* Add to methods
  + The original study included wildtype (Col-0) *A. thaliana* hosts, as well as a knockout to the salicylic acid response pathway (npr1-1) and jasmonic acid sensitivity (coi1-1).
  + Inoculation in a randomized complete block design, 2x replication per experiment, 2 independent experiments
  + 85% of the 23,898 hosts genes were differentially expressed among the B. cinerea isolates, as such we included all transcripts in our eQTL analysis.
* Figure legend and results: Vertical stripes of SNPs are indicative of *trans*-eQTL hotspots; loci which modulate expression variation across many genes in the pathogen.
  + Could plot and describe the same thing in the host
* Four A. thaliana networks: 131 genes in four co-expression networks with >= 5 genes. Network architecture altered around a constitutive core across A. thaliana genotypes. We included the largest networks (npr1-1) to estimate all possible ties. Network I: camalexin biosynthesis, Net IV chloroplast function
* Average LD decay in the B. cinerea genome is <1kb, so we can be fairly confident of the SNP tagging particular hotspot peak genes {Atwell 2018}.
* Gene annotation thoughts (some of this may move to discussion)
  + Many of the *B. cinerea* eQTL hotspot target genes are enzymatic (34%, 140/412 genes with functional annotation), indicating a role of *B. cinerea* metabolic shifts as the fungal infection progresses *in planta*.
    - 11 TFs. Suggestive of eQTL hotspots controlling major networks of B. cinerea gene expression variation; the targets of these eQTL are also regulatory genes modulating expression of downstream genes. eQTL hotspots could be near the top of a hierarchical regulatory structure.
    - 2 genes with a peak of expression during the outset of colonization (BcPIO5, BcPIO10). Gioti et al 2006. Also, gene (HOX TF) induced in early infection stage (BcHOX8,BcPIE1). Expressed early in infection as well as at a later intermediate stage- these genes may play an ongoing role in the infection progress, or may remain active at the newly colonized cellular edge of the growing lesion.
    - 2 mitogen-activated protein kinases: active signaling cascades in the fungus, acting to regulate major cell functions. Decision-making in the progression of infection.
  + eQTL hotspot annotations
    - Short-chain dehydrogenase/reductase SDR; Glucose/ribitol dehydrogenase; NAD(P)-binding domain // Glycoside hydrolase, family 63 // Phosphatidate cytidylyltransferase // GTP cyclohydrolase I (4 At peaks): these enzymes may cause metabolic changes to B. cinerea that elicit an A. thaliana response, or this could be a secreted enzyme functioning in digestion of host polysaccharides. Either would likely stimulate major host responses and thus and expression response.
    - NACHT and HET (2 At peaks): Genes of this class have been previously shown to predict compatibility and mating type in B. cinerea. These loci may structure the pathogen diversity and as such could be major vectors of the pathogen variation affecting host response.
      * How to test this?
    - Protein kinase-like domain; Fructosamine/Ketosamine-3-kinase // Topoisomerase II-associated protein PAT1 // 6-phosphogluconate dehydrogenase, C-terminal-like; Ketopantoate reductase; NAD(P)-binding domain // SNF2-related; Helicase, superfamily 1/2, ATP-binding domain; P-loop containing nucleoside triphosphate hydrolase (4 Bc enzyme peaks): These enzymes may alter major branches of B. cinerea metabolic pathways active during infection of A. thaliana.
      * Test this: are targets often enzymes?
    - For B. cinerea TFs affecting At expression: likely indirect. It might be mediated by the proteins these TFs interacts with or could be a consequence of epigenetic modifications., of other genes in*B. cinerea*.
    - Bcin16g01950 (At peak) (Glycoside hydrolase, family 63): A secreted glycoside hydrolase which gives some idea of the direct mechanism of targeting A. thaliana gene expression.
    - Bcin12g00330 (Topoisomerase II-associated protein PAT1) and Bcin09g06590 (Helicase) are eQTL hotspots, which make sense as these genes would have a direct influence on the transcription machinery of B. cinerea. However, I would have assumed that these genes are more conserved in the B. cinerea genomes. Again, I would think that these two eQTL hotspots might be conserved in the coi1 and npr1 as well.
      * Helicases and topoisomerases could play a role in how many nuclei are present in each cell, potential to alter virulence.
      * Bcin09g6590 (Bc) eQTL targets:
        + Many enzymes (28/ 129 genes) + TFs (5)
      * Bcin12g0330 (Bc) eQTL targets:
        + BcMIMP1 expressed in planta, BcPIO5 and Bc PIO10 genes with expression peaks at outset of colonization
        + BcSAK1 MAPK
        + Many enzymes (24/101 genes)
      * Across all At eQTL, top terms from all targets:
        + Protein binding, ATP binding, metal ion binding, DNA binding, oxidation-reduction process, DNA binding TF activity, regulation of transcription, kinase activity, response to salt stress
      * Bcin04g00830 (At) eQTL targets:
        + Lots of DNA and RNA binding, possible TF activity
        + Defense responses (8), PCD (2), ROS, SAR
        + Signaling pathways (ABA, ethylene, GA)

