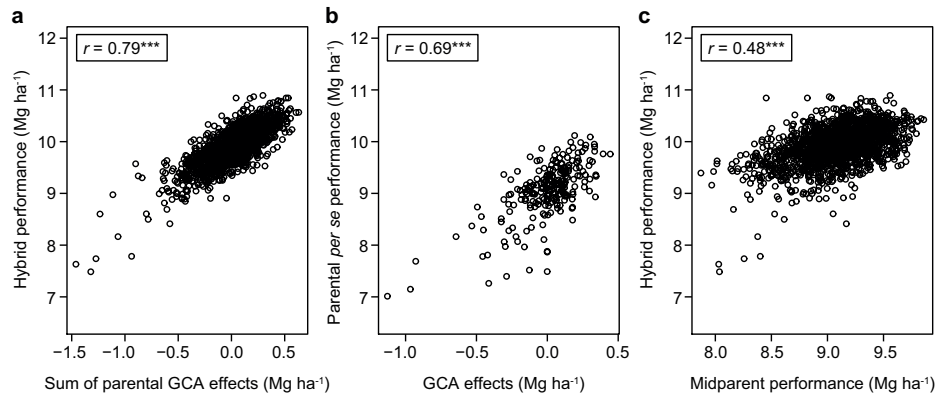
Figure 5: Prediction of hybrid performance. **a** Association between hybrid performance and sum of parental GCA effects (not cross-validated). **b** Association between parental line *per se* performance and GCA effects. **c** Association between hybrid performance and midparent performance. Data from Chapter 4 based on a separate analysis of "Elite" hybrids and parents.

simulated annealing algorithm is then used to search for a high yielding heterotic pattern. Finally the long-term success of the selected heterotic groups is evaluated based on quantitative genetic parameters. Applying this framework to a panel of 1,604 wheat hybrids and their 135 parents, Zhao et al. (2015) suggested that a starting group size of about 16 individuals offers a good compromise between short-term and long-term selection gain for grain yield in European winter wheat. Interestingly, for a heterotic group size of 16 individuals they found on average 81 % overlapping genotypes when comparing the result of the three-step approach with an approach assigning genotypes based on their GCA effects, which can again be explained by the strong effect of parental *per se* performance on hybrid performance in wheat. Moreover, the results by Zhao et al. (2015) gave evidence that the identified heterotic pattern is accompanied with a higher relevance of σ^2_{GCA} leading to a lower ratio of σ^2_{SCA} to σ^2_{GCA} and enhancing recurrent selection gain as expected based on quantitative genetic theory (Reif et al. 2007).

The elaborated three-step approach is a perfect tool for the decision support when starting to establish heterotic groups in wheat, which might then be continued by following *HyBFrame*. Nonetheless, the three-step approach by Zhao et al. (2015) neglected relevant hybrid seed production issues when assigning individuals into theoretical heterotic patterns and assumed that a few major genes control required male or female floral characteristics. This, however, seems not to be the case as revealed by recent studies dissecting the genetic architecture underlying male floral traits in wheat (Boeven et al. 2016a; Muqaddasi et al. 2016; 2017a, b, c). Moreover, as also followed by Zhao et al. (2015), genetic distance itself is not sufficient but definitely required as a supporting element for the establishment of heterotic groups. Likewise, as reviewed by Melchinger and Gumber (1998), molecular marker based genetic distance is not suitable for the prediction of hybrid performance but displays a perfect tool for grouping of germplasm and a pre-identification of promising germplasm for heterotic groups. Hence, *HyBFrame* requires genetic distance analyses and integrates this knowledge in a unified approach for the establishment of heterotic groups.

We, therefore, studied the genetic diversity in a panel of 1,110 winter wheat lines released during the past decades in 35 countries. This analysis revealed no clear population structure within Western European material, but some main trends in global wheat genetic diversity became evident. Briefly, some genetic backgrounds tended to cluster together: Western European lines, Eastern European lines, lines released in China and lines from the US clustered together with Eastern European lines (Boeven et al. 2016b). Interestingly, some lines assigned to male lines due to their favorable floral traits also tended to cluster together. The main diversity patterns were associated with the geographical origin and most likely evolved from long-term domestication, evolution and adaption to different environmental conditions (Cavanagh et al. 2013; Boeven et al. 2016b).

Not surprisingly, the global wheat diversity was also accompanied with differentiating phenotypic variation. For instance, lines from Eastern Europe, France or the US tended to earlier heading compared with lines from Germany or Great Britain. The wide range in heading date and hence in flowering time can be explained by adaptation to different environmental conditions and the resulting selection of favorable genotypes by breeders. In line with our phenotypic results, Langer et al. (2014a) observed that the photoperiod insensitive allele *Ppd-D1a* of the major photoperiod regulator *Ppd-D1* is more frequent in French, Eastern European and Russian wheat lines resulting in earlier flowering to escape heat and drought stress in regions of lower latitude. In addition to major genes, Díaz et al. (2012) demonstrated that copy number variation (CNV) at the *Ppd-B1* and *Vrn-A1* loci affects flowering time in wheat, as increased *Ppd-B1* and *Vrn-1* copies were accompanied by early flowering, day neutral phenotypes and increased vernalization requirement, respectively. In Chapter 3, we analyzed our global wheat collection of 1,110 winter wheat lines also for CNV at *Ppd-B1* and *Vrn-A1* and found that CNV reflects environmental conditions of the different origins. For instance, we observed a North to South trend within Europe for *Ppd-B1* resulting in a higher frequency of the two or three copy variants in France and in lines assigned to the Eastern European group. In a global context, US

lines showed a great similarity with South Eastern European lines and Chinese lines possessed the highest frequency of the three copy variant reflecting the high pressure for photoperiod insensitivity.

Likewise, plant height is an important aspect for hybrid wheat seed production since male lines should be taller than female lines to facilitate high out-crossing rates. We observed a wide range for plant height in our global wheat collection. British and French varieties were on average about 10 cm shorter compared to German varieties. This finding can be explained by a higher frequency of major semi-dwarfing genes *Rht-B1b* and *Rht-D1b* in varieties from Great Britain and France (Würschum et al. 2015b). Historically, semi-dwarfing genes dominated UK wheat production since the 1980s (Angus 2001). Eastern European lines were only slightly shorter compared to the German material. This is also consistent with results by Würschum et al. (2015b) since they found that the weaker *Rht-B1b* allele, which explained about two and a half times less genotypic variance compared to *Rht-D1b*, was more frequent in Eastern European lines, being the main source for plant height reduction within this material. Moreover, Würschum et al. (2017a) revisited the genetic control of plant height in our global wheat collection of 1,110 lines and identified a major *Rht* locus on chromosome 6A, *Rht24*, and found a temporal trend as the height-reducing allele *Rht24*b was more present in recent high yielding material released after 1990.

As illustrated by the *HyBFrame* approach, heterotic pools are not closed groups but should be continuously broadened by new germplasm to increase allelic diversity. The great variation in flowering time and plant height are two examples underscoring that interesting germplasm sets to widen established heterotic groups are restricted by phenotypic adaptation (Boeven et al. 2016b). In addition, bread-making quality traits are of essential importance in wheat breeding and must also be considered in the context of hybrid wheat. Thus, most of the promising lines identified by their predicted GCA or genetic distance cannot be directly used to perform testcrosses due to adaptation issues, lacking abiotic or biotic stress tolerance or due to constraints in floral traits required for hybrid breeding. Therefore, some of these

interesting lines first need to be crossed with adapted lines from the respective heterotic group into which they should be introgressed, followed by a selection for the above-mentioned traits. For some traits where major QTL are known, like *Vrn*, *Ppd* or *Rht*, marker-assisted selection can effectively support this process. Then, progenies are again genotyped to predict their GCA and the most promising candidates can enter the respective heterotic group and support their improvement by reciprocal recurrent selection as outlined in more detail by Boeven et al. (2016b).

In the context of genetic diversity and hybrid wheat breeding, Akel et al. (2018) recently investigated the usefulness of spelt wheat as a heterotic group for bread wheat. Spelt and bread wheat are genetically clearly separated and hybrids between these groups show also an interesting amount of heterosis. However, much lower hybrid performance and baking-quality compared to pure bread wheat hybrids as well as free-threshing issues make spelt wheat rather unattractive for hybrid wheat breeding (Akel et al. 2018).

