Introduction

Plant-pathogen interactions can be classified in two groups; qualitative interactions, in which few genetic variants interact to determine binary disease outcomes, or quantitative, in which a spectrum of interactions may occur due to genetic variation between the host and pathogen. The genetic basis of quantitative plant-pathogen interactions is less understood and is being explored through the study of phenotypic variation across genetically diverse hosts and pathogens, finding links to genetic variation. Interactions between plants and generalist pathogens, which do not specialize on particular hosts, are more often quantitative due to a lack of reciprocal co-evolution, and generalist pathogens often harbor higher genetic diversity {Williamson 2007}.

Within a generalist pathogen, there are several theories for how one species can adapt to a broad host range. Perhaps the pathogen has evolved a singular resistance gene of broad effect that disables core defense pathways across diverse plant host species; a sort of silver bullet. This mechanism would predict low diversity in the pathogen as the silver bullet gene is fixed in the population. However, we see very high diversity in *B. cinerea* and no evidence of a single gene with high linkage to virulence, so we can eliminate this hypothesis. A second prediction for an extreme generalist species is specialization at the level of the individual. This would predict high population structure and moderate diversity. However, we observe low population in *B. cinerea*, high diversity, and a lack of evidence for individual specialization to hosts. Our final prediction is specialization at the gene or allele level, which would select for very high diversity and low population structure as the different genetic strategies are intermixed within individuals. This is consistent with the SNP diversity and low population structure observed in previous studies of *B. cinerea*, due to a combination of random mating and frequent recombination {}.

There is a lack of evidence for qualitative virulence/ resistance genes underlying quantitative disease outcomes in plant-pathogen interactions. Rather, the genetic basis of plant resistance in these interactions is highly polygenic (Glazebrook 2005, Nomura, Melotto et al. 2005, Goss and Bergelson 2006, Rowe and Kliebenstein 2008, Barrett, Kniskern et al. 2009, Corwin, Copeland et al. 2016, Fordyce, Soltis et al. 2018). X to X loci are implicated with relatively small effect sizes, and these genes have varied functions including X.

On the pathogen side, recent studies have accumulated evidence for a polygenic basis of virulence as well {Corwin 2016; Zhang 2017; Atwell 2018; Soltis 2019}. These studies provide many candidate loci for pathogen resistance, with diverse functional annotations. Some of these loci appear to modulate virulence across multiple virulence phenotypes, including lesion size across multiple hosts and independent phenotypes of lesion growth (Corwin, Copeland et al. 2016, Fordyce, Soltis et al. 2018, Soltis, Atwell et al. 2019). However, thus far we know little about the molecular mechanism of action by which these genes affect virulence outcomes.

Many measurable phenotypes result from the interaction of plant and pathogen, including gene expression responses. Each expression profile may be considered a unique indicator of the progression of the interaction between host and pathogen. As such, analysis summarizing information across transcriptomes can elucidate the common and specific genetics underlying virulence phenotypes and hypothesize causal relationships between genetic variation and expression responses. Individual gene expression profiles can be treated as phenotypes for analysis such as genome-wide association (GWA).

Expression quantitative trait loci (eQTL) are the markers correlated with variation in transcripts’ expression profiles and are hypothesized as points of direct or indirect genetic control over expression variation. Locally linked (*cis*) eQTL may indicate regulatory variation within the expressed gene itself, or nearby. Additional markers distant from the responding gene are classified as *trans*-eQTL. *Trans*-eQTL may be due to genes present in a common regulatory network, or transcription factors acting upon the expressed gene. *Trans*-eQTL hotspots (loci linked to expression variation across many transcripts) may point to master regulators, with extensive pleiotropy across many genes.

