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Circadian regulation of maize transcriptomes in B73 and Mo17 inbreds and their reciprocal hybrids.

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Circadian rhythms have been shown in nearly all forms of life, ranging from algae to plants to mammals. These rhythms include oscillations of gene expression. We recently surveyed the maize transcriptome for diurnal rhythmicity and found >23% of all leaf measured transcripts displaying diurnal regulation under field conditions at the R1 stage of development [1]. This diurnal study involved environmental light and temperature variation and thus was different from classical “circadian” studies, which typically use constant conditions of light and temperature.

A molecular clock model was recently proposed that links heterosis to the improved or better-tuned circadian clock in Arabidopsis hybrids [2]. To assess this hypothesis in maize and to examine classical circadian transcription, leaf tissues of inbred lines B73 and Mo17 along with their reciprocal hybrids (BxM and MxB) were assayed under constant conditions over three days by microarray mRNA profiling.

Briefly, plants were grown to the V3 stage under normal greenhouse conditions and then transferred to a growth chamber with constant light and temperature (25ºC). After 24 hours of acclimation, leaves from three plants per genotype were collected every four hours for 72 hours. Additionally, B73 whole-root tissue was collected at each time point. Messenger RNA was prepared from each genotype~time point and assayed using an Agilent microarray representing ~43.000 ESTs. The data were normalized via the Quantile Normalization method and assessed for rhythmicity via the GeneTS package in the R Statistical Programming Language as previously described [1].

The leaf results indicate that overall the number of detected circadian cycling transcripts was lower than in leaves from the field diurnal study [1], which may be due to the absence in the controlled environment of other environmental cues that reinforce the diurnal pattern (Table 1). Mo17 leaf tissue displayed slightly lower numbers of cycling genes and lower amplitude waves than B73, which may in part be due to probe affinity as the oligos on the microarray were designed largely according to the B73 genome. There is evidence of a slight attenuation or ‘run down’ of the cycling pattern, because the median peak/trough values decreased each successive day. However the third day median peak/trough values were still approximately two-fold different, indicating good persistence of circadian rhythms (Table 1 and all Figures). No significant numbers of cycling transcripts were detected in roots.

Both reciprocal hybrids display markedly lower numbers of cycling transcripts than the parents (Table 1). The possibility exists for allelic offsets in peak times to cause broader or lower amplitude waves that by additive interference diminish those scored as cycling in hybrids. To resolve this issue shorter time period resolution and allelic specific expression should be used. Among those cycling transcripts that do appear to cycle in hybrids, they tend to show an additive pattern between both inbreds (Figure 1). In many cases the hybrid diurnal patterns could be mimicked by taking the arithmetic means of the signal from the parent inbreds (Figure 1). In other words the amplitudes of the hybrid cycling genes correspond to the mid-parent values. The mid-parent values of cycling transcripts, and the reduced overall number of cycling transcripts, both indicate an additive pattern likely prevails in hybrids.

Maize core clock oscillators such as *ZmCCA1* and *ZmTOC1b* (Figure 2) did not show consistent increased amplitude patterns in the reciprocal hybrids as predicted by the molecular clock model of heterosis for Arabidopsis [3]. Rather, the circadian clock oscillators also appeared to more closely match the mid-parent value in hybrids as observed for the other circadian cycling transcripts.

These results do not support the model for the circadian machinery having a major impact on heterosis in maize. The results also indicate that rhythmic gene expression patterns appear to be mostly additive and do not show epistatic interactions. These results are consistent with other studies that showed additive gene expression as the major pattern observed in RNA profiling experiments of maize hybrids [4, 5].

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2. Ni Z, Kim ED, Ha M, Lackey E, Liu J, et al. (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature 457: 327–331.
3. Chen ZJ. (2010) Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci. Feb;15(2):57-71.
4. Guo M, Rupe MA, Yang X, Crasta O, Zinselmeier C, Smith OS, Bowen B. (2006) Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis. Theor Appl Genet. 113 (5):831-45.
5. Springer NM, Stupar RM. (2007) Allelic variation and heterosis in maize: how do two halves make more than a whole? Genome Res.17(3):264-75.