As mentioned before, heterotic groups normally must be established through breeding. Reciprocal recurrent selection is a breeding method for population improvement in hybrid breeding and was used for the establishment of heterotic groups in maize (Wricke and Weber 1986; Duvick et al. 2004). It has been shown that this breeding method is effective when overdominance or at least partial dominance are present (Comstock et al. 1949; Schnell 1961; Wricke and Weber 1986). Likewise, long-term reciprocal recurrent selection might give evidence for the gene action of heterosis (Lamkey and Edwards 1999). The genetic architecture of heterosis is therefore affecting reciprocal recurrent selection, and consequently also the establishment of heterotic groups.

Recently, Jiang et al. (2017) elaborated a quantitative genetic framework to elucidate the genetic architecture in a diverse wheat population. This new framework enables the analysis of epistasis, which normally requires the use of special mating designs (Melchinger et al. 2007). Jiang et al. (2017) found

pronounced additive-by-additive epistatic effects underlying grain yield heterosis in wheat while dominance played a less important role. In Chapter 4, we applied the same quantitative genetic framework in a large panel of 2,046 wheat hybrids exploiting different levels of genetic diversity between parental lines, using elite germplasm, historic lines released during the past decades and a random sample of gene bank accessions. Although we found less significant effects mainly due to the lower number of SNP markers, our study confirmed the predominant role of additive-by-additive epistatic effects for grain yield heterosis in elite hybrid wheat. This result is, to a certain extent, not surprising, since it mirrors favorable additive-by-additive epistatic interactions which are also fixed in hexaploid wheat lines. This phenomenon is also known as so called fixed heterosis in wheat (Kaeppler 2012). Thus, also wheat lines profit not only from additive but also from epistatic effects (Goldringer et al. 1997). Likewise, under absence of inbreeding depression, which is true for wheat, quantitative genetic theory generally suggests that a great amount of heterosis is due to favorable additive-by-additive epistasis (Lamkey and Edwards 1999). However, the prominent role of epistasis underlying heterosis challenges the establishment of heterotic groups even more as it limits reciprocal recurrent selection based on the exploitation of dominance effects between gene pools (Jiang et al. 2017). It remains an open question if favorable epistatic interactions can be fixed in a pool concept. Moreover, it can be speculated if a strict long-term selection for combining ability instead of line *per se* performance would lead to a change in the genetic architecture of heterosis enriching dominance effects in wheat.

We also studied the genetic architecture of grain yield heterosis in crosses between exotic lines and elite testers. Here, we found a differentiating pattern where dominance effects played a more prominent role accounting for about one quarter of the genotypic variance of grain yield heterosis (Boeven et al. to be submitted). This finding was attributed to less breeding and selection within the exotic panel, likely resulting in a lower accumulation of additive-by-additive epistatic effects and explaining the more prominent role of dominance. It further gives evidence that the genetic architecture of

grain yield heterosis in wheat is greatly depending on the genetic background. However, as hybrid performance in wheat is driven by parental *per se* performance, exotic lines are of no direct use for hybrid wheat breeding and the establishment of heterotic groups. It nevertheless supports the notion that a modification of the genetic architecture of heterosis towards dominance is feasible.

The establishment of heterotic groups are always long-term projects. It is also important to note that heterotic groups are never "finished", but have to be developed and continuously improved. Since the middle of the 2000s, for instance, hybrid varieties in rapeseed have almost completely replaced line varieties in the German market, but clear heterotic groups like in maize are still not established. Since hybrid breeding in wheat is even more complex than in rapeseed, it can be expected that it will take several decades until clear heterotic groups are established, under the assumption that breeding companies will focus on hybrid wheat. The cornerstone based on quantitative genetic theory has been laid, and even if it appears challenging due to the genomic complexity of wheat, interested breeding companies are now asked to take the next step. Due to the aforementioned reasons, the joint establishment of heterotic groups and patterns across breeding companies is highly recommended.

Genetic diversity and heterosis in wheat: Is the upper limit reached already?

The amount of about 10 % midparent heterosis in elite wheat appears to be sufficient to seriously consider the implementation of a hybrid breeding program. Nevertheless, a higher amount of heterosis would make this decision much easier due to an improved long-term competitiveness of hybrid versus line breeding (Longin et al. 2014). One could ask, if the amount of heterosis in wheat will stay constant or might change in the future.

Heterosis is a derived trait depending on the variation of two parameters, the midparent value as well as the performance of the F_1 generation. For instance, the long history of hybrid breeding in maize led to a substantial increase in genetic differentiation between established heterotic pools (van Heerwaarden et al. 2012), while the amount of midparent heterosis was decreasing over the years due to two contrasting events: strong inbreeding depression in early years and a continuously improved line *per se* performance during the past decades (Duvick 1999). A similar phenomenon was observed in early hybrid breeding in rye (Hartwig H. Geiger, pers. commun.). Thus, allogamous crops tend to have very high midparent heterosis in early hybrid breeding stages, but show a decreasing trend with time (Melchinger and Gumber 1998). In contrast, wheat has a self-pollinating nature and is therefore almost not affected by inbreeding depression. Hence, we speculate that midparent heterosis would increase with time when selection would completely focus on combining ability instead of line *per se* performance.

In addtition, heterosis and hybrid breeding should benefit from increased diversity between parental lines, which is in line with quantitative genetic theory under simplifying assumptions not considering epistasis (Falconer and Mackay 1996). This trend was already observed in very early hybrid maize breeding (Hallauer 1999) and became also obvious in a study by Moll et al. (1962) classifying parental populations based on geographic distance (Figure 6a). However, in a similar study exploiting a higher level of parental divergence, Moll et al. (1965) observed a decrease of heterosis in extremely wide crosses with large genetic divergence (Figure 6b). This finding can be explained by the disruption of favorable allelic and non-allelic co-adapted gene complexes resulting in negative dominance and epistatic interactions and leading to outbreeding depression (Lynch 1991; Falconer and Mackay 1996; Mohamed and Pirchner 1998; Aspi 2000). Besides genetic distance, Melchinger and Gumber (1998) also stressed, that heterosis depends on the adaptation of parental lines.

Consequently, this kind of experiment would ideally be performed with adapted lines of a similar yield level that cover the entire range of genetic
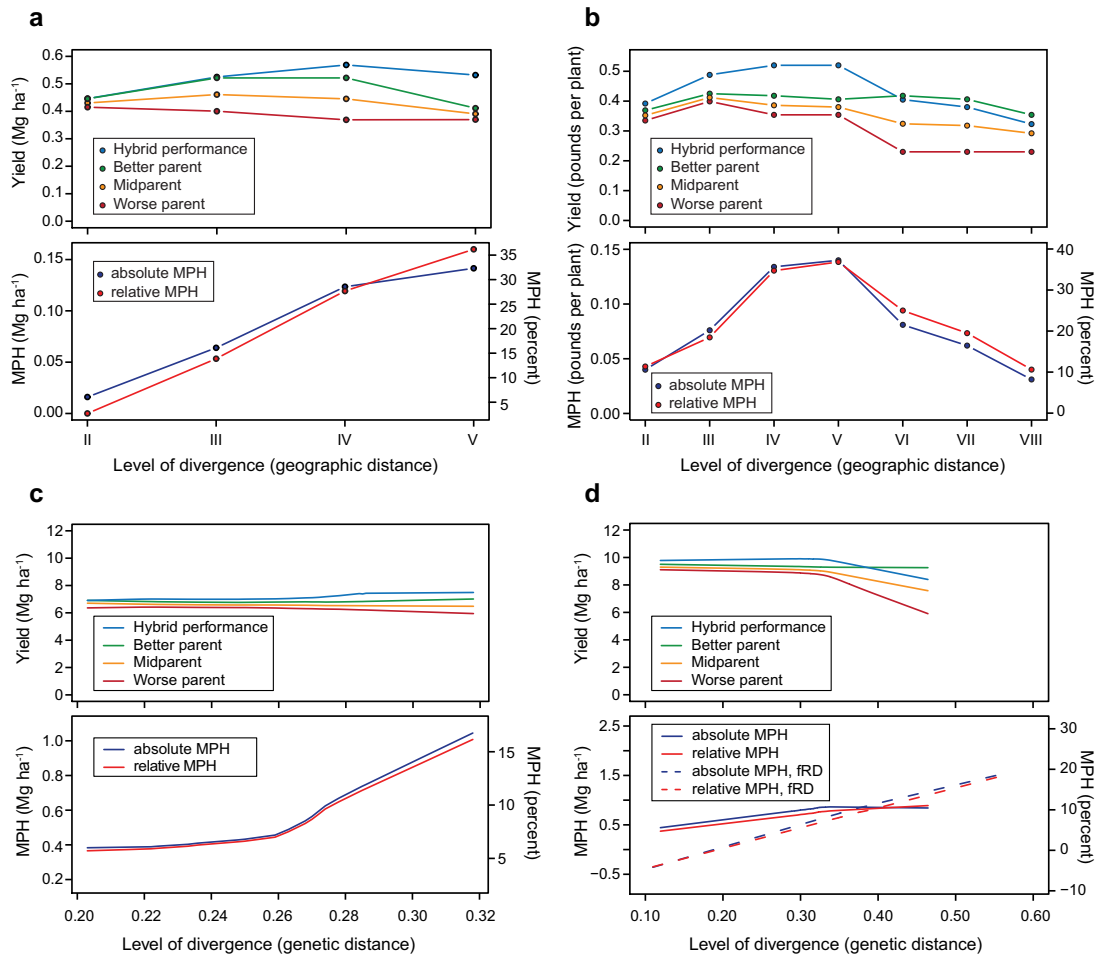
Figure 6: Visualization of different datasets showing the relationship between heterosis and genetic divergence. **a** Maize grain yield and midparent heterosis (MPH) based on data from Moll et al. (1962), levels of divergence (II–V) are according to the original study and are based on geographic distance and ancestral relationships. **b** The similar analysis but showing data from Moll et al. (1965), levels of divergence (II–VIII) are according to the original study and are based on geographic distance and ancestral relationships. **c** Relationship between heterosis and genetic divergence based on data in tropical maize populations from Reif et al. (2003). Genetic distance was estimated based on 85 SSR markers. Relationship was visualized by fitting natural smoothing splines. **d** Relationship between heterosis and genetic divergence in wheat (based on data from Chapter 4, modified). Genetic distance was estimated based on a 15k SNP array. Relationship was visualized by fitting natural smoothing splines.