Previous studies have examined eQTL in pathogens…

Studies encompassing transcriptomic variation in both a host and pathogen, and genomic variation within one of the interacting organisms, can look for signs of interspecific *trans*-eQTL; loci in the pathogen that modulate expression in the infected host, or loci in the host that modulate expression in the infecting pathogen. A few studies have examined variation in host-pathogen interactions in this way, validating the ability of this approach to identify pathogen loci modulating host expression levels, and thus candidate loci for interspecific signals (Wu, Cai et al. 2015, Guo, Fudali et al. 2017). A small number of previous studies have identified cross-species trans-eQTL, as a way to hypothesize causal relationships between individual genes in the interspecific interaction (Wu, Cai et al. 2015, Guo, Fudali et al. 2017). eQTL identified through this approach include one locus in a plant pathogenic nematode which modulates expression of >60 genes in its host *Medicago truncatula* (Guo, Fudali et al. 2017). Further, in *M. truncatula* a total of 213 genes in the host were linked to one or more loci in the pathogen, and functionally enriched for transcription factors as well as defense-related enzymes and enzymes involved in essential amino acid biosynthesis (Guo, Fudali et al. 2017). In mouse, 1054 host genes were linked to one or more loci in the pathogen, across a total of 208 pathogen eQTL (Wu, Cai et al. 2015). These host genes were enriched for ATP response, metabolic functions, and antimicrobial and inflammatory immune responses, and genes from the same host network often shared the same eQTL {Wu 2015}. Many of the host genes were linked to multiple parasite eQTL {Wu 2015}. They were able to validate 14 out of 15 host genes selected based on predicted immune activity {Wu 2015}.

On the host side, similar methods can identify human host genetic polymorphisms affecting bacterial parasite gene expression; three bacterial genes were regulated by these identified host eQTL (Guo, Fudali et al. 2017).

For this work, we focus on an extreme generalist pathogen with high genetic diversity, *B. cinerea*, and the model plant host, *A. thaliana*. *B. cinerea* exhibits highly quantitative virulenceThese interactions are well-characterized phenotypically, and we have previous information on some of the potentially relevant genetic factors on both the pathogen and host side. This also gives us the opportunity to connect our findings, particularly in plant genetic targets and affected pathways, to many previous datasets.

Previous studies in the *A. thaliana* - *B. cinerea* pathosystem point to control of expression variation on the host side of the interaction … Detached leaves of wildtype *A. thaliana* and major immune pathway mutants were inoculated with 96 genetically variable isolates of *B. cinerea,* and at 18 hours post inoculation, mRNA was collected. Variation in expression of *A. thaliana* genes was very sensitive to pathogen genetic variation; expression of host genes was under approximately equal regulation from genetic variation across the *B. cinerea* isolates, and host immune-pathway responsive variation across the *B. cinerea* isolates {Zhang 2017}. This far exceeded the contribution of the major host immune pathway variants to variation in gene expression {Zhang 2017}. The host-pathogen genetic interactions target four major host response networks; jasmonic acid and salicylic acid signaling and camalexin biosynthesis, defense and cell cycle, and two photosynthesis networks {Zhang 2017}.

The authors analyzed co-expression of genes across the *B. cinerea* isolates and *A. thaliana* immune pathway mutants. Genes were condensed into co-expression networks, which hypothesize causal links between many genes in an interacting web. However, these analyses do not untangle the directionality of effect from one gene, one pathway, or one genome to another.

In this study, we ask how genetics within the pathogen may modulate expression variation over the course of infection. We work with the gene expression data from Zhang *et al*., performing genome-wide association (GWA) of variation in individual transcript expression profiles with SNP level variation within the *B. cinerea* genome when infecting the wildtype host Col-0 *A. thaliana*. This gives us a hypothesis of directionality; any locus in *B. cinerea* linked to expression variation in the host or pathogen is directly or indirectly modulating expression. With numerous traits in this analysis, we focused on general patterns of eQTL distribution across the genome, and identification of major hotspots of eQTL.

Any genes linked to expression variation of many members of the previously described *A. thaliana* and *B. cinerea* virulence co-expression networks both affirms the biological relevance of the pathway and suggests a genetic control factor in pathway-level expression variation. Determining the pathogen genetic control of both host and pathogen gene expression over the course of infection can give us inference into points of genetic control over virulence pathways in the pathogen. Further, it can elucidate the sensitive host pathways, to inspire a search for potential resistance alleles among host variants. If we consider the full transcriptome of host and pathogen, this provides us thousands of phenotypes to test in genome-wide association, and we can deepen our search for loci which control multiple phenotypic measures of the progression of the plant-pathogen interaction. We can build inference on which genes in the pathogen are core factors in the virulence interaction, and which are uniquely controlling specific attributes of the interaction.

