12/06/18

750 words / start 12:26pm / end 12:55pm

I gave lab meeting and it went well. I opted in to meet with Dan Runcie separately the Monday after. It all went fine. I should actually follow up on the ideas that Dan suggested to me, but so far I’ve mostly put them aside. It’s one of those semi-frustrating moments when I’m skeptical of an answer Klieb seems satisfied with, where I want to dig in and try another method to convince myself (and future reviewers?) that our methods are valid and our results make sense. Then again, maybe that’s the kind of analysis I should add into the manuscript later, if the dissertation chapter is being developed into a manuscript for publication. Maybe I only have 3 reviewers I need to impress at this point, and it’s the Dans and Bo. Then again, do I need to try some of it to appease Runcie? I don’t know him well enough to know how tightly he holds onto things. I would hope he’d support whatever Klieb wants to do to get me out the door… but I don’t know for sure. Anxious waffling ensues. I’ll probably not actually work on his suggestions, just keep letting them quietly nibble at my confidence. For that reason alone, it could be worth a look. Hopefully it won’t take ages to pull off.

So now all I have left is the exit seminar, and writing the damn thing. I’m feeling more motivated to stare at the exit seminar than the writing. I guess it’s okay to go with that- it feels more urgent, and maybe once I have a draft I can let it sit for a minute until I get a seminar date from Stacey. It’s not Dan’s priority, but at least it’s me doing work that needs to happen.

When I first opened up the tomato talk to copy slides over, I felt overwhelmed and undirected. The last time I presented on it was ages ago, in terms of the layers of edits I forced the manuscript through. Who knows if any of my data slides are useful as-is. Who knows if I’ll have to redraw them all in color. But it also feels good to have a presentation that’s way too long (let’s see, 100 slides right now! Oops!) and know that it’s just editing and culling and refining my script and transitions that needs to happen. No major new chunks to throw in.

After seeing Alex’s exit talk I’m having some anxiety/ remorse over my work feeling too simple and repetitive. Which is a little wild- it’s been plenty of work and plenty hard for me. But his talk (to me) looked like an impressive array of different techniques, in which he developed tons of skillsets in service of one big, exciting (to him) research question. But maybe that’s okay, for me to see that contrast. Alex is smart, but he also likes his work and wants to continue in this type of research. It’s okay if my dissertation looks a little simplistic or boring from my own perspective- I’m merely trying to get done and get out. And I don’t even intend to continue in this work. So, for real this time, Enough is Enough.

Here goes trying to write some kind of story about Botrytis to frame the exit talk.

1. Plant pathogens are classically defined by a few of their lifestyle traits, including the extent to which they specialize for survival on only a single host species or clade (obligate relationship), versus live as generalists with a wide range of suitable hosts. Additionally, pathogens are described by the mechanisms by which they infect and feed upon their host plants. Necrotrophs XX something about killing tissue as they consume cells, brush up on this XX and biotrophs XX living tissue XX. Botrytis cinerea is interesting in that it is intermediate between biotroph and necrotroph, but also because it is an extreme generalist.
   1. Necrotrophs can infect living tissues, and extract nutrients from dead or senescing plant materials

Botrytis cinerea is one member of a genus of fungal plant pathogens. However, because all of my research focuses on this species, I’ll refer to this pathogen as Botrytis for short throughout the remainder of my talk.

1. Botrytis cinerea is an extreme generalist in that it can infect hosts throughout the plant kingdom. The majority of its hosts are within the eudicots, but Botrytis is pathogenic on hosts as distantly related as (pine? Spruce?) and (mosses?) This poses several biological questions, including, what about this pathogen facilitates such an exceptionally broad host range?
2. There are several hypotheses for the biology of an extreme generalist.
   1. One hypothesis predicts a sort of silver bullet gene, /750/ which would effectively disarm conserved plant defenses across many species. Diversity would be low, as this gene would not need to specialize.
   2. Alternately, the pathogen may be specialized to particular hosts, but at the isolate level rather than the species level. Individuals may specialize to different hosts, such that on the pathogen species level, it appears to have general host targets.
   3. Finally, the generalist pathogen may specialize at the allelic level. This would account for a highly diverse isolate, in which unique alleles are optimized for attack of different host species.

By the way, we work with Botrytis individuals as single-spore isolates collected from individual host tissues. As such, to refer to a genetically distinct individual of Botrytis, I will use the term “isolate”.