diversity. This, however, is not possible in practice, as genetic distance will inevitably be confounded with adaptation and *per se* performance and genetically distant lines will be less adapted and lower yielding. In line with this, Moll et al. (1965) observed a decrease in heterosis in the most divergent crosses including populations from Mexico. Interestingly, these Mexican populations were also poor performing in their original environment. In practice, it is impossible to disentangle the confounding effects of genetic divergence, adaption and *per se* performance. Reif et al. (2003) revisited the "Moll concept" analyzing genetic distance and heterosis in tropical maize. In contrast to earlier studies, Reif et al. (2003) investigated genetic distance based on molecular markers. Interestingly, these authors found no evidence for decreasing heterosis under maximized genetic distance and attributed this finding to the similar adaptation of parental lines and the lack of extremely divergent crosses (Figure 6c).

In wheat, a number of studies assessed heterosis and genetic distance but not in extremely diverse material and always in the context of hybrid prediction (Boeven et al. 2016b). As hybrid wheat breeding is still in its infancy and hybrid breeding programs need to be planned in an optimal manner, profound knowledge about the relationship between heterosis and genetic distance in a broad diversity space is of utmost important. We exploited a maximum level of genetic diversity in wheat without leaving the primary gene pool. Interestingly, when not considering crosses between closely related material where heterosis is naturally limited, we observed an average grain yield midparent heterosis of about 10 % in elite crosses as well as in crosses between elite and exotic lines under maximized diversity. We found no evidence for a breakdown of heterosis under maximized genetic distance as postulated by Moll et al. (1965) in maize (Figure 6d). Moreover, enabled by the growing toolbox of genomic approaches, we incorporated a new functional Rogers' distance (fRD) which gives special weight to dominance effects estimated in a genome-wide prediction framework. This distance measure accounts for the genetic architecture of the trait of interest. Heterosis increased linearly with an increasing heterotic genetic distance between par-

ents (Figure 6d, Boeven et al. to be submitted). Thus, genome-wide neutral markers are most likely diluting the true association between heterosis and genetic distance.

Interestingly, average absolute grain yield heterosis in the set of hybrids between elite and exotic lines was consistently on a lower level throughout the entire genetic distance space compared to the set of hybrids between elite and elite lines, also when considering a similar extent of midparent value in both sets. This pointed again to the fact that the extent of heterosis was specific within each hybrid set. We dissected the genetic architecture underlying grain yield heterosis and found more negative dominance and dominance-by-dominance epistatic effects in the set of hybrids between elite and exotic lines compared with the set of elite hybrids. This finding might explain the lower extent of absolute grain yield heterosis within the set of hybrids between elite and exotic lines. The fewer negative dominance and dominance-by-dominance epistatic effects in the set of hybrids between elite and elite lines can be explained by purifying selection in early stages of pedigree line breeding programs, where dominance is still present when loci are not yet fixed.

We might have to refine our proposed *HyBFrame* approach and might have to re-think the organization of germplasm in hybrid breeding programs. Since not all markers contribute to heterosis, maintaining genetic distance based on genome-wide markers between heterotic groups might hamper the introgession of favorable alleles from the other group leading to sub-optimal *per se* as well as hybrid performance. Following from this, it can be hypothesized that heterotic groups should not diverge on a genome-wide level but only for the subset of the genome contributing to (positive) heterosis (Boeven et al. to be submitted). However, heterosis is quantitative and practical breeding needs to consider many traits besides grain yield making this approach very challenging and complex. Thus, future research needs to validate whether this approach can be profitable for hybrid breeding.

Our study revealed two important results for current hybrid wheat breeding programs. First, a substantial increase in genetic distance to the elite

parent is always accompanied with a lower *per se* performance of the exotic parent. This leads to a lower hybrid performance compared with elite crosses. Second, we did not observe any additional gain in heterosis which might be tapped and transferred to the elite pool by fast genomics-assisted adaptation of exotic lines. Thus, a maximized diversity of parental lines in the sense of leaving the elite pool has no direct benefit for initial hybrid breeding phases.

Redesign of the wheat floral architecture: A perfect scenario for genomics-assisted breeding

The self-pollinating nature of wheat is exploited in wheat line breeding programs but it displays a major bottleneck for hybrid wheat seed production as it greatly hampers cross-pollination. From an economic perspective, the additional benefit of grain yield heterosis in commercial hybrid wheat is therefore often eliminated by the higher hybrid seed production costs. Thus, competitive hybrid wheat breeding requires the redesign of the wheat flower, regardless of the used hybrid mechanism (Whitford et al. 2013).

The compatibility of male and female parents in hybrid wheat is generally dependent on different environmental factors such as temperature or wind velocity as well as on the genotype itself. The realized seed set in hybrid crossing blocks is finally the most important factor as it determines hybrid seed costs. Seed set in hybrid crossing blocks is influenced by some main requirements on the female and male side as schematically illustrated in Figure 7. These requirements lead to a number of indirect male and female floral traits influencing the trait seed set itself.

We used a special crossing block design, originally elaborated in hybrid rye breeding, to test 31 male lines for their seed set on two female testers.
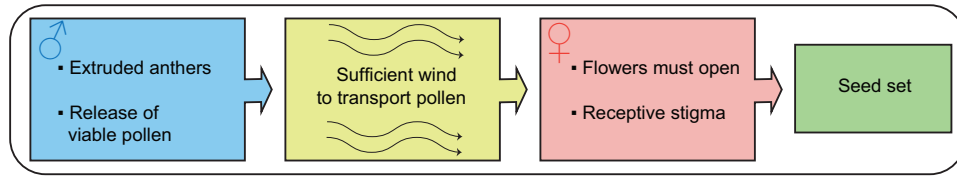
Figure 7: Main parameters influencing seed set in hybrid wheat seed production, adapted from Pickett (1993).

The use of isolation walls allowed us to test this relatively high number of male lines on a relatively small area. However, we observed that field trials for seed set are very challenging. We pre-selected male and female lines for a compatible flowering time based on data for heading date from the previous year. The selected females were expected to flower about two days earlier than the males. However, our study showed that slight deviations in flowering time largely influence hybrid seed set and this nicking effect was underscored by the moderate positive correlation between heading date and seed set (Boeven et al. 2018). Consequently, nicking issues can lead to an underestimation of good male lines in their seed set as it was the case for the variety *Apache* (Boeven et al. 2018).

