So, what’s happening at Stillwater?

During our call this week, we went through flowering time mash results for the diversity panel at the fourway sites. Stillwater (STIL) and Kingsville (KING) had the most unusual SNP effects in the mash results. Often, KING had antagonistic pleiotropy with the other sites, and STIL had a much larger effect than the other sites. Here’s a mash SNP that overlapped Li’s fourway QTL on Chr02N:

A screenshot of a cell phone

Description automatically generated

I spent time looking through the data, and eventually formed the hypothesis that the Midwest and Gulf populations are responding to extremely different environmental signals across the planting locations.

**Here is why I think the Midwest and Gulf populations need different environmental indexes**. If you plot cumulative GDD between greenup and flowering *(or photothermal time, or days to flowering, for any base temperature, it doesn’t matter – so I picked 13C as Li has used that base temperature in previous work)* and create a reaction norm plot, where cumulative GDD is on the y-axis, and the sites are ordered by latitude on the x-axis, you get the following:

A close up of a map

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The blue lines are the linear trends for each subpopulation *(smoothed curves are very similar).* The green lines are Gulf clones, and the purple lines are Midwest clones.These two subpopulations are the most distinct on this kind of plot, including the 8X, admixed 4X, and Atlantic material.

So here you see that cumulative GDD is a pretty good index for the Midwest population. Regardless of the site, Midwest individuals in general are just looking for ~600 cumulative growing degree days before they flower. *(Gulf individuals virtually always need more cumulative GDD than Midwest individuals)*.

The same is not true for the Gulf population. There does seem to be an interaction with latitude, but not a perfect one, and it’s particularly messy at Temple (TMPL – unfortunately we don’t have a fourway population here) and Stillwater.

There is lots of antagonistic pleiotropy evident in the Gulf population in KING relative to the other sites. Oh, and we see some strange flowering signatures at STIL and TMPL for the Midwest population. It seems plausible that this is causing STIL SNP effects to be much larger than they are other sites for some SNPs.

Because of the imperfect interaction with latitude, I looked at various features of daylength as contributors to flowering time in Gulf individuals. Only when I plotted daylength at each site relative to greenup and flowering times did I start to get a theory about what was going on. So here, day of the year is on the x-axis, and daylength for each site is indicated by the black curve. Then, the points below the black curve indicate date of greenup (50%, GR50) and flowering (1%, FL1; and 50%, FL50). The green points are from Gulf individuals, and the purple points are from Midwest individuals. And the dashed vertical line is the longest day of the year.

A close up of a map

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Basically my hypothesis is that 1) Midwest individuals flower once they’ve accumulated about 600 growing degree days, and 2) north of Kingsville, **Gulf individuals flower once daylength starts decreasing** and they’ve accumulated at least ~800 growing degree days. This explains the massive difference in cumulative growing degree days until flowering between PKLE and TMPL on the previous plot – at both sites, Gulf individuals wait until after the summer solstice to flower (and may wait even longer at TMPL due to unknown other environmental factors – maybe drought). *(And if you’re worried about possible collection gaps in the data – don’t be. Emergence was being scored at all of these sites between greenup and flowering and nicely spans the gap between GR50 and FL1 – data was being collected at all of these sites the entirety of this period.)*

And Kingsville – well, Kingsville is strange. Clearly, if there is some dependency on daylength in the Gulf population, it’s broken for at least some Gulf individuals due to some other environmental factor at KING (and PKLE/TMPL to much lesser extents).

In the STIL panel, you can see the cause of the days to flowering GxE and reaction norm crossovers at that site. At TMPL, the Midwest subpop greens up last. At STIL, GR50 timing overlaps in the two subpopulations. And once you get to CLMB, the Midwest subpop greens up first. STIL is clearly an important transition site for both subpopulations.

Here’s what I propose as an outline for the Results/Discussion for the paper.

1. First, introduce the diversity panel at the common gardens, and introduce what we know or hypothesize about environmental indices triggering flowering in the different subpopulations – maybe even discuss Gulf, Midwest, Atlantic, and 8X subpopulations. Point out that the Gulf and Midwest subpopulations have the most distinctive flowering responses out of all of the populations.
2. Next, introduce the fourway cross – a genetic resource we have to break up the genes involved in the distinctive flowering responses in the most distinctive subpopulations.
3. Show the QTL regions and GxE patterns in the fourway – maybe looking for daylength sensitivity and for cumulative GDD requirements in this cross, if we can figure out how to model that. We could use all five years of data here. Maybe a model like the GWAS model below if possible.
4. Then go back to the diversity panel. GWAS for Gulf, Midwest, and Gulf & Midwest individuals. Phenotypes could be BLUPs from a model where
   1. FL50 ~ PLANT\_ID+ covariate(ave\_GDD\_per\_day\_GR\_to\_FL) + fixed(Site, Site:Subpop).
5. Use GWAS association overlaps with fourway to narrow intervals affecting flowering in the fourway. Discuss candidate genes from these regions, gene expression data, and allele frequencies and effect sizes of these SNPs in the two different subpopulations.
6. Later in discussion: discuss other environmental factors that could influence flowering time in our dataset – including drought (LINC fourway flowering, maybe TMPL 2019 in diversity panel) and weirdness at KING.