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Heterosis and growth in a developing maize plant

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Abstract

Heterosis has been extensively studied for nearly a century, yet genetic and biological mechanisms underlying the phenomenon remain elusive. This study extends our understanding of heterosis in maize (*Zea mays* L) by examining heterosis in the language of growth and development–growth rate, duration of the linear phase, and final trait value. By utilizing a set of phenologically uniform genetic materials the confounding effect of differences in development were eliminated. Using two parental inbred lines and the F1 hybrid, heterosis was examined using logistic growth curves for a series of vegetative and reproductive traits across stages of development (V-stages). Vegetative and reproductive traits examined in this study displayed the classic sigmoidal growth curve. More importantly these curves were occurring at the same developmental time points in the F1 and parental inbred lines. In short, heterosis confers an advantage to the F1 that occurs early in development in terms of growth rate and while the growth rate of the F1 and parental lines eventually coalesce, that initial advantage due to heterosis is maintained throughout the lifecycle of the plant resulting in a larger final trait value

Keywords: heterosis, growth, development

Abbreviations: QTL: quantitative trait locus, PPFD: photosynthetic photon flux density, DAE: days after emergence, PC: principal component

Introduction

Over 100 years have elapsed since Shull and East published the results of their inbreeding and outcrossing experiments on maize (*Zea mays* L), and the term «heterosis» was coined to describe the superiority of the F1 over its parents (Shull, 1908; 1909; 1952; East, 1908). Heterosis or hybrid vigor is an observable phenomenon that can be described as an increase in the vigor, size, yield, rate of development, or resistance/tolerance to stress that can be attributed to hybridization (Weaver, 1946; Leng, 1954; Heimsch et al, 1950; Echarte and Tollenaar, 2006; Tollenaar et al, 2004). The extraordinary success of the commercial maize breeding industry is due, in part, to the intensive breeding system (i.e., inbred-hybrid) made possible because of heterosis (Lee and Tracy, 2009; Crow, 1998). Heterosis in the modern commercial maize germplasm pool is relatively predictable, as breeding and selection over seven decades has resulted in the creation of heterotic groups (Lee and Tollenaar, 2007; Lee and Tracy, 2009).

Accompanying the commercial impact of heterosis in maize has been an equally intense interest in understanding the genetic/biological causes underlying heterosis. The two main genetic models for heterosis, the dominance model (Davenport, 1908; Bruce, 1910; Keeble and Pellew, 1910) and the over-dominance model (Shull, 1908; East, 1908), both require that the parents differ in gene frequency. However substantial genome-wide heterozygosity is not a requirement for the expression of heterosis, as heterosis can be observed between pairs of closely related inbred lines (Lee et al, 2007). In general though, there is a negative relationship between the level of genetic relatedness and heterosis, meaning that the more alleles that two inbred lines shared in common, the less heterosis that is observed (Moll et al, 1965; Lee et al, 2007; Flint-Garcia et al, 2009). QTL mapping approaches have been utilized in attempts to reconcile the two competing genetic models (Garcia et al, 2008; Xiao et al, 1995; Ishikawa, 2009). Specific enzymatic processes or biochemical pathways have been studied as possible underlying biological causes of the phenomenon (Hollick and Chandler, 1998; Scandalios et al, 1972; Rood and Larsen, 1988; Dixon et al, 1999). And most recently in the era of big data and genomics technologies, high throughput genomics (Stupar et al, 2008; Fu and Dooner, 2002; Guo et al, 2006; Stupar et al, 2007) and proteomics approaches (Zhang et al, 2012) have been applied. However all of these heterosis studies face fundamental experimental challenges at the germplasm-level, the genomic-level, the phenotypic-level, and the trait-level. Most heterosis studies involve inbred lines from different heterotic groups. At the genomic-level, parental lines originating from different heterotic groups are structurally quite distinct. Vast expanses of non-colinearity in non-genic regions of DNA (Fu and Dooner, 2002; Brunner et al, 2005) and presence/absence differ-

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ences in the genic regions (Fu and Dooner, 2002; Springer et al, 2009) exist. At a phenotypic-level, parental lines originating from different heterotic groups tend to exhibit gross differences in phenology and plant architecture that confound the interpretation of heterosis studies. And finally, when studying heterosis there is the tendency to use discrete or final trait values (e.g., grain yield or plant height), rather than examining the developmental progression of the trait (i.e., growth and development).