1. Previous work from our research group negates some of these hypotheses.
   1. First, genetic variation for measures of Botrytis virulence is highly quantitative, which negates the hypothesis of a single silver bullet gene with minimal diversity.
   2. For example, in our collection of 96 Botrytis isolates, the host from which we isolated each Botrytis genotype was not a major factor determining the genetic distance of the isolates. This suggests that individual isolates are not specialized to particular hosts, but that Botrytis is a generalist even at the individual level.
   3. Third, we find that the geography of where Botrytis individuals were isolated does not predict the genetic distance between them. This lack of isolation-by-distance hints at a panmictic population, with common interbreeding between diverse isolates.
2. Together, our evidence so far seems to rule out the hypothesis of a single universally-effective virulence gene, or the hypothesis of specialization at the isolate level. This leads us to the hypothesis of allele specialization: which genes have allelic variation that contribute to virulence on specific hosts, or subsets of hosts?

11/16/18

Sacramento and Davis have been blanketed in smoke from the Camp Fire for days… UC Davis campus has been closed from Tuesday – Friday this week. It has me feeling melancholy (extra?) and tired and lazy. I’ve mostly worked from home all week, though today I changed it up and headed to SeanR’s work. The change of space and office type environment kind of helped. I like working at home, but maybe not every day for weeks on end.

I’m at a weird moment. I’ve had some momentum in trying to rush to finish by December, but we recently extended my deadline to March. And I’m tiring of the job application phase, with so few interviews or responses. I want to maintain the momentum but am not really feeling it- feeling some fatigue, but more than that, I’m feeling malaise and apathy. Ideally I’d have some looming new assignment that helps me value this phase as my only focused writing opportunity… but it’s hard to fool myself into thinking this exists.

Plus, there’s this annoying level of mini-deadlines pushing certain projects forward. It’s looking like I’ll need to give lab meeting on 11/30, so I need to push figure generation. But what I want to be doing right now, oddly, is writing. And even figure generation was slowed down- by a job application deadline, a job interview, a science communication day, reading through Dan’s new grant proposal, reading through Vivian’s paper draft. I take longer than needed to do these tasks when they’re not what I want. Therefore delaying even further on the work I care about in the moment. I’m not sure how best to balance these things. Maybe I can (oddly) reward myself for doing the yucky work efficiently by scheduling a block of the work I want to do? I don’t know.

11/10/18

On Saturday I attended a workshop on dissertation writing, titled “getting started – getting done”. And it was and wasn’t very useful. The best workshop sessions I could find were in writing a literature review, and in grammatical editing. The literature review provided some nice structure and guided thinking about what I wanted for my chapter 2 introduction. But the meat of it, the strategy for how to take notes as you read, for how to do a good literature comb… I figured all of that when I wrote my masters’ thesis. And when I wrote my metabolism review paper with Dan. The grammar lecture also provided some more structure than I had previously been aware of… but the best resource offered was a handful of links to grammatical editing resources. Part way through, I realized how little information there was about what the dissertation, this great body of work, was even supposed to look like. I searched for my grad group’s guidelines, and for the guidelines of the school. There was practically nothing. No length expectations, no content expectations. Just a publication-quality body of work that indicates that the student has the ability to conduct independent research. So I asked Lauren if her department had guidelines… she recommended checking out the dissertations of previous students. I did- Jason’s, Rachel’s, Jenna Gallegos’. They were, for the most part, shorter than I expected. Jenna’s had only 2 data chapters. So I came away from the day with something- a bit more certainty that I know as much about this process as I can, given where I am along the way. That I’m not missing some big piece. That the work I plan to complete will be sufficient. That the only real block between me and the end is, well, me. It comes down to my willingness and motivation to complete the work. The skills are there. They have to be.

11/09/18

Less writing this week. I backed off a little bit on writing for its own sake. We finally heard from Suzi and got her Botrytis genome manuscript. I realized that it basically should have been published 2 years ago. I’m a teeeensy bit irritated at her for holding us data-hostage. I want a lot of her data for follow-up analysis on my stuff (or at least for Celine’s sake for the eudicot stuff) but question how much she’ll ever share. I hope she’s okay, whatever is going on.

Also, I had health (IUD) problems which made for 4 doctor’s appointments this week. Holy hell. Lauren reminded me to consider all of this “work time” to stave off the guilt and feel productive. So there we are. Hot yoga felt super good- I was more awake, more calm, kinda energized after the class. But every day is hard right now and seems to involve crying or emotional distress at some point. I’m a little exhausted.

I’ve committed (today) to the 6 day trip to Oman and feel pretty darn surreal about it. So far I’m pretending it’s not happening. I need a strategy to notify Dan of it when he’s back after next week. I’m thinking I’ll call it another wedding. Close enough? I AM committing to a) getting enough sleep b) working in transit c) being responsible. Sure, 3 days off I can do. Not a full 6.