Sorted list approach, top terms: protein binding, DNA binding, ATP binding, metal ion binding, regulation of transcription, DNA binding TF activity

* + - * Bcin04g04700 (At) eQTL targets:
        + Lots of DNA-binding TF activity
        + GTP/ ATP binding
        + Enzymes: kinases, hydrolases
        + Signaling: ABA, JA, redox, SA response
        + Chloroplast, chlorophyll, photosynthesis (LOTS), plastid
        + Defense responses (26), ISR, innate immune (3), PCD, wounding

Sorted list approach, top terms: DNA, regulation of transcription, ATP, TF… photosynthesis, defense response

* + - * Bcin10g05900 (At) eQTL targets:
        + DNA-binding, a few TFs
        + A little ATP binding
        + Some ABA, defense, JA, redox

Sorted list approach, top terms: protein, metal, transcription, TFs, redox, ABA response, stress response

* + - * Bcin16g01950 (At) eQTL targets:
        + ATP binding, GTP binding
        + DNA binding & TFs
        + A little defense, ABA, JA regulation & response, SA response
        + Photosynthesis

Sorted list approach, top terms: TFs, redox, photosynthesis, bacterium defense response, JA response, ABA response

* Additional analysis options
  + Wei 2017 table 2 is highly connected nodes (hub genes) and bottlenecks (high centrality hubs)- check this list for any overlaps!!
  + Polygenicity in B. cinerea eQTL for A. thaliana transcripts: are most genes only linked to one SNP/ locus? Or multiple?
  + Could also look at gene ontology (GO) and tissue/ ontogeny expression (Atlas) for A. thaliana genes linked to B. cinerea eQTL
    - Also, can do GO enrichment across full gene list or across individual clusters (if condensing eQTL and targets into interaction networks)
    - Mostly done (REVIGO, TAIR) but could also do GOrilla and atlas
  + Could filter A. thaliana transcripts by high B. cinerea heritability prior to further analysis (optional filtering step)
    - Could do the same for B. cinerea genes with high B. cinerea heritability
  + Look at the total number of significant locus-transcript connections ~ eQTL “edges”
    - For A. thaliana
    - For B. cinerea
    - Have the option of clustering into linked networks based on these. Could look at network structure, gene ontology, look for overlap with Wei’s networks, etc.
  + Which chromosomes are the hotspots on?
    - Where on the chromosomes?
    - Any trend toward center/ teleomere?
    - Any physical linkage between hotspots?
  + Could add a map of workflow- see Wu 2015 Figure 1A for inspiration
  + In the future, could use Wu 2015 GPLS approach rather than coexpression network analysis- may have higher predictive power