Introduction outline

1. ~~Plant-pathogen interactions~~
   1. ~~Qualitative: binary disease states~~
   2. ~~Quantitative: continuous disease states~~
   3. ~~Little information on quantitative disease genetics~~
      1. ~~So far: evidence for lack of qualitative R genes in plants/ virulence genes in quantitative disease pathogens~~
      2. ~~Patterns of quantitative disease genetics~~
         1. ~~Quantitative genetic basis on plant side of virulence as lesion size: Corwin (and earlier), Fordyce 2018, Zhang 2017~~
            1. ~~Highly quantitative/ polygenic~~
            2. ~~Patterns of which loci are involved~~
            3. ~~Other species/ labs… LIT REVIEW~~
         2. ~~Quantitative genetic basis on pathogen side of virulence as lesion size: Soltis 2019, Atwell 2019~~
            1. ~~Also highly quantitative/ polygenic~~
            2. ~~Patterns of which loci are involved~~
            3. ~~Other species/ labs… LIT REVIEW~~
         3. ~~Shared loci across multiple phenotypes? Larger emerging patterns of genetic players in quantitative virulence outcomes~~
            1. ~~Soltis 2018, commonalities across plant hosts~~
            2. ~~Fordyce 2018 (multiple measures of lesion), Corwin/ Zhang for lesion size or expression on multiple host genotypes…~~
            3. ~~Other species/ labs…~~
2. ~~Transcriptome variation (gene expression profiles) as many quantitative phenotypes describing the interaction between host and pathogen.~~ 
   1. ~~Insight into shared and unique bases of virulence phenotypes?~~
   2. Quantitative genetic basis on plant side of expression variation: Zhang
      1. Patterns of how many loci
      2. Patterns of which loci
      3. Something about networks, idk
   3. Other species/ labs… LIT REVIEW
3. ~~eQTL to learn genetic control of expression phenotypes~~
   1. ~~cis eQTL: regulatory variation within target gene, or closely linked~~ 
      1. ~~(promoter, local structure, ~ operon analogue?)~~
   2. ~~trans eQTL: regulatory variation distant from target gene.~~ 
      1. ~~Mechanism? Shared network/ transcription factor/ other reasons for correlation…~~
   3. ~~Trans eQTL hotspot: “master regulator” idea~~ 
      1. ~~could be transcription factor/ core of interacting pathway/ ???~~
4. need a logical link here
5. ~~eQTL in plant-pathogen interactions~~
   1. eQTL in plant host
      1. LIT REVIEW, major findings – number of loci, function?
         1. Any hotspot analyses/ validation?
   2. eQTL in pathogen
      1. examples from Malaria…
         1. Gonzales 2008
         2. Zhu 2018
      2. do a deeper LIT REVIEW… is there anything here? Haven’t really found it
6. ~~interspecific trans eQTL in plant-pathogen interactions~~
   1. ~~very few studies~~
      1. Major patterns?
      2. ~~Guo 2017~~
         1. ~~Medicago + parasitic nematode (parasite eQTL, host expression)~~
         2. ~~Human + Salmonella (host eQTL, parasite expression)~~
      3. Wu 2015
         1. Mouse + Plasmodium (parasite eQTL, host expression)
   2. What would these hits mean?
      1. Vaguely, signs of network-network crosstalk between plant and pathogen
         1. Network x network coevolution rather than gene x gene
            1. Do I talk about generalists here? Or earlier? Or never?
7. Our study system for cis eQTL, trans eQTL hotspots, and interspecific trans eQTL
   1. Botrytis
      1. quantitative virulence… cite
      2. quantitative genetics of virulence… cite
   2. Arabidopsis
      1. Model host, efficient system – cite plant path studies
      2. Well defined genetics for validation, pathway information
   3. Botrytis-Arabidopsis pathosystem
      1. Botrytis genetic component describing Arabidopsis infection phenotypes
         1. Zhang
8. Our study methods
   1. Detached leaf assay
   2. mRNA isolation from infected leaf, 18hpi
   3. transcript expression levels
   4. GWA to X Botrytis SNPs
   5. Selection for top SNP per transcript

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