In the good work news, the tomato resubmission is ready to go. I need to upload it tomorrow and then poof! I will not touch it again for a bit. Hope to GODS that it gets accepted this time. My fragile little psyche needs it.

Plus, completed some data analysis for the eQTL paper and had plenty of new thoughts. But ugh, I wish I could convince myself that this method is valid.

I \*am\* feeling more comfy with letting Dan in on my thoughts as they happen, less protective of my work and ideas. Less anxious about whether I’m working enough (cause I’m working just about as much as I can manage). Here’s hoping I can keep it up.

11/05/18

I decided that devoting time to catchup on chores etc. on the weekends is fine. It has to happen, and weekends are the best time for that. So on Sunday I did just that- house cleaning, turtle tank, voted, cooked a little. Caught up on emails. Worked on job apps a bit. Went for a walk.

Misplaced my notebook at home or at SeanR’s. Urgh. I’ll make a little to-do list on wunderlist.

Thoughts from yoga class last night on how to get p-values (SNP effects) for the Top At (Bc < top hits = 0) / Top Bc (At < top hits = 0) SNP lists? Ideally, as follows (using top At hits as example):

1. Read in each transcript GWA for Bc genes
2. Get p-value for all SNPs from the At list
3. If transcript 1, keep these values. If transcript > 1, keep only max value between current & previous. Match on SNP+Chr location.
4. Now, you should have the max SNP fx at each of those loci.

This would be a bit time-intensive, but worthwhile I think.

So… somehow rand2 is identical to the (old) rand1?? Checked phenotype inputs (FAM files) as well as association file outputs for individual (randomized) gene profiles. They are identical between rand1 and rand2, though rand2 certainly ran independently (a month later). I re-randomized phenotypes for rand2 and now am re-running GEMMA for these.

FYI, this probably means that sample() in R starts from the same seed each time. BE CAREFUL OF THIS. Fortunately, permutations 1 and 3:5 were unique from each other.

11/03/18

Already weekends are harder and more tiring. I’m finding it difficult to chug along with work without regretting missing the weekends as time to catch up on chores + snuggles + downtime + socializing + job applications + scicomm work. This balance feels difficult. I’m sticking to my minimums for now though—compliance is, oddly, the only thing keeping me complying. Just intense adherence to arbitrary rules.

I finished the permutations and I’m not exactly sure what to do with them. First thought is manhattan plot all 5. Just take a look to see how consistent they are. Take the max (100% threshold) across ALL of them. Do I have any SNPs above this for the actual data? If not, how about the 99.9% threshold? Who cares if it’s imprecise as heck. It’s just a first look.

But then, how do I want to summarize across all 5? Manhattan plot of the max value per SNP? Sure. Or maybe some sort of sliding window mean +- SE line across the genome, to get a sense of the variation and where our real hits land. IDK.

11/02/18

I’m starting a dissertation journal to keep me writing, even on the data analysis days. I’m trying to impose structure: to find a way to feel accomplished on a regular basis, to track actual progress toward a nebulous goal, to nudge myself toward staying focused and productive even on the bad days. I’m only 5 days in but so far it’s helping.

Writing so far feels empty, as normal. The plots I have so far are a little too sketchy- I don’t feel confident in calling patterns of hotspots without the thresholding done. I’m worrying over the problem of thousands of statistical tests – does a 5x permutation feel sufficient? I might try calculating an FDR correction just to help me feel confident… that none of my SNP hits are real. Haha.

Also I still can’t trust my R results at first pass, but maybe I’m getting better at troubleshooting through plotting. Or at being skeptical of my results in a semi-productive way. Hopefully.

I’m still afraid that I have a huge blind spot in this project. I’m partially reading the literature to convince myself that GWA to find “eQTL” for the full transcriptome is anything other than crazy. That damn multiple-testing problem. I feel like I should do some sort of informed pre-selection of which transcripts to examine, or which SNPs to include. It bothers me to not first a) select for only the transcripts with significant *B. cinerea* genetic effects and b) split out cis-loci from trans-loci. I’m trying to trust Dan that lumping it all together and seeing what jumps out is okay. I’m not convinced. What if \*somehow\* through random chance and bad luck those transcripts with no *B. cinerea* genetics are sticking around in our top hits?

Oh wait, I could test that. I’m going to look at which transcripts end up in my \*highly significant SNP associations\* club and see how they looked in Wei’s heritability data. DO IT.