Table 1. Number of diurnally cycling genes and their median amplitude at 30% FDR (false discovery rate)

Genotypes	Number of Cycling Genes	Median Amplitude (Fold)
B73	2191	2.44
Mo17	1549	1.74
BxM	465	1.92
MxB	203	1.97

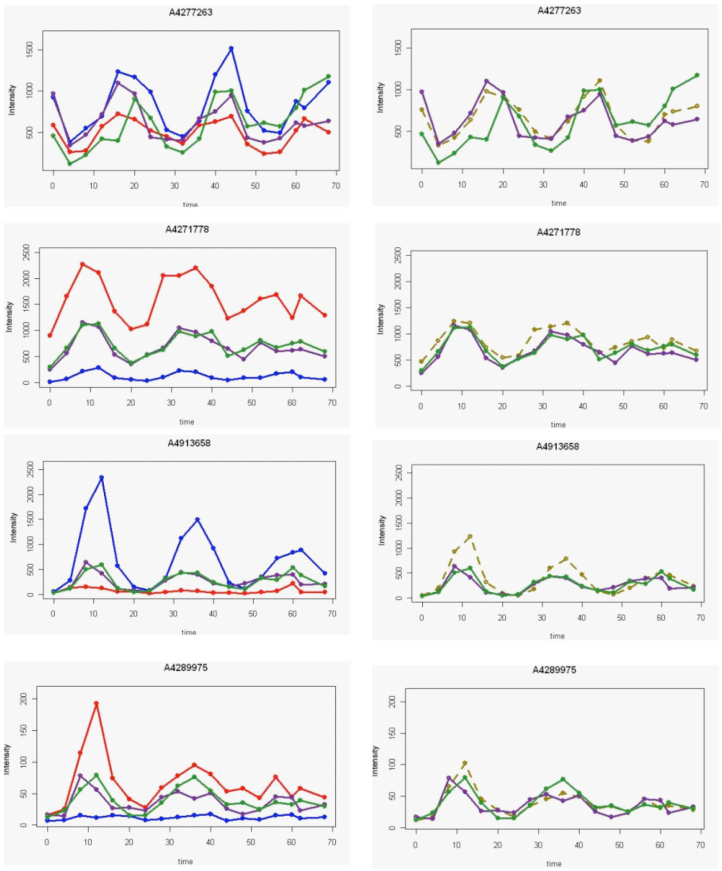

Figure 1. Four examples of additive rhythmicity of reciprocal hybrid circadian cycling transcripts. Inbred and hybrid gene expression are shown B73 (blue), Mo17 (red), MxB (green) and BxM (purple). Calculated mid-parent values in hybrids are gold-dotted lines. The probe ID on the Agilent microarray is written on the top of each graph.

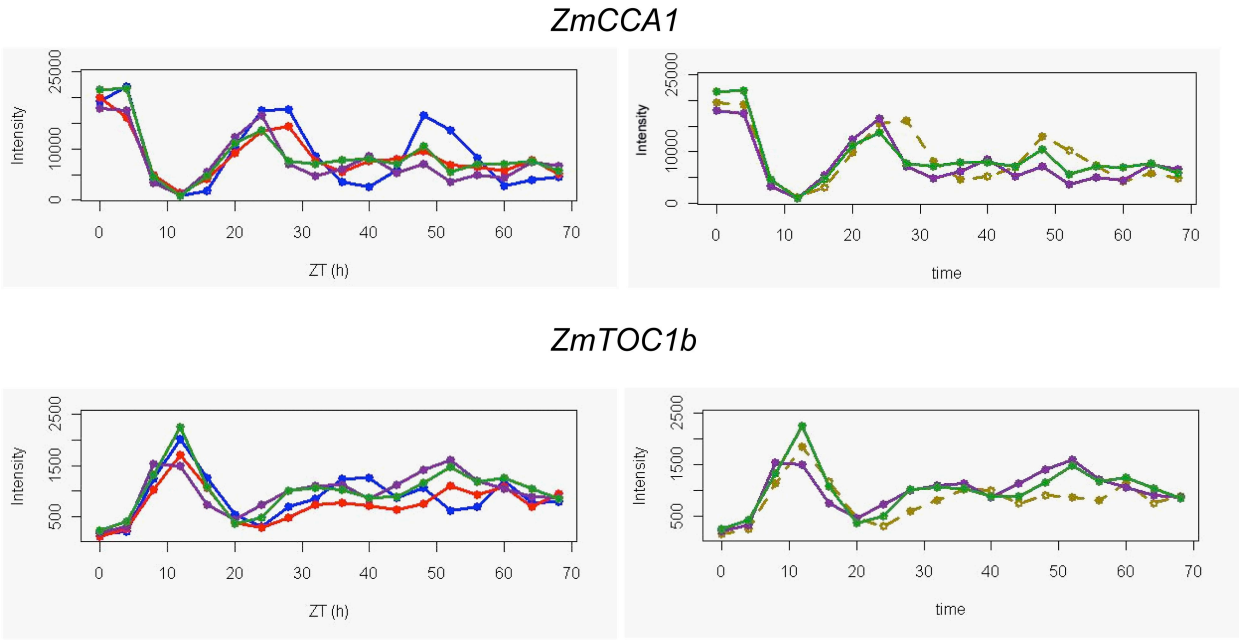

Figure 2. Circadian expression of the core clock oscillators *ZmCCA1* and *ZmTOC1b* in inbreds and reciprocal hybrids.
The color lines are the same as in Figure 1