**Probing how tomato domestication affected virulence genetics in a population of *Botrytis cinerea***

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**Abstract**

Plant domestication alters variation in many phenotypes, including pathogen resistance. It is unclear what effect plant domestication has on quantitative plant resistance and generalist pathogen virulence. Many plant-pathogen studies focus on qualitative resistance loci in plants against pathogens; relatively little is still known about quantitative resistance in plants, and the corresponding virulence loci in pathogens. To study quantitative virulence in a generalist pathogen like *Botrytis cinerea*, we must work with genetic variation within the pathogen, and diverse plant hosts. In this study, we quantified variation in lesion size of 91 *B. cinerea* isolates on 6 domesticated *Solanum lycopersicum* and 6 wild *S. pimpinellifolium* genotypes. We find that lesion size variation is controlled by plant genotype, plant domestication status, and pathogen genotype. Through genome-wide association (GWA) of lesion size variation to *B. cinerea*, we find a highly quantitative basis of virulence on tomato, with thousands of genes contributing. We find a subset of genes that control lesion size variation across tomato genotypes, and many genes that uniquely associate with few host genotypes.

**Introduction**

The progression of a plant disease is mediated by the complex interaction of diverse molecular pathways of the host and pathogen. The resulting disease outcome is the sum of pathogen virulence/sensitivity and host plant susceptibility/resistance mechanisms, specific to the interacting genotypes of the host and pathogen. A key aspect controlling the genetic architecture of these traits is the host range of the pathogen. Specialist pathogens are a major focus in plant pathology; virulent on a narrow range of hosts, and often limited to a single species or genus. This narrow and often obligate host range for the pathogen can enhance co-evolution between host resistance genes and pathogen virulence mechanisms. Most known genes for plant resistance to specialist pathogens confer qualitative resistance through plant innate immunity, via large-effect loci that enable the recognition of the pathogen by the plant {Dodds 2010; Pieterse 2012; Dangl 2001; Jones 2006}. These recognition signals can be conserved pathogen patterns such as cell-wall polymers or flagellin, or alternatively, specific virulence factors that block perception of the pathogen, but in turn are detected by plant proteins that guard the signaling networks {Ferrari 2007; Dodds 2010; Jones 2006; Boller 2009; Bittel 2007}.

In contrast to specialist pathogens, generalist pathogens are virulent across a wide range of diverse plant hosts. Generalist pathogens may have less stringent co-evolution in connection to specific hosts and their accompanying resistance mechanisms, because these pathogens can easily shift to more favorable niches by moving from host to host. Thus, generalist pathogens can evade the rapid evolution of new resistance mechanisms within specific hosts, and re-infect specific hosts upon evolution of any new virulence mechanism. This niche-shifting ability may partly explain the observation that most naturally variable plant genes for resistance to generalist pathogens are quantitative in their effect, rather than qualitative. For example, there are no known naturally variable large-effect resistance loci against generalist pathogens such as *Botrytis cinerea* {Rowe 2008; Corwin 2016; Glazebrook 2005; Goss 2006; Barrett 2009; Nomura 2005}. Modern genomic approaches are rapidly identifying the causal genes controlling plant quantitative resistance to generalist pathogens. Unlike qualitative resistance loci that predominantly involve genes in signaling cascades, the quantitative resistance genes also include a broad array of direct defense genes like those involved in secondary metabolite production, cell wall formation, and defense proteins {Ferrari 2007; Poland 2009; Denby 2004; Zipfel 2004; Walz 2008; Zheng 2006; Rowe 2008; Corwin 2017}. The effect of these quantitative plant resistance loci is highly dependent upon the infecting pathogen’s specific genotype. For example, the ability of *Botrytis* to infect *Arabidopsis* is partly dependent on whether the specific isolate is sensitive or resistant to a key defense compound, camalexin {Kliebenstein 2005}. However, very little is known about the number of virulence loci within generalist pathogens that contain causal polymorphisms, or the genetic architecture of these loci. There are no reported naturally variable large-effect virulence loci in generalist pathogens, suggesting that virulence is controlled by quantitative genetic variation in these pathogens. Thus, to truly understand quantitative host-pathogen interactions, we need to work with genetic variation in both the host and pathogen.