The photoperiod regulator *Ppd-D1* has a major effect on flowering time control in European winter wheat and generally affects wheat adaptability and yield (Worland 1996; Snape et al. 2001; Langer et al. 2014b) Moreover, we showed in Chapter 3 that also different copy number variants at *Ppd-B1* reflect global adaptation in wheat (Würschum et al. 2015a). Thus, even though we know quite a bit about the genetic control of the highly heritable trait flowering time in wheat, the effect of the individual year can be large and it is highly challenging to select male and female lines optimally fitting in the short hybrid seed production nicking window of wheat. Therefore, effective breeding for improved seed set requires a number of female testers covering broad nicking windows. Moreover, these testers should also optimally represent the respective heterotic groups in the future (Longin et al. 2007; Boeven et al. 2016b).

We were able to include two female testers to assess seed set under field conditions and observed no statistically significant differences in seed set between the two lines (Boeven et al. 2018). However, this was rather a sampling effect of the two female lines and we experienced in other hybrid seed production crossing blocks that the female parents can have great effect on seed set. Female receptivity generally depends on environmental conditions during flowering time as high temperature substantially above 20°C as well as too cool temperatures or high relative humidity can have harmful effects on receptivity and seed set (Imrie 1966; Pickett 1993). Likewise, the length of stigma receptivity is also affected by the environment and site effects, but can vary from a few days up to almost two weeks (De Vries 1971). Knox et al. (1986) suggested to evaluate stigma receptivity as seed set after pollination at different times relative to flower opening, but a precise experiment like this under field conditions would be very challenging. Previous studies also reported about variation for stigma length of about three to four millimeters in wheat, which is about two millimeters less compared to rye or triticale (Pickett 1993; Blouet et al. 1999; Singh et al. 2007). Since larger stigma sizes lead to more exposed stigma branches, selection for stigma size would have positive effects for cross-pollination and seed set (Virmani and Edwards 1983; Blouet et al. 1999). More detailed knowledge about the phenotypic variation and genetic control of female wheat organs like stigma size, stigma exsertion or stigma receptivity would facilitate the more targeted improvement of female hybrid parents in European winter wheat. However, we conclude that the final test for the trait seed set indirectly combines all relevant characteristics of female organs and is for now easier to implement in hybrid breeding programs. Finally, a large number of female lines would need to be screened for their seed set and this phenotypic effort could then be used to establish genomic prediction models for this difficult-to-measure trait.

Moreover, the female spike morphology can affect the cross-pollination in hybrid crossing blocks. It has been discussed that spikes with closely packed spikelets are disadvantageous for cross-pollination since lemma and palea are

appressed to one another (Campbell et al. 1983; Pickett 1993). This phenomenon is sometimes referred to as "clubbing". On the other hand, wheat spikes with spikelets spaced more loosely on the rachis can be beneficial for pollen capture in hybrid seed production since the floret can open with less physical restriction (Kofoid 1991; Pickett 1993). This characteristic is often referred to as "laxness" of a spike. Laxness or compactness of a spike is moderately positively correlated ($r = 0.48^{**}$) with the number of spikelets per spike (SPS) (Würschum et al. 2018a). We performed a genome-wide association study for SPS and identified a major QTL on chromosome 7A explaining almost 17 % of the genotypic variance and weighted ridge-regression best linear unbiased prediction was promising and yielded a cross-validated prediction ability of 0.69 (Boeven et al. 2016b). In line with our results, a number of other studies also found medium- or major-effect QTL for this trait (Ma et al. 2007; Buerstmayr et al. 2011; Echeverry-Solarte et al. 2014; Zhai et al. 2016). Thus, genomics-assisted breeding is feasible and would allow to reduce SPS and indirectly increase laxness in the female pool to improve cross-pollination. However, SPS is also an important yield component in wheat (Tian et al. 2015), where too lax spikes have negative effects on yield *per se* performance and on general combining ability (Borghi et al. 1988; Pickett 1993). Consequently, this asks for an optimal compromise between lax *versus* compact spikes on the female side, which requires further research. In general, the partitioning of different yield components in the male and female pools seems interesting to support hybrid seed production as well as heterosis.

Cross-pollination in wheat is dependent on sufficient viable wind-borne pollen (Figure 7). Wheat pollen is known to be relatively heavy and tests for terminal fall velocity suggest a rather short distance of a few meters between male and female parents (Lelley 1966; D'Souza 1970; De Vries 1971). In Chapter 6, we also tested the effect of the distance between male and female lines on seed set. The locations showed a great effect on the seed set between the more close and distant female plots. On average, the closer female plots had a slightly higher seed set but this was only barely statistically significant

(Boeven et al. 2018). The effect of spatial distance on seed set was not as high as expected. This suggests that wheat pollen can reach longer distances under certain environmental conditions. For instance, Virmani and Edwards (1983) reported that the distance of wheat pollen dispersal could reach up to a few hundred meters in very extreme cases. Based on our results, however, a width of about 3 m for female strips can be accepted for commercial CHA based hybrid seed production to optimize the male to female ratio. However, even wider distances are not recommended due to the declining gradient of seed set in the more distant female plots as demonstrated by Brears and Bingham (1989) and also supported by the findings of Khan et al. (1973).

Our data supported the fact that not all locations are equally suitable for efficient hybrid seed production. In particular, wind is favored for wheat pollen flow and a careful selection of potential hybrid seed production locations based on multiannual climatic conditions and test seed production is recommended to increase efficiency. Moreover, even the use of tractor-mounted fans or helicopters as wind support for hybrid wheat seed production have been discussed in the literature (Virmani and Edwards 1983; Koekemoer et al. 2011). These approaches sound interesting but are definitely unrealistic, as they would further boost the already high hybrid wheat seed production costs. Consequently, genotypes with favorable male floral traits giving enough seed set under natural wind pollination are required.

The large genotpyic variation and high heritability for seed set suggest that this trait can be improved by recurrent phenotypic selection. However, conducting male screenings for seed set under field conditions is cumbersome and definitely not feasible in early stages of breeding schemes for male pool improvement (Boeven et al. 2018). Nonetheless, seed set is the most important trait hybrid wheat breeders should use to establish superior male lines. Hence, this phenotypically difficult-to-measure trait displays an ideal situation for genomics-assisted breeding approaches. This would require a much larger diversity panel of a few hundred potential male lines and phenotypic effort to conduct a genome-wide association study for seed set and to calibrate

and test genomic prediction models. However, such a multi-environmental large-scale experiment for seed set would be really challenging due to the already mentioned nicking issues and resulting choice of female testers. Alternatively, the identification of easier-to-screen male floral traits associated with seed set opens up the possibility of indirect selection in early breeding stages of male pool development. Then, the cumbersome assessment of seed set could be conducted only in later stages with fewer candidates to be tested.

During the first big wave of hybrid wheat breeding between the 1960s and late 1980s, many studies focused on flowering and floral traits in wheat. The trait anther extrusion showed a wide phenotypic variation and appeared quite promising for breeding for cross-pollination capability in wheat (Joppa et al. 1968; D'Souza 1970; Milhonic and Jost 1970; De Vries 1971, 1973; Johnson and Patterson 1973; Barnett and Patterson 1974; Sage and Isturiz 1974; Atashi-Rang and Lucken 1978). Langer et al. (2014b) evaluated two approaches to assess anther extrusion: (i) anther extrusion count (AEX) based on the remaining anthers in the lateral florets; and (ii) visual anther extrusion (VAEX) based on a 1–9 scale (Figure 8a). Both traits show large genotypic variation, have high heritability estimates and possess a high phenotypic trait correlation of about 0.7 (Langer et al. 2014b; Boeven et al. 2016a; Boeven et al. 2018). The scoring of VAEX is mainly affected by weather conditions such as wind or rainfall, by daytime and by the personal effect of the breeder during the scoring. VAEX was therefore considered less accurate than AEX, which is less affected by weather conditions and more objective due to the counting procedure. On the other hand, scoring AEX takes more time per genotype, about 10 minutes for four spikes, but the possibility to deep-freeze spikes and assess AEX after peak work periods makes it more flexible than VAEX. Interestingly, VAEX had a much higher association with seed set than AEX (Boeven et al. 2018). This might be explained by the effect of the "personal impression". Very likely, further floral characteristics like filament length or size and the color of anthers are unconsciously taken into account during the scoring of visual anther extrusion. For instance, filament length itself is also positively correlated with seed set (Boeven et al. 2018) and Beri

Figure 8: Anther extrusion of wheat lines. **a** Low, medium and high level of anther extrusion in wheat, from left to right. **b** Spike during flowering of the well-known super male *Piko*.

and Anand (1971) found an positive association between filament length and pollen grain shed outside the floret. Moreover, AEX is only considering the two lateral florets and the effect of the central florets is not considered, which might also explain the lower correlation with seed set. Consequently, VAEX appears more promising and should be treated with higher priority when following the concept for male pool development suggested by Boeven et al. (2016a).