Increased vigor is an attribute that is both intuitively obvious, yet difficult to explicitly define. In the context of heterosis in the elite maize germplasm pool, increased vigor is equivalent to a greater rate of development (i.e., rate of the shoot apical meristem moving through the reiterative process) and increased growth (i.e., mass or volume). Growth for most traits can be modeled with a sigmoidal shaped growth curve (Hunt, 1979; 1982). While the upper asymptote (i.e., final trait value) of this curve tends to be the focus of most heterosis studies, it is the rate of the growth, the changes in the rate of growth over time, and the duration of the linear phase that determines the final trait value. And while final trait values of two genotypes may be fairly similar, the growth curves leading to those trait values may be very different. For example hybrids representing conventional heterotic combinations (e.g., Stiff Stalk x Lancaster) generally have the same number of leaves as the parental inbreds. However, the hybrids reach anthesis (i.e., flowering) significantly earlier than the parental inbred lines, meaning the F1 is moving through the vegetative developmental stages more quickly than the parental inbred lines (Tollenaar et al, 2004).

In this paper, heterosis in the context of growth and development of vegetative and reproductive features is examined in terms of rate of growth, duration of growth, and final trait values. Specifically, is heterosis of a final trait value due to heterosis for rate of growth, duration of growth, or is it due to a heterotic advantage in both the rate and the duration of growth? However unlike heterosis studies that only concentrate on final trait values, gross differences in phenology and plant architecture among the parental inbred lines and the F1 hybrid, must be accounted for in a growth and development study. Rather than attempting to model the impact of these phenological and plant architecture differences, we chose to minimize or eliminate them. This study utilizes a sister-line hybrid, meaning that the F1 is the result of crossing two closely related inbred lines. While the parental inbred lines and the F1 share numerous phenological and architectural attributes, the genotypes are distinct from one another. And most importantly, the F1 exhibits heterosis for grain yield (Lee et al, 2007) despite the relatively large blocks of genome shared between the two inbred lines (64% identical-by-descent (Singh et al, 2011). Utilizing this novel approach will lead to a better understanding of phenotypic and trait-level impact of heterosis, and may offer insight into optimal growth stages from which to evaluate inbred line combinations, or optimal growth stages for more in-depth investigation of metabolic processes, transcriptomic changes, or genome-wide phenomena.

Materials and Methods

Genetic materials, experimental designs, and growing conditions

Two Iodent sister inbred lines (CG60, CG108) and the F1 hybrid (CG60xCG108) were used in this study (Lee et al, 2000; 2001). Genotypes were grown in growth chambers (Conviron model PGW36; Winnipeg, Manitoba) using a randomized complete block design (RCBD). Briefly, experimental units were plants (one plant per pot, nine pots per genotype per growth chamber). Each growth chamber was considered a block, which contained 9 plants of each genotype. Three growth chambers were used simultaneously, with three replications in time, for a total of nine blocks. Growth chamber conditions consisted of 16 hour days, day/night temperatures of 25°C and 20°C, and a PPFD between 600 - 900 µmol m^{-2} s^{-1} at the top of the canopy depending on the position within the chamber. Plants were grown in 3-gallon pots in Turface MVP (Profile Products LLC, Buffalo Grove, Illinois). Pots were rotated daily within each chamber and were watered daily to soil capacity with a dilute nutrient solution (0.4 g l^{-1} 28-14-14, 0.4 g l^{-1} 15-15-30, 0.4 g l^{-1} $MgSO_4.7H_2O$, 0.5 g l^{-1} $Ca(NO_3)_2$, 0.2 g l^{-1} NH_4NO_3, 0.04 g l^{-1} Micronutrient Mix (Plant Products Co Ltd, Brampton, ON), 0.03 g l^{-1} Fe-chelate, 0.03 g l^{-1} Mn-chelate, 0.002 g l^{-1} $ZnSO_4.7H_2O$, and 0.002 g l^{-1} $CuSO_4.5H_2O$, pH = 5.8).