A key evolutionary process in plants that has affected resistance to specialist pathogens is domestication from wild plants to crop plants. Domesticated plant varieties are typically more sensitive to specialist pathogens than are their wild relatives {Smale 1996; Couch 2005; Rosenthal 1997; Dwivedi 2008}, and pathogens may evolve higher virulence on domesticated hosts {Stuckenbrock 2008}. Further, domestication typically imposes a strong genetic bottleneck that reduces genetic diversity in the crop plant, and often decreases the availability of resistance alleles against specialist pathogens in the crop plant germplasm. This loss of diversity in resistance alleles is assumed to extend to all domesticated varieties, particularly if cultivated plants experience reduced selective pressures from pathogens. These general evolutionary patterns, of lower resistance and allelic diversity found when studying the interaction of specialist pathogens with crop plants, are assumed to similarly hold for generalist pathogens and their domesticated hosts. However, we have less information about how crop host domestication affects disease caused by generalist pathogens, when the resistance to these pathogens is quantitative and polygenic rather than qualitative and monogenic. As such, there is a need to conduct a detailed analysis of how domestication may alter the interaction of a plant with a broad generalist pathogen.

*Botrytis cinerea* provides a model generalist pathogen for studying quantitative interactions with plant hosts, and underlying evolutionary processes for this generalist in contrast to specialist pathogens. *Botrytis cinerea* is a broad generalist pathogen that can infect most tested plants from bryophytes to eudicots and causes pre- and post-harvest crop losses in many plant species {Nicot 1996; Elad 2007; Fillinger 2015}. Individual isolates of *B. cinerea* display the same broad host range as the generalist species {Deighton 2001; Finkers 2007; Ten Have 2007; Corwin 2016} in contrast to pathogens like *Fusarium oxysporum* where the species can infect a number of hosts, but each isolate is highly host specific {Katan 1999; Barrett 2012}. Even though *B. cinerea* isolates have broad host ranges, individual isolates display significant variation in virulence phenotypes. Genetic variation between pathogen isolates in the production of the phytotoxins, botrydial and botcinic acid, differentially controls virulence on host plants including tomato {Siewers 2005; Dalmais 2011}. More recently, natural variation in VELVET, a gene involved in development and secondary metabolism, led to quantitative variation in virulence on multiple host plants {Schumacher 2012}. Additionally, *B. cinerea* has genetic variation in virulence genes that can control degradation of different plant cell walls {Rowe 2007}. In combination, the genetic variation in diverse virulence mechanisms can contribute to the formation of quantitative differences in virulence between the isolates {ten Have 1998}. In support of this is genomic sequencing of diverse *B. cinerea* isolates, showing that they contain a high level of genomic sequence diversity spread across the genome. The polymorphism rate in *B. cinerea* is 6.6 SNP/kb in this study which is more variable than previously studied plant pathogens (1-2 SNP/kb in *Blumeria graminis*, 5.5 SNP/kb in the compact genome of the obligate biotroph Plasmodiophora brassicae) {Hacquard 2013; Wicker 2013}, and on par with the genetic diversity found in the human pathogen *Mycobacterium tuberculosis* (2.9 to 6.2 SNP/kb){Power 2017; Farhat 2013; Desjardins 2016}. Further, these isolates show that the species has a high level of recombination and genomic admixture. As such, this collection of *B. cinerea* isolates contains genetic variation in a wide range of virulence mechanisms, offering the potential to challenge the host with a blend of diverse virulence mechanisms, and the potential to identify the pathogen variation controlling quantitative virulence even in non-model plant systems.

On the plant side, resistance to *B. cinerea* is dominated by quantitative and highly polygenic resistance, with no evidence for qualitative resistance {Rowe 2008; Corwin 2016}*.* A model pathosystem for studying quantitative interactions is the tomato-*B. cinerea* system, where the pathogen causes crop loss due to both pre- and post-harvest infection. Resistance to *B. cinerea* is a quantitative trait in tomato, with identified tomato QTLs each explaining up to 15% of phenotypic variation for lesion size on stems {Finkers 2007}. Tomato is a model system for study of the impact of domestication upon plant physiology and resistance {Panthee 2010; Bergougnoux 2014; Tanksley 2004; Bai 2007}. This includes evidence that tomato domestication has altered the circadian clock phase {Muller 2016}, which can modulate resistance to *B. cinerea* {Sauerbrunn 2004; Weyman 2006; Bhardwaj 2011}. Further, *B. cinerea* infection can influence the amplitude of circadian oscillations in clock gene expression {Windram 2012}, and both the plant and pathogen clocks impact *B. cinerea* virulence {Hevia 2015}. This suggests that host plant domestication within tomato can alter traits known to influence *B. cinerea* resistance from other systems. Thus, the tomato-*B. cinerea* pathosystem allows us to directly test how genetic variation in a generalist pathogen may be influenced by domestication in a crop plant.