Male lines possessing extraordinary high pollination capability probably combine a large number of favorable male floral traits. During the last five years, the State Plant Breeding Institute screened about 5,000 wheat lines for VAEX plus addtitional indirect male floral traits. These lines included old and new released varieties as well as large collections from international gene banks. A few lines showed comparable or even higher VAEX than the already well-known males such as *Apache* or *Piko* (Figure 8b). This illustrates that lines with favorable male floral characteristics are rare and

that breeding schemes for male pool development are required (Boeven et al. 2016a). It also suggests that high VAEX is required but not sufficient for good outcrossing capability. For instance, the variety *Piko* had the highest value for seed set but not for all of the assessed indirect male floral traits including VAEX (Boeven et al. 2018). We conclude that traits like pollen viability, pollen longevity or the time course of pollen release, and flowering duration are of importance and require further research to unravel the "Piko secret". One part of this secret might be that *Piko* is able to release pollen over a longer period of time compared to other high performing VAEX lines. However, this hypothesis needs to be tackled in future experiments taking a closer look at flowering duration in wheat.

We demonstrated the importance of indirect male floral traits to breed for outcrossing capability in wheat. Indeed, indirect male floral traits are easier to assess than the trait seed set. However, the assessment of visual anther extrusion or pollen mass are also very time-consuming, depending on climatic conditions and daytime, and hence, are impractical for early breeding stages of male pool development with many candidates to be tested (Boeven et al. 2016a). Thus, genomics-assisted breeding approaches could greatly accelerate breeding schemes for improving male floral characteristics. In Chapter 5, we employed a panel of 209 diverse winter wheat lines and performed a genome-wide association (GWA) study to dissect the genetic architecture of relevant indirect male floral traits. The genome-wide scan revealed a complex genetic architecture underlying anther extrusion and pollen mass. A complex genetic architecture underlying anther extrusion was also found in other winter and spring wheat collections (Muqaddasi et al. 2016, 2017a, c). We identified the dwarfing gene *Rht-D1* as the only major QTL and our results provided strong evidence that *Rht-D1b* (mutant type, short phenotype) leads to negative effects for male floral traits. Thus, it is reasonable to consider *Rht-D1* in breeding schemes for male pool development. Future linkage mapping studies in bi-parental populations might detect additional QTL for male floral traits. Up to now, however, the complex genetic architecture

underlying male floral traits limits the potential of classical marker-assisted selection.

Therefore, we tested the potential of genomic prediction for male floral traits and found moderate to high prediction abilities for anther extrusion, visual anther extrusion and pollen mass. The great potential of genomic prediction for anther extrusion was also confirmed in spring wheat (Muqaddasi et al. 2017c). Collectively, our results suggest that genomic prediction is a powerful tool for the pre-selection of promising male lines. However, breeders must first invest phenotypic effort in screening large populations for difficult-to-measure male floral traits in multi-environmental field trials to establish robust training sets for genomic prediction models. Then, it displays a perfect scenario where genomics-assisted breeding can effectively support recurrent selection breeding schemes for male pool development as suggested by Boeven et al. (2016a). One should keep in mind, however, that any breeding scheme for male pool development should have a high focus on general combining ability and must include final field-tests for the ultimate target trait seed set.

Plant height is a crucial trait in wheat breeding as it has large effects on the harvest index, lodging and grain yield (Hedden 2003). In hybrid wheat breeding, relative plant height of male and female components is a crucial parameter for an efficient hybrid seed production. Due to the pollen characteristics of wheat, male lines should be slightly taller than female lines to support pollen shed and reception of pollen on the female flower (Virmani and Edwards 1983; Pickett 1993). Consequently, the positive effect of the wild-type *Rht-D1a* (tall phenotype) on male floral traits is beneficial and fits in the framework for hybrid wheat seed production. Wheat plant height in Northern European countries including Germany, France and the UK is controlled by *Rht-D1* (Worland and Snape 2001; Le Couviour et al. 2011; Würschum et al. 2015b; Würschum et al. 2018b). Thus, the mutant-type *Rht-D1b* could be fixed in the female pool to achieve a desired reduced height. However, wheat hybrids show positive heterosis of about 10 % for plant

height (Longin et al. 2013; Boeven et al. to be submitted). Consequently, the use of reduced height loci in the male and female parents greatly depends on the desired and accepted stature of the hybrids in the respective wheat markets. Würschum et al. (2017a) reported the strong height-reducing effect of *Rht24* and its relevance in global wheat breeding. Interestingly, *Rht24* appears to have no negative effects on male floral traits (Würschum et al. 2018b). This suggests that good outcrossing capability can be combined with short height on the male side, too. A prominent example is the variety *Apache* (Boeven et al. 2016a; Boeven et al. 2018). In addition, the use of *Rht24* is very interesting in a CMS hybrid system, as female maintainer lines need to possess a sufficient cross-pollination ability, too. These findings greatly expand the *Rht* portfolio for hybrid wheat breeders and allow the pre-selection of parental lines with the aid of molecular markers for major *Rht* loci.

To tackle another open question, we conducted a small experiment regarding the longevity of wheat pollen under field conditions in the 2015/16 growing season. To this end, we used two female testers and four released wheat varieties including *Piko*, *Hermann*, *Henrik* and *Naturastar* as pollen parent. We used two female spikes for each cross and spikes were hand-emasculated following standard protocols. Emasculated spikes were directly covered with paper bags. As a control, we performed all crosses as it is routine in wheat breeding. Afterwards, we collected pollen of five ears per male and stored the pollen in petri dishes under field conditions. The following crosses were then performed after 3, 7, and 24 hours, respectively, using a paintbrush to apply the pollen. All spikes were harvested and we counted the seed set. While the standard crossing procedure led to seed set in all cases, we did not find any seed set for crosses where the pollen was stored for 3 or 7 hours. Interestingly, we observed up to two kernels for some crosses after storing the pollen for 24 hours. Genotyping for the polymorphic *Rht-1* loci revealed, however, that all kernels harvested from the 24 hour version resulted from unintended selfing. Consequently, our results suggest that wheat pollen generally has a rather short longevity. This is in line with previous

studies from the 1960s and 1970s which reported a maximum longevity of 30 minutes under field conditions and up to 3 hours under optimal temperature and humidity (for review see Pickett 1993).

Commercial hybrid seed production generally requires logistical efforts in planning of the crossing-blocks. To produce GCA trial seeds in breeding programs, however, it would be sometimes advantageous to have more flexibility regarding the seed production. We did some pre-tests for two alternative methods as illustrated in Figure 9. We used the same two females testers and isolation walls as described by Boeven et al. (2018). A portable vacuum cleaner was used to collect pollen from four flowering male lines and each vacuum cleaner bag was then opened and shaken above the emasculated female testers. The second approach was a bit more simplistic. Male spikes with extruded anthers were cut and shaken above the emasculated female testers. Both approaches led to substantial seed set in a similar range as natural wind pollination in crossing-blocks (Boeven et al. 2018). Interestingly, the vacuum cleaner method tended to have lower seed set than the shaking method. However, our results should be taken with caution since it was just a pre-test and a more thorough experiment is required. Nonetheless, we could already demonstrate that an improvement of hybrid seed production on a small-scale sufficient to produce novel hybrid combinations for evaluation in breeding programs seems feasible.