The genotypes were also grown in a field trial for assessing mature ear characteristics. The field trial was a RCBD grown for two years (2009-10) at two locations (Elora and Waterloo, ON) with four replications in 2009 and two replications in 2010. Genotypes were planted in 2-row plots at a plant density of 74,000 plants ha^{-1} using an Almaco SeedPro 360 precision planter, with 3.2 m rows in length and 0.76 m spacing between rows. Primary ears from 10 plants per plot were hand-harvested from the field trial, and the number of rows of kernels and the number of kernels per ear were recorded.

Traits measured

Stage of development was followed using the leaf-collar method (i.e., V-stage; Ritchie et al, 1986). Both destructive and non-destructive measurements were made on each genotype from the time the coleoptile emerged from the soil until five to seven days prior silking. On a nearly daily basis following the start of V3, measurements were taken on each plant within each growth chamber (i.e., block) for: (1) V-stage, (2) leaf-tip stage, (3) distance from the soil to each visible leaf collar, (4) distance from the soil to the tip of each leaf, and (5) the minor diameter of the elliptical

stem at each exposed leaf-collar. Measurements of lengths were made using a tape measure, and width measurements were made using a digital caliper. From V6 to V14 randomly selected single plants of each genotype within each block were harvested on the first day the plant attained a new V-stage. Destructive sampling measurements were made on: (1) the distance from node five to each subsequent node and used to determine stem length, (2) fresh weight of the above ground portions of the plants, and (3) length of the tassel measured from the final leaf node to the tip. Leaf lengths used for analysis were derived by taking the distance from the soil to the tip of the leaf and subtracting it from the distance of the ground to the node of the respective leaf at each V-stage. The primary developing ear initial was dissected and placed in Karnovsky's fixative (Ruzin, 1999) for a period of not less than four weeks. Digital imaging of developing ear initials was done using a tri-nocular stereo microscope with a 90X magnification capacity (Cyber Scientific Inc, Kitchener, Ontario; modelV434B) and a three megapixel camera (Cyber Scientific Inc, Kitchener, Ontario, model A1530). Prior to imaging, the developing ears were immersed in a solution of 90% EtOH, 1% glycerin and 0.5 mg ml^{-1} basic fuchsin to enhance contrast (Bonnett, 1940). The number of florets, spikelet meristems, or spikelet pair meristems per row, and the number of rows of florets were recorded for each ear initial.

Data analysis

Growth curves for all traits were fit to the logistic growth model with either the procedures NLMIXED or NLIN using SAS Version 9.1 (SAS Institute Inc, Cary, NC, USA). The logistic growth function is a three-parameter model:

$$y_{ij} = \frac{b_1 + u_{i1}}{1 + \exp[-(v_{ij} - b_2)/b_3]} + e_{ij}$$

where y_{ij} represents the jth measurement on the i^{th} plant, v_{ij} is the corresponding V-stage, b_1, b_2, and b_3 are fixed-effects parameters, u_{i1} are the random-effect parameters assumed to be normally distributed around a mean of zero, and eij are the residual errors assumed to be normally distributed with a mean of zero and independent of the random-effect parameter (Lindstrom and Bates, 1990; Pinheiro and Bates, 1995). The random-effect parameter was tested as if associated with all possible combinations of fixed-effects parameters and the parameters generated from the model with the best fit – as determined by the output listed under "fit statistics" that lists the maximized value of the log likelihood as well as the Akaike and Bayesian information criteria – was maintained. When the model was applied to an individual genotype-block combination – as when measurements were taken non-destructively – treatment replicates were considered as the random-effect parameter. When the model was applied to an individual genotype, blocks were considered as the random-effect parameters. The first derivative of the growth curve provides absolute growth rates over time, while the minimum and maximum values of the second derivatives were used to define the beginning and end of the approximately linear phase of growth.

Percent mid-parent heterosis was calculated as:

$$\text{Percent Heterosis} = \frac{\text{Hybrid Value - Midparent Value}}{\text{MidParent Value}}$$

Paired t-tests ($\alpha = 0.05$) between the mid-parent value and the hybrid value were used to test for heterosis at each V-stage. Differences among genotypes at a given V-stage were detected in PROC MIXED SAS Version 9.1 with genotype as a fixed effect and block and the block by genotype interaction being considered random effects ($\alpha = 0.05$).