In this study, we conducted genome-wide association (GWA) in *B. cinerea* to test how it broadly responds to host phenotypic variation, and more specifically to domestication. We examined the contributions of tomato variation, domestication, and *B. cinerea* genetic variation to lesion size in on detached leaves. Lesion size of *B. cinerea* is a quantitative trait, controlled by genetics in both the plant and the pathogen {Rowe 2008}. In our analysis of lesion images at 72 hours post inoculation, both host and pathogen genotypes contribute to virulence. Our findings suggest that individual isolates are generalists across tomato genotypes and across domestication in *Solanum.* A subset of single isolates, however, are sensitive to tomato domestication. We do not find evidence for host specialization; *B. cinerea* isolates collected from tomato tissues are not within the most-virulent isolates on tomato. At the genetic level, virulence of *B. cinerea* is highly quantitative, with hundreds of significant SNPs with small effect sizes associated with lesion area on each tomato genotype.

**Methods**

**Tomato genetic resources**

We obtained seeds for 12 selected tomato genotypes in consultation with Dr. Roger Chetelat at the UC Davis TGRC. These include a diverse sample of 6 genotypes of domesticated tomato’s closest wild relative (*S. pimpinellifolium*) from throughout its native range (Peru, Ecuador) as well as 6 heritage and modern varieties of *S. lycopersicum*. We bulked all genotypes in long-day (16h photoperiod) greenhouse conditions at UC Davis in fall 2014. We grew plants under metal-halide lamps using day/night temperatures at 25°C/18°C in 4” pots filled with standard potting soil (Sunshine mix #1, Sun Gro Horticulture). We watered plants once daily. We pruned plants and staked them upright, and collected fruits as they matured. We stored fruits at 4°C in dry paper bags until seed cleaning. We incubated seeds and locule contents at 24°C in 1% protease solution (Rapidase C80 Max) for 2h, then rinsed them in dI H2O and air-dried. We then stored seeds in a cool, dry, dark location until further plantings.

To grow plants for detached leaf assays, we bleach-sterilized all seeds prior to germination on paper in growth chambers, in flats covered with humidity domes. At 7 days we transferred seedlings to soil (SunGro) and grew all plants in growth chambers in 20°C, short-day (10h photoperiod) conditions with 180-190 uM light intensity and 60% RH. We bottom-watered with dI H2O every two days for two weeks, and at week 3 watered every two days with added nutrient solution (0.5% N-P-K fertilizer in a 2-1- 2 ratio; Grow More 4-18-38). We used the plants for detached leaf assays 6 weeks after transferring seedlings to soil.

**Botrytis genetic resources**

We sourced the *B. cinerea* collection from single-spore isolates from fruit and vegetable tissues as described by Atwell {Atwell 2015}. We extracted DNA from *B. cinerea* hyphal cells and sequenced by Illumina GAIIx or HiSeq as described by S. Atwell {Atwell 2015}. We cleaned and aligned the sequencing data and made variant calls as previously described {Atwell 2015}. For the 91 isolates used in this study, we utilized a total of 272,672 SNPs with MAF 0.20 or greater, and less than 10% missingness (SNP calls in at least 82/ 91 isolates). Successful GWA studies have been completed in other pathogens with as few as 75 individuals, and as few as 3,000 SNPs due to the small size of many microorganism genomes {Power 2017}.

**Botrytis growth**

We maintained *B. cinerea*  isolates as conidial suspensions in 30% glycerol for long term storage at -80°C. For regrowth, we diluted spore solutions to 10% concentration in filter-sterilized 50% grape juice, and then inoculated onto 39g/L potato dextrose agar (PDA) media. We grew isolates at 25°C in 12h light, and propagated every 2 weeks.

**Detached leaf assay**

To study the effect of genetic variation in host and pathogen on lesion formation, we infected detached leaves of 12 diverse tomato varieties with the above 91 *B. cinerea* isolates. We used a randomized complete block design for a total of 6 replicates across 2 experiments. We placed leaflets on 1% phytoagar in seed flats, with humidity domes on top. For each plant genotype, we placed leaflets from each of 10 individual plants onto agar in blocks. We selected eaves by a random sample of 5 leaves per plant, and 2 leaflet pairs per leaf.

We collected spores from mature (1-2 week old) *B. cinerea* cultures, and diluted to 10 spores/ µL in filter-sterilized 50% grape juice. We inoculated 4µl droplets of spore suspensions onto detached leaves at room temperature with 24h light. We mock-inoculated control leaves with 4µL of grape juice without spores. We measured lesion development using digital photos of all leaflets at 24, 48, and 72 hours post inoculation in combination with downstream automated image analysis.