Secondary applications of the hybrid technology in wheat

The hybrid technology itself offers some secondary applications besides hybrid breeding in its actual sense. For instance, van Ginkel and Ortiz (2017) proposed the hybrid-enabled line profiling (HELP) approach, which aims to identify best parents and cross combinations using the hybrid technology and inbred lines derived from the 3–5 % top performing F_1s. This strategy
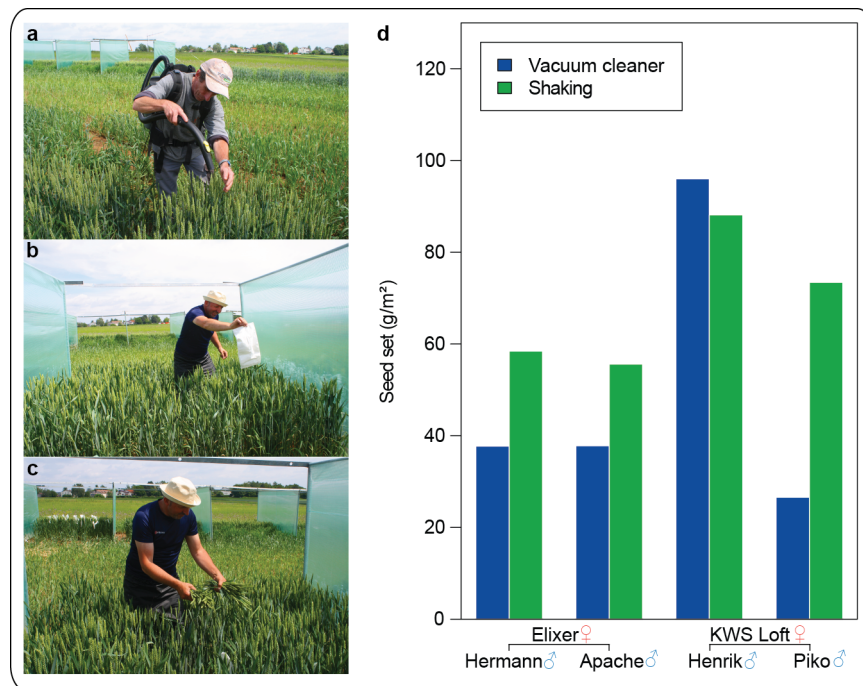
Figure 9: Alternative methods for pollination in hybrid wheat seed production.
a, **b** Pollen collection with a portable vacuum cleaner and pollination. **c** Male spikes
with extruded anthers are cut and directly shaken above emasculated female plots. **d**
Results of the two methods.

transfers knowledge from hybrid to line breeding and was already applied in
earlier wheat breeding programs where line and hybrid breeding were per-
formed in parallel. In that case, when it is applied as a spin-off strategy and
the data is gathered anyway, the proposed method seems reasonable and
breeders would probably intuitively perform HELP or modified versions of
this approach. However, it is questionable if that approach pays off due to
increased costs and longer breeding cycles when the hybrid technology is not
applied on a routine basis.

In addition, the hybrid technology offers chances to leverage the exploita-
tion of untapped genetic diversity of gene bank accessions for grain yield
(Longin and Reif 2014). It is highly challenging to screen genetic resources
for their yield potential due to lodging issues or lack of major resistance or
adaptation genes. Longin and Reif (2014) proposed a theoretical concept to

unmask the yield potential of genetic resources by crossing them with elite testers based on the CHA hybrid technology. The elite testers must be chosen in such a way that they are able to eliminate masking effects for example by carrying dwarfing genes. The true breeding value of the genetic resources for grain yield is then evaluated in the F_1 hybrid background and delivers an estimate for their combining ability in hybrid breeding as well as a starting point to mine for novel grain yield alleles in wheat line breeding programs.

We applied the framework proposed by Longin and Reif (2014) and crossed a number of "Historic" and "Exotic" lines with elite testers (Boeven et al. to be submitted). For example, one "Historic" hybrid derived from a cross between a male tester and *Maris Kinsman*, a British variety from the 1970s, yielded 10.72 Mg ha^{-1} and was among the top 15 performing elite hybrids of the whole study (Figure 10) (Boeven et al. to be submitted). The "Historic" hybrids significantly ($P < 0.001$) outperformed their parents with an average yield advantage of 0.95 Mg ha^{-1}, and seven "Historic" hybrids showed a higher yield than the best commercial check variety.

Grouping the "Historic" lines according to their year of release revealed a clear temporal trend of increased yield line *per se* performance over the last six decades ($r = 0.79, P < 0.001$) (Figure 10). The corresponding hybrids showed a similar but weaker trend ($r = 0.62, P < 0.001$) and performed on a higher level. Focusing only on the period between 1960 until 1989 revealed a significant time trend for the "Historic" lines ($r = 0.43, P < 0.01$) but not for their hybrids ($r = 0.20, P = 0.17$). There was no significant difference in yield between elite checks and "Historic" hybrids generated with comparably recent lines released between 1990 and 2016, while the line *per se* performance of these lines was significantly ($P < 0.001$) outperformed by the elite checks. In general, the observed temporal trend is typical for breeding germplasm and reflects the progress in line breeding during the past decades (Laidig et al. 2014; Losert et al. 2017). Our results proved that the theoretical concept suggested by Longin and Reif (2014) is practicable and of interest for wheat breeding programs. However, we only focused on grain yield and
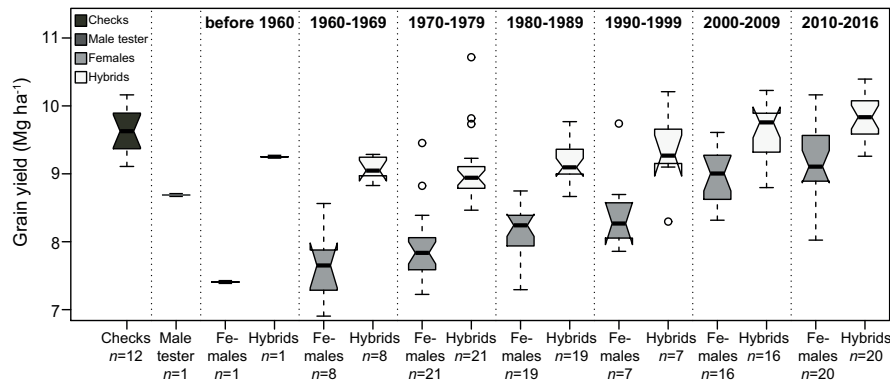
Figure 10: Temporal trend in grain yield performance exploiting "Historic" varieties applying the approach suggested by Longin and Reif (2014). Boxplots showing grain yield of commercial check varieties and "Historic" lines and their hybrids, as well as their male elite tester. Lines are ordered according to their year of release. Checks are *Colonia, Elixer, Hybred, Hystar, JB Asano, Julius, KWS Loft, KWS Smart, LG Alpha, RGT Reform, Rumor* and *Tobak*. Based on data from Chapter 4.

the final validation of this concept requires more than competitive F_1 grain yield performance. Moreover, we conclude that the choice of optimal testers for this approach warrants further research.

Future challenges and an outlook for hybrid wheat

The present findings show that hybrid wheat holds great potential to boost global productivity of wheat. Previous research as well as our own studies have shown that the production of hybrid wheat seed is currently the major challenge and bottleneck. The success of hybrid wheat will rely on the availability of a cost-efficient system for hybrid seed production including a satisfactory hybrid mechanism and an improved outcrossing capability. Most of the important floral traits show large genotypic variances and high heritabilities. We outlined genomics-assisted breeding strategies to support the

redesign of the wheat flower. Nevertheless, this is not an easy task and requires long-term commitment of the public and private sector. Since hybrid wheat is still a niche product and not established in the market, detailed studies and robust numbers about hybrid wheat economics are lacking. A number of authors suggested that a commercial heterosis of about 1 Mg ha^{-1} is required to counterbalance higher seed production costs (Schachschneider 1997; Weissmann and Weissmann 2002; Koekemoer et al. 2011). Recent hybrid wheat seed costs are about 2.5 times higher compared to certified seed of line varieties in Germany. Taking a reduced sowing rate of 220 kernels per square meter for hybrids into account, we estimated a minimum required hybrid yield advantage of about 4 % to counterbalance only the higher seed costs and to reach a break-even. Again, this highlights the great need for a more cost-effective hybrid seed production system in wheat.