Principal component analysis was conducted using PROC PRINCOMP SAS Version 9.1. Genotypes at individual V-stages were used as subjects. The fitted values for a trait and the absolute growth rate at that timepoint in development (i.e., first derivative of the growth curve) were used without standardization as variables. An eigenvalue of one was used as a cut-off for the retention of principal components for further comparisons. The standard equation for finding the distance between two points on a plane:

$$d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

was used to calculate the distance between CG60x-CG108 and the mid-parent on the principal component bi-plot. Comparisons between field and growth chamber values were made using Chi-square tests for homogeneity for kernel bearing ears (observed values) and spikelet number from developing ears (expected values).

Table 1 - Ear characteristic means and standard errors and expression of heterosis (%) for the characteristics from growth chamber grown plants (rows of florets, total florets per row and total florets per ear) compared to ear characteristics of field-grown plants (rows of kernels, kernels per row and kernels per ear).

	Rows of florets	Rows of kernels	Total florets per row	Kernels per row	Total florets per ear	Kernels per ear
CG60	12.9±0.2	13.0±0.1	40.4±1.3	18.9±0.2	521±23	244±2.0
CG108	15.5±0.3	14.7±0.1	39.4±2.1	18.7±0.1	611±32	274±2.0
F1	14.3±0.2	14.0±0.1	44.4±1.3	26.6±0.1	635±21	369±1.9
Heterosis (%)	n.s.	n.s.	11	41	12	42

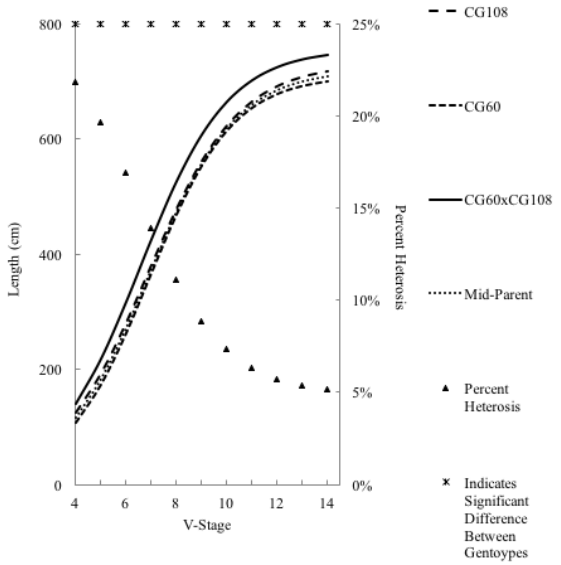

Figure 1 - Growth curves for cumulative leaf length (cm) of the parental inbred lines (CG60, CG108), the F1 (CG60 x CG108), and the mid-parent across developmental stages (V4 – V14). When significant ($p<0.05$) mid-parent heterosis (▲) and differences between genotypes (*) are indicated at each V-stage.

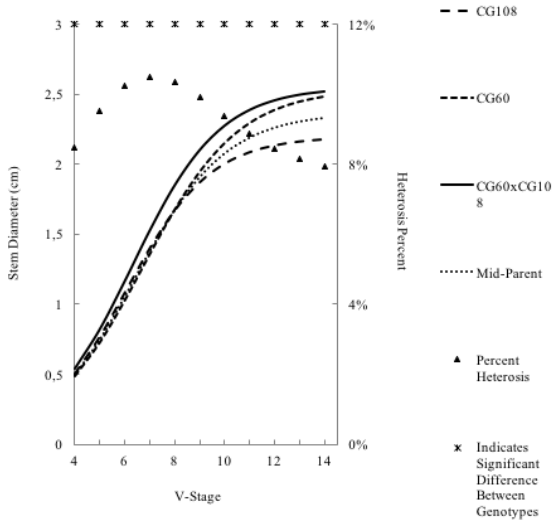

Figure 2 - Growth curves for stem diameter of the third leaf collar (cm) of the parental inbred lines (CG60, CG108), the F1 (CG60 x CG108), and the mid-parent across developmental stages (V4 – V14). When significant ($p<0.05$) mid-parent heterosis (▲) and differences between genotypes (*) are indicated at each V-stage.

Results

The three genotypes used in this study meet the criteria of exhibiting of heterosis while not exhibiting differences in phenology. In terms of rate of development, there were no differences among the genotypes. On average the V4 stage was reached 13 days after emergence (DAE), V6 was reached 20 DAE, and V14 was reached 45 DAE. In terms of final leaf number and floral initiation, again no differences were detected among the genotypes. All genotypes exhibited 14 leaves, tassel initiation occurred at V6, and the primary female floral meristem (i.e., upper most ear initial) was initiated at V6. Yet despite the phenological uniformity, two years of field data consistently showed that the F1 exhibits heterosis for kernel number of the primary ear (Table 1). While there are significant differences among genotypes for all three ear traits, heterosis is only present for kernels per row and kernels per ear (Table 1).

Heterosis during vegetative growth

The characters followed during vegetative growth were those most frequently associated with descriptions of increased vigor: leaf length, stem length and diameter, and fresh weight. Heterosis for leaf length growth rate exhibited the same dynamics for each individual leaf examined (leaves #6 through #14). In general heterosis was detected early in development (i.e., V4 – V6), but was not evident after V6 (Supplementry Table 1). This pattern was also observed in the growth rates for cumulative leaf length (Supplementry Table 1). Since cumulative leaf length tends to be reflective of each individual leaf length, we will only discuss the cumulative leaf length results unless otherwise noted. The F1 exhibited the largest cumulative leaf length over all V-stages with heterosis reaching a maximum at the V4 (18%) and declining to 5% by V12 (Figure 1). Approximately 50% to 60% of a leaf's length was accumulated during the linear phase of growth (Supplementry Table 2). While there were no differences between the genotypes for the duration of the linear phase of growth, significant heterosis for growth rate over the linear phase was observed in leaves #6 and #7 (10% and 9% heterosis, respectively).

Of the vegetative characters, stem length is the character that clearly distinguished the inbred parents from one another, both in terms of absolute values and in terms of growth rate (Supplementary Figure 1; Supplementary Table 1). This was also the character that was the least informative in terms of heterosis. Mathematically heterosis was present for stem length as the value of the F1 was significantly greater than the mid-parent value; but the F1 value fell between the two parental values (Supplementary Figure 1). Likewise, while significant differences were present between genotypes for the absolute growth rate of stem length accumulation from V4 to V11, the F1 value fell between CG108 and CG60 and no significant differences were detected between F1 and the mid-parent (Supplementary Table 1). The stem length results are not entirely surprising as during the development of CG108 biomass per se was the selection criteria (Lee and Kannenberg, 2004), with taller inbred lines having a competitive advantage.

Unlike stem length, stem diameter exhibited biologically meaningful heterosis. Stem diameter data

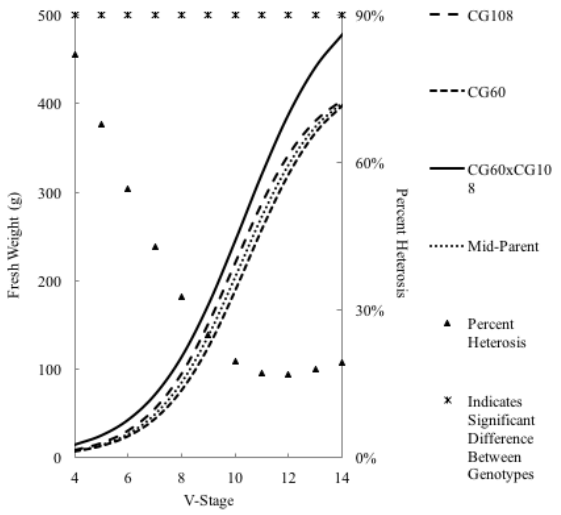

Figure 3 - Growth curves for fresh weight accumulation (g) of the parental inbred lines (CG60, CG108), the F1 (CG60 x CG108), and the mid-parent across developmental stages (V4 – V14). When significant ($p<0.05$) mid-parent heterosis (▲) and differences between genotypes (*) are indicated at each V-stage.