Hybrid breeding is more complex compared to line breeding in wheat. Besides the aforementioned floral constraints of wheat, breeders need to reorganize their germplasm in a completely different way, inverting the present line breeders' way of thinking. Hybrid breeding generally asks for more resources compared to line breeding. A possible CMS system requires backcrosses with the respective cytoplasm and restorer genes need to be introgressed in promising male lines. It is very likely that a minimum of two to three restorer genes are required in wheat to achieve full restoration, which inflates the size of backcross populations. In addition, efficient tools for hybrid prediction are required as the number of possible single-cross hybrids is a quadratic function of the number of parental lines (Zhao et al. 2015; Boeven et al. 2016c). Both examples underscore that effective hybrid wheat breeding asks for more resources, for example using molecular markers to aid backcrosses, to predict untested hybrids, and finally to increase the response to selection (Longin et al. 2015; Zhao et al. 2015; Liu et al. 2016; Würschum et al. 2017b). Although there is a lot of progress in genotyping technology and marker costs were decreasing during the last years (Rasheed et al. 2017), it still remains questionable if smaller breeding companies will be able to take

that effort. The fate of these smaller companies will therefore highly depend on the competitiveness of line versus hybrid varieties in the wheat market.

Generally, before launching a hybrid wheat breeding program, breeding companies must carefully consider a number of parameters. The final decision about the usefulness of hybrid wheat is then driven by its genetic potential as well as by general economic and political conditions. Longin et al. (2014) compared the expected selection gain and long term competitiveness of hybrid versus line breeding under simplifying assumptions and only considering grain yield. They found that hybrids would be superior in the early years due to an initial advantage of heterosis, but line breeding would catch up eventually due to a larger annual selection gain, which is strongly influencing the competitiveness of hybrid versus line breeding (Longin et al. 2012; Longin et al. 2013; Longin et al. 2014). This finding illustrates that line breeding programs and line varieties will likely be able to compete with hybrids, especially because a large number of traits other than grain yield need to be considered. Thus, economic and political factors mainly regarding the problem of farm saved seeds might be decisive reasons and a major driving force for wheat breeding companies to shift from line to hybrid breeding.

Nevertheless, it does not need to be a black or white decision. It seems realistic that both hybrids and lines could co-exist in the future wheat market because of their different agronomic characteristics. Due to the heterozygous state of hybrids, it is assumed that they can better buffer environmental effects and are more yield stable compared to inbred lines, which is also of interest in the context of climate change. Mühleisen et al. (2014) observed a higher yield stability in hybrid wheat compared to lines but as indicated by recent results from Liu et al. (2017) differences between lines and hybrids are not always statistically significant. Further research is required to reveal the agronomic performance of hybrids and lines under different environmental conditions. However, it is likely that hybrid wheat possesses a beneficial performance in more marginal environments while line varieties might maintain the first choice for wheat production under optimal conditions. Moreover, it

is likely that line varieties are advantageous for the production of wheat with very high bread-making quality. In contrast, hybrids are more interesting for breeding companies in countries where high amounts of farm saved seeds or the collection of royalties are problematic. These examples underscore a possible co-existence of lines and hybrids in the wheat market.

To conclude, the exploitation of heterosis in wheat is possible and promises many advantages. A number of issues still need to be solved but the basis for hybrid wheat is laid. The near future will show if the theoretical potential of hybrid wheat could lead to superior varieties boosting global wheat productivity, and dealing with increasing food demand and climate change.

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8 Summary

Wheat is one of the top three global staple crops, possesses the largest global cultivation area, and plays a key role for the world's future food security. However, its projected yield increase is insufficient to meet the future food and feed demand of an ever-growing world population. Consequently, the rate of breeding progress and productivity of wheat must be increased. Unfortunately, current wheat line breeding has a low return on investment mainly due to high levels of farm saved seeds, which makes wheat less attractive for the plant breeding industry and leads to lower investments and progress compared to other crops where the hybrid technology is established. Hybrid breeding is a worldwide success story in many crops but is not yet established in wheat. Hybrid wheat promises increased yield gain due to the exploitation of heterosis, higher yield stability and stabilized return on investments for breeding companies which warrants further investment and breeding progress in this important stable crop.

The self-pollinating nature of wheat is a major bottleneck for hybrid seed production and efficient hybrid wheat breeding requires the redesign of the wheat floral architecture to enhance cross-pollination. Furthermore, the long-term success of hybrid wheat is crucially dependent on the establishment of heterotic groups, on the identification of a high yielding heterotic pattern, and finally, on the realized amount of heterosis and hybrid performance. Therefore, the main objectives of my thesis research were to: (i) analyze the genetic diversity and adaptation in a global winter wheat collection and

evaluate how diversity trends could be used to support the development of heterotic groups in wheat; (ii) assess the relationship between heterosis and genetic distance under maximized diversity and evaluate the usefulness of exotic germplasm for hybrid wheat breeding; (iii) dissect the genetic architecture underlying male floral traits in wheat to enable genomics-assisted breeding approaches and investigate the trait seed set which is most crucial for an efficient hybrid seed production.

The analyses of genetic diversity in a large worldwide panel of 1,110 winter wheat varieties released during the past decades showed no major population structure but revealed genetically distinct subgroups. Most of the global diversity trends could be explained by breeding history and were associated with geographical origin and long-term domestication. We found that the frequency of the copy number variants at the *Photoperiod-B1* (*Ppd-B1*) and the *Vernalization-A1* (*Vrn-A1*) loci reflect wheat adaptation to the environmental conditions of the different regions of origin. Thus, adaptation issues add an additional layer of complexity and hamper the direct introgression of genetic diversity to support the genetic divergence between heterotic pools. Based on all these analyses, we proposed *HyBFrame*, a unified framework illustrating how global wheat genetic diversity can be used to support and accelerate reciprocal recurrent selection for the development of genetically distinct heterotic groups in wheat.

In a second experiment, we produced 2,046 wheat hybrids by crossing elite with elite lines as well as elite with exotic lines and performed multi-environmental field trials. Interestingly, we found an average midparent heterosis of about 10 % in elite crosses as well as in exotic crosses and observed no evidence for a breakdown of heterosis under maximized genetic distance among the hybrid parents. Genetic distance based on genome-wide molecular markers revealed only a very weak association with midparent heterosis for grain yield. Here, we elaborated a functional Rogers' distance giving weight to heterosis loci and observed a strong positive association between heterosis and this novel distance measure. Hence, considering the genetic architecture

of heterosis revealed a more accurate picture of the relationship between heterosis and genetic distance. In addition, the genetic architecture of heterosis in wheat is crucially dependent on the genetic background. We found that a higher number of negative dominance and dominance-by-dominance epistatic effects can reduce the level of absolute heterosis in wide crosses between exotic lines and elite testers. Moreover, hybrid performance in wheat is mainly driven by parental *per se* performance. Thus, elite lines are favorable for hybrid breeding and should be employed as the starting material for heterotic grouping.

Hybrid seed production is the major bottleneck for hybrid wheat breeding and explains the low market share of hybrid wheat varieties. Seed set on the female plants in crossing blocks is the most crucial trait for hybrid seed production in wheat. We tested 31 male lines and evaluated the hybrid seed set on two female tester lines in crossing blocks. Seed set showed a large genotypic variation and a high heritability suggesting that recurrent selection for increased seed set is feasible. The major problem is the synchronized flowering between male and female lines, making the evaluation of seed set in large panels very complex and difficult. Hence, indirect male floral traits with high correlation to the trait seed set would be promising to breed for improved hybrid seed production. We found a strong association between seed set and visual anther extrusion, underscoring that indirect male floral traits have a high potential for preliminary male screenings. We also dissected the genetic architecture underlying promising male floral traits and assessed the potential of genomics-assisted approaches for their improvement. We employed a panel of 209 diverse wheat lines and found a complex genetic architecture underlying all male floral traits. The *Reduced height* gene *Rht-D1* was identified as the only major QTL, for which the commonly used height-reducing allele showed negative effects on male floral traits. This genetic architecture with many moderate- or small-effect QTL limits classical marker-assisted selection. In contrast, genomic prediction yielded moderate to high prediction abilities for anther extrusion. Finally, we proposed a breeding scheme to in-

crease cross-pollination in wheat based on a combination of phenotypic and genomics-assisted selection.

Taken together, hybrid breeding in wheat is a very promising approach and the next years will show if all of the current issues can be solved. This thesis research contributed to breeding strategies for hybrid wheat breeding and to the general understanding of heterosis in crops.