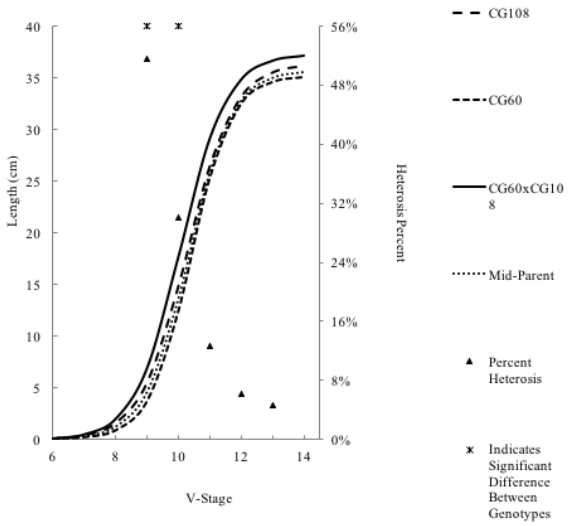

Figure 4 - Growth curves for tassel length (cm) of the parental inbred lines (CG60, CG108), the F1 (CG60 x CG108), and the mid-parent across developmental stages (V4 – V14). When significant ($p<0.05$) mid-parent heterosis (▲) and differences between genotypes (*) are indicated at each V-stage.

was collected at all exposed leaf collars throughout development (V4 – V14). Since the growth curves are similar across leaf collars (data not shown), only the growth curve for the stem diameter at leaf collar three will be presented as it is representative of what is occurring at all leaf collars (Figure 2). Heterosis was detected at all V-stages, ranging from 8- 12% (Figure 2). And heterosis for the absolute growth rate was observed, ranging from 11-12% from V4 to V7 to 8% at V8 (Supplementary Table 1). Interestingly significant differences between genotypes were also found for the duration of the linear phase of growth with the linear phase being longer in CG60, shortest in CG108 and the F1's value being between the two parental values (i.e., no heterosis for duration) (Supplementary Table 2).

Heterosis for fresh weight was evident across all V-stages, with the F1 exhibiting significantly greater fresh weights than either of parental inbred (Figure 3). Unlike the levels of heterosis observed for leaf length and stem diameter, percent heterosis for fresh weight was considerably larger and more dynamic, reaching a high value of 82% at V4 and then declining to 20% by V10 (Figure 3). While there was no heterosis detected for duration of the linear phase (Supplementary Table 2), growth rate for fresh weight accumulation exhibited significant heterosis, ranging from 59% at V4 to 24% by V7. However, heterosis for growth rate was only present early in development, as there were no significant differences between the genotypes for the absolute growth rate of fresh weight accumulation at the later V-stages (Supplementary Table 1).

Heterosis during reproductive development

Ear development and tassel development share many similar developmental attributes (Bonnett, 1954; Cheng et al, 1983). The ear is agronomically and economically of greater interest than the tassel, and a previous study suggested that heterosis has a greater impact on the ear than on the tassel (Meghji et al, 1983). Therefore, we chose to follow ear development in greater detail and only document tassel length. Heterosis for tassel length exhibited the same pattern of expression as the vegetative characters, peaking at V9 (51%) and declining rapidly to 6% by V12 (Figure 4). Heterosis for the absolute growth rate of tassel length was only detected at one developmental stage, V9 (36%, Supplementary Table 1). And no differences in either the duration of or the slope of the linear phase of growth were observed (Supplementary Table 2).

Genotypic differences in the progression of ear development are minimal, yet the final number of florets formed is significantly different among the genotypes and heterosis is detected for final floret number. Final floret number was attained for all genotypes at the V14 stage. Initiation of the primary female floral meristem (i.e., uppermost ear initial) occurred for all genotypes at the V6 stage. The duration of ear development was consistent across genotypes: 25 days, nine V-stages. Yet, despite these similarities, differences between genotypes in female inflorescence development were present. While the number of rows of florets was significantly different between genotypes, heterosis was not present, as the F1 and the mid-parent values were not significantly different (Table 1). Differences in the number of florets per row were detected starting at V9 (Figure 5), with a significant difference between the F1 and the mid-parent