9 Zusammenfassung

Weizen ist eines der drei weltweit wichtigsten Grundnahrungsmittel, besitzt die größte globale Anbaufläche und spielt eine Schlüsselrolle für die zukünftige Ernährungssicherheit der Welt. Die vorhergesagten Ertragssteigerungen sind jedoch zu gering um der zukünftigen Nachfrage nach Nahrungs- und Futtermitteln einer stetig wachsenden Weltbevölkerung gerecht zu werden. Folglich müssen der Züchtungsfortschritt und die Produktivität des Weizenanbaus gesteigert werden. Die aktuell im Weizen angewandte Linienzüchtung hat jedoch durch den hohen Anteil des Nachbausaatgutes eine geringe Rentabilität, was die Attraktivität des Weizens für Züchtungsunternehmen mindert und zu geringeren Investitionen und Fortschritt, verglichen mit Hybridkulturarten, führt. Die Hybridzüchtung ist eine weltweite Erfolgsgeschichte, aber im Weizen nicht etabliert. Hybridweizen verspricht erhöhte Ertragszuwächse durch die Ausnutzung der Heterosis, eine höhere Ertragsstabilität und eine stabile Rendite für Züchtungsunternehmen, was weitere Investitionen und Züchtungsfortschritt für dieses wichtige Grundnahrungsmittel gewährleistet.

Die selbstbefruchtende Natur des Weizens ist eine entscheidende Einschränkung für die Hybridsaatgutproduktion und eine effiziente Hybridweizenzüchtung ist auf die Umgestaltung der Weizen-Blühbiologie zur Verbesserung der Fremdbefruchtung angewiesen. Darüber hinaus ist der langfristige Erfolg des Hybridweizens entscheidend von der Etablierung heterotischer Gruppen, der Identifikation eines ertragreichen heterotischen Musters, sowie der letztendlich realisierten Heterosis und Hybridleistung abhängig. Die

Hauptziele meiner Dissertation waren daher: (i) die genetische Diversität und Adaptation in einer globalen Winterweizen Kollektion zu analysieren und zu evaluieren wie Diversitätstrends für die Etablierung von heterotischen Gruppen im Weizen unterstützend genutzt werden können; (ii) das Verhältnis zwischen Heterosis und genetischer Distanz, unter der Berücksichtigung maximaler Diversität, zu beurteilen und den Nutzen exotischen Zuchtmaterials für die Hybridweizenzüchtung einzuschätzen; (iii) die genetische Architektur männlicher Blühmerkmale im Weizen zu untersuchen, um genomisch unterstützte Zuchtverfahren zu ermöglichen und darüber hinaus das Merkmal Saatgutansatz zu untersuchen, welches die entscheidende Rolle für eine effiziente Hybridsaatgutproduktion spielt.

Die Analyse der genetischen Diversität einer weltweiten Weizenkollektion mit 1,110 Sorten, zugelassen in den letzten Jahrzehnten, zeigte keine Hauptpopulationsstruktur, aber genetisch unterscheidbare Untergruppen. Viele dieser globalen Diversitätstrends konnten durch die Züchtungsgeschichte erklärt werden und waren mit der geographischen Herkunft und der langfristigen Domestikation assoziiert. Wir konnten zeigen, dass die Kopienzahlvariation an den *Photoperiode-B1* (*Ppd-B1*) und *Vernalisation-A1* (*Vrn-A1*) Loci die Anpassung des Weizens an verschiedene Umweltbedingungen und die unterschiedlichen Herkunftsregionen widerspiegelt. Adaptationsprobleme führen daher zu einer erhöhten Komplexität und verhindern die direkte Einkreuzung genetischer Diversität, um die genetische Divergenz zwischen heterotischen Gruppen zu unterstützen. Basierend auf diesen Ergebnissen wurde von uns der *HyBFrame* Ansatz vorgeschlagen, ein umfassender Rahmen der beschreibt, wie die globale Diversität des Weizens genutzt werden kann, um die reziproke rekurrente Selektion zur Entwicklung genetisch unterschiedlicher heterotischer Gruppen im Weizen beschleunigend zu unterstützen.

In einem zweiten Versuch wurden von uns insgesamt 2,046 Weizenhybriden von Kreuzungen zwischen Elite-Linien und Kreuzungen zwischen Elite- und exotischen Linien produziert und anschließend in Feldversuchen in mehreren Umwelten getestet. Interessanterweise fanden wir eine durch-

schnittliche Heterosis zum Elternmittel von ungefähr 10 % in den Elitekreuzungen sowie in den exotischen Kreuzungen und beobachteten keinen Hinweis für einen Rückgang der Heterosis unter maximaler genetischer Distanz zwischen den Eltern. Die genetische Distanz basierend auf genomweiten molekularen Markern zeigte nur eine sehr schwache Assoziation mit der Heterosis zum Elternmittel. Hier wurde von uns eine funktionale Rogers' Distanz entwickelt, welche Heterosis Loci besonders wichtet und eine stark positive Korrelation zwischen Heterosis und dieser neuen Distanzmethode beobachten ließ. Die Berücksichtigung der genetischen Architektur der Heterosis führte daher zu einem deutlich klareren Bild zwischen Heterosis und genetischer Distanz. Zudem ist die genetische Architektur der Heterosis im Weizen entscheidend vom genetischen Hintergrund des Materials abhängig. Wir beobachteten, dass ein höheres Ausmaß an negativen Dominanz- und epistatischen Dominanz × Dominanz-Interaktionseffekten das Niveau der absoluten Heterosis in weiten Kreuzungen zwischen exotischen Linien und Elitetestern reduzieren kann. Darüber hinaus wird die Hybridleistung im Weizen maßgeblich von der elterlichen Eigenleistung bestimmt. Folglich sind Elitelinien für die Hybridzüchtung zu favorisieren und sollten als Startmaterial für die heterotische Gruppierung genutzt werden.

Die Hybridsaatgutproduktion ist die entscheidende Einschränkung in der Hybridweizenzüchtung und erklärt den geringen Marktanteil von Hybridsorten. Der Saatgutansatz auf den weiblichen Pflanzen in Kreuzungsblöcken ist das entscheidende Merkmal. Wir haben 31 Väterlinien für ihren Saatgutansatz auf zwei Müttertestern in Kreuzungsblöcken untersucht. Das Merkmal Saatgutansatz zeigte eine große genotypische Variation und eine hohe Heritabilität, was den Einsatz der rekurrenten Selektion zur Verbesserung dieses Merkmals ermöglicht. Das Hauptproblem ist die Synchronisation der Blüte zwischen männlichen und weiblichen Linien, was die Evaluierung des Saatgutansatzes in großen Kollektionen sehr komplex und schwierig gestaltet. Daher wären indirekte männliche Blühmerkmale mit einer hohen Korrelation zum Saatgutansatz sehr vielversprechend, um für eine verbesserte Hybridsaatgutproduktion zu züchten. Wir fanden eine starke Assoziation zwischen

Saatgutansatz und visueller Antherenextrusion, was das hohe Potenzial indirekter männlicher Blühmerkmale für erste Bonituren zur Vatereignung unterstreicht. Zudem haben wir die genetische Architektur vielversprechender männlicher Blühmerkmale untersucht und das Potenzial von genomisch unterstützten Ansätzen zur Verbesserung dieser Merkmale evaluiert. In einer Kollektion von 209 diversen Weizenlinien fanden wir eine komplexe genetische Architektur für alle männlichen Blühmerkmale. Das Kurzstrohgen *Rht-D1* wurde als einziges Major-Gen (QTL) identifiziert und das häufig verwendete Kurzstroh-Allel zeigte negative Effekte für männliche Blühmerkmale. Diese genetische Architektur, mit vielen mäßig bis wenig wirkenden QTL, limitiert die klassische markergestützte Selektion. Im Gegensatz dazu zeigte die genomische Vorhersage mäßige bis hohe Vorhersagefähigkeiten für Antherenextrusion. Schließlich wurde von uns ein erstes Züchtungsschema, basierend auf phänotypischer und genomisch-unterstützter Selektion, zur Verbesserung der Fremdbefruchtung des Weizens vorgeschlagen.

Zusammenfassend ist der Hybridweizen sehr vielversprechend und die nächsten Jahre werden zeigen, ob alle aktuellen Probleme gelöst werden können. Die vorliegende Arbeit liefert einen Beitrag zu Züchtungsstrategien für die Hybridweizenzüchtung und zum allgemeinen Verständnis von Heterosis in Kulturpflanzen.

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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.

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Braunschweig, im Februar 2018

Philipp Boeven