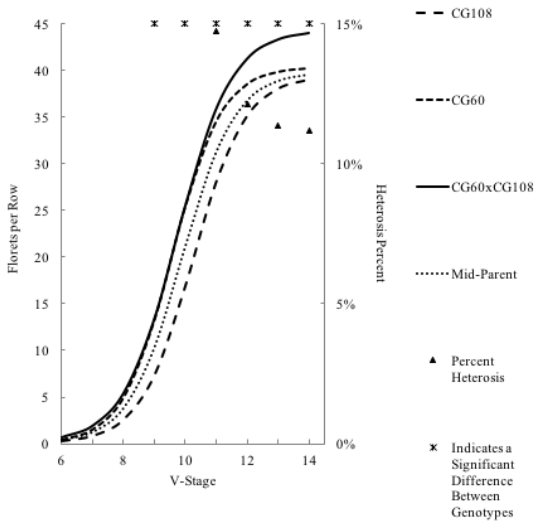

Figure 5 - Growth curves for number of florets per row of the parental inbred lines (CG60, CG108), the F1 (CG60 x CG108), and the mid-parent across developmental stages (V4 – V14). When significant ($p<0.05$) mid-parent heterosis (▲) and differences between genotypes (*) are indicated at each V-stage.

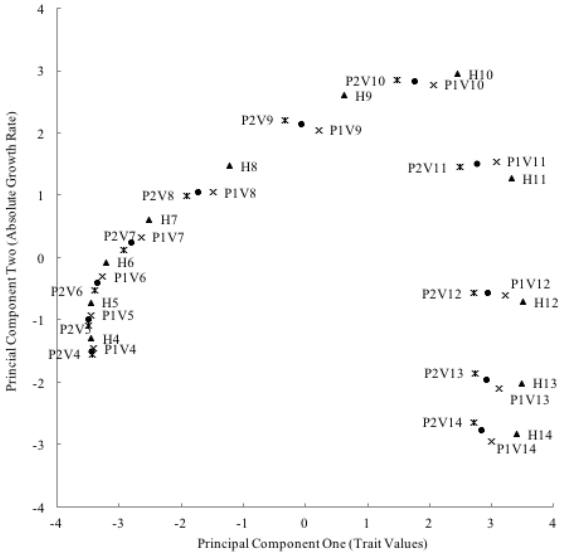

Figure 6 - Bi-plot of principal component two (PC2) relative to PC1 across developmental stages (V4 through V14) for the parental inbred lines CG60 (P2, *) and CG108 (P1, x), the F1 (H, ▲), and the mid-parent (•).

value for florets per row being first detected at V11, resulting in 15% heterosis for florets per row at V11 that declined to 11% at V14. Only at the V8 stage was there a significant difference between genotypes in the absolute growth rate of florets per row; however, no heterosis for the absolute growth rate of florets per row was detected (Supplementary Table 1). There were no differences between genotypes for the duration of or the slope of the line over the linear phase of floret per row accumulation, the period when 55-58% of the florets were produced (Supplementary Table 2).

Discussion

Increased rate of development is not required for the expression of heterosis.

The homogeneity in the time to V-stages and in the time of floral initiation in the genotypes of this study is in contrast to material used in other studies where differences among the inbred lines and the resulting hybrids in either rate to leaf appearance (e.g., Tollenaar et al, 2004), or in timing of floral initiation (e.g., Siemer et al, 1969) were detected. However, despite the lack of variation in phenology and a high degree of common ancestry, the presence of heterosis for kernel number and other attributes is not entirely surprising. The relationship between degree of relatedness and the magnitude of heterosis is predictable and linear (Moll et al, 1965; Lee et al, 2007; Flint-Garcia et al, 2009). In other words the heterosis observed between the two sister-lines in this study lies on this continuum. Heterosis for grain yield had previously been documented in this F1 (Lee et al, 2007). While Lee et al (2007) did not measure kernel number, kernel weight tends to be the less variable component of grain yield and kernel number the more elastic component (Tollenaar et al, 1992; Echarte et al, 2000). And finally, two very different breeding methodologies were used in the development of CG60 and CG108, which potentially led to fixing different alleles (Lee et al, 2000; 2001; Lee and Kannenberg, 2004).

Heterosis for growth is both cumulative and dynamic

By examining heterosis in terms of growth and development of a single trait, several general themes emerge. The expression of heterosis does not require a change in the general progression of growth (i.e., the shape of the growth curve does not change). Differences between the F1 and the inbred parents are greatest early in development (i.e., V-stage) with heterosis declining over subsequent V-stages (e.g., Figures 1-5). But the first instance of significant differences between the F1 and the mid-parent is not consistent for all traits. For example, some traits like fresh weight and leaf length heterosis was observable immediately (e.g., V4). While other traits such as florets per row and tassel length exhibited a delay in the expression of heterosis as it was not detected until at least four V-stages following tassel and ear initiation. Probably the most striking theme is that describing the expression of heterosis of the individual plant character in terms of growth generally does not fit the perceived expectation of what heterosis should look like - that there is an obvious advantage in the F1 that occurs early and that advantage is maintained throughout the lifecycle of the plant. The one exception is fresh weight accumulation (Figure 3), which is

not surprising as fresh weight, or biomass accumulation, is a composite character in that it integrates the other vegetative characters followed in this study (i.e., stem diameter and leaf length).

Simultaneously examining the expression of heterosis for all characters, both in terms of growth rate and trait value, captures the dynamic nature and cumulative effect of heterosis. Using trait values and absolute growth rates for each genotype–V-stage combination, the first two principal components (PC) collectively accounted for 93% of the variation (68% and 25%, respectively) (Figure 6). Based on eigenvectors, PC1 captures trait values while PC2 captures absolute growth rates (Supplementary Table 3). Early in development (e.g., V4) the differences between the F1 and inbred parents are small and due exclusively to absolute growth rates (i.e., PC2). As development progresses, these differences in absolute growth rate result in differences in trait values and are at a maximum at V9. By latter stages of development (e.g., V14) heterosis for absolute growth rate declines yet the cumulative heterotic advantage of the F1 for the trait value is maintained (Figure 6; Supplementary Table 4). Using this integrative approach the growth and development data does fit the perceived expectation of what heterosis should look like - that there is an obvious advantage in the F1 that occurs early and that advantage is maintained throughout the lifecycle of the plant.

Conclusions

While most of the scientific effort regarding heterosis has focused on the genetic and genomic mechanisms underlying it, speculation regarding the biological basis for heterosis has occasionally been the subject of discussions. Many if not all of those discussions focused on finding the cause. For example, size of the adult plant was attributed to the superior size of the hybrid embryo and primordia, with this advantage simply being maintained throughout development (Ashby, 1932; 1936). Heterosis for grain yield has been attributed to a hybrid advantage in light interception (i.e., greater leaf area) and partitioning of carbohydrate to the grain (i.e., harvest index) (Tollenaar et al, 2004). However as noted by Whaley (1952) «structural differences between inbreds and heterotic hybrids [as are the embryo size noted by Ashby (1932, 1936) and the leaf area indices observed by Tollenaar et al. (2004)] … are apparently to be regarded as results of heterosis rather than as causal factors.»

In this study we have purposely avoided ascribing a biological cause to the phenomenon of heterosis. Rather using growth curves for a series of vegetative and reproductive traits across stages of development (V-stages) in a phenologically uniform set of genetic materials we demonstrate several attributes of heterosis that will hopefully help shape future heterosis studies. We demonstrate that increased rate of development is not a requirement for the expression of heterosis. Nor does expression of heterosis require a change in the general progression of growth. In short, heterosis confers an advantage to the F1 that occurs early in development in terms of growth rate. And while the growth rate of the F1 and parental lines eventually coalesce, that initial advantage due to heterosis is maintained throughout the lifecycle of the plant resulting in a larger final trait value. This study demonstrates the challenges of examining heterosis. Not all traits are influenced by heterosis. And of the traits influenced by heterosis, small changes in growth rate during brief periods of development are sufficient to result heterosis of the final trail value. Designing experiments targeting these brief periods where differences in growth rates exist between the F1 and parental lines may be the key to identifying genes and molecular mechanisms driving heterosis.

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