**Automated Image Analysis**

We measured lesion areas using the EBImage and CRImage packages {Pau 2010; Failmezger 2010} in the R statistical environment {R Development Core Team and Team 2009}. Leaflets were identified as objects with green hue, and lesions were identified as low-saturation objects within leaves. Images masks were generated for both the leaf and lesion, then manually refined by a technician to ensure accurate object calling. The area of these leaves and lesions were then automatically measured as pixels per lesion and converted to area using a 1 cm reference within each image.

**Data analysis**

We analyzed by F-test the linear model for the full experiment, including the fixed effects of isolate genotype, plant domestication (*S. lycopersicum* or *S. pimpinellifolium*), plant genotype (which is nested within species), experiment, and block (nested within experiment) on lesion area. We next included terms for the interactions of plant domestication with isolate, plant genotype with isolate, and experiment with isolate, plant domestication, or plant genotype as fixed effects. Adding terms for individual plant, leaf, and leaflet position did not significantly improve the full model, so we omitted them from further analysis. We also tested a mixed model with random effects of experiment and block, but this did not affect our interpretation of the fixed effects. Next we calculated the least-squared means of lesion size within each tomato genotype. For the within-genotype model we included the fixed effect of isolate, and the random effects of experiment, the isolate by experiment interaction, and leaflet pair (nested within leaf, nested within individual plant). We then used these means as the phenotype input to our custom bigRR script for GWA. SNP data included 272,672 loci at MAF 0.20 or greater and <10% missingness as described above. We imputed missing SNPs in bigRR. Because bigRR provides an estimated effect size, but not a p-value, we performed permutation analyses to determine effect significance. We permuted the phenotypes 1000x and re-ran bigRR, to establish 95%, 99%, and 99.9% thresholds for significance. We performed SNP annotation using SNPdat {Doran 2013} with gtf construction from the T4 gene models for genomic DNA ([http://www.broadinstitute.org](http://www.broadinstitute.org/), {Staats 2012}), annotating SNPs with genes within 2kb windows.Functional annotations are based on the T4 gene models for genomic DNA (http://www.broadinstitute.org, *B. cinerea*; {Staats 2012}). Additional genes of interest were taken from NCBI (https://www.ncbi.nlm.nih.gov/) and included by mapping sequence to the T4 reference using MUMmer v3.0 {Kurtz 2004}. We used the program InterProScan within BLAST2GO for functional annotation of the gene models (http://www.blast2go.com).

**Results**

**Experimental Design**

To directly measure how tomato domestication affects quantitative resistance, we infected a collection of 91 diverse *B. cinerea* isolates (genotypes) on 6 wild and 6 domesticated tomato genotypes. Previous studies have examined *B. cinerea* resistance between domesticated and distantly related wild tomato species using single isolates of pathogens {Egashira 2000; Nicot 2002; Guimaraes 2004; Ten Have 2007; Finkers 2008}. These previous studies typically used individual wild and domesticated tomato genotypes that were the founders of mapping populations, and found a wide range of pathogen resistance levels both within and between tomato species. However, it is still unknown how domesticated and closely related wild tomatoes compare for *B. cinerea* resistance using multiple plant genotypes and a population of the pathogen. We selected 6 domesticated *Solanum lycopersicum* cultivars and 6 wild *S. pimpinellifolium* genotypes, the closest wild relative of *S. lycopersicum*, to directly study the selection associated with the impact of domestication {Peralta 2008}. We used a previously collected sample of 91 *B. cinerea* isolates obtained from various eudicot plant hosts, including tomato stem tissue (2 isolates; T3, KT) and tomato fruit (3 isolates; KGB1, KGB2, Supersteak). We infected all 91 *B. cinerea* isolates onto each of the 12 plant genotypes in 3-fold replication across 2 independent experiments in a randomized complete block design, giving 6 measurements per plant-pathogen combination, for a total of 3,276 lesions. We digitally measured the area of the developing lesion at 72 hours post infection (HPI) (Figure R1). At 72 hours significant lesion growth was visible, but no lesions had spread to infect over half of the leaflet. Digital measurement of the area of the developing lesion provides a composite phenotype controlled by the interaction of host and pathogen genetics. This measurement of the plant-*Botrytis* interaction has been used successfully in a number of molecular and quantitative genetic studies {Ferrari 2003; Denby 2004; Kliebenstein 2005; Ferrari 2007; Rowe 2008}.

**Comparison of Pathogen Genetics, Plant Genetics and Crop Domestication Effects on Resistance**

To measure the relative contribution of genetic diversity in both the plant and the pathogen to variation in the virulence/ resistance phenotype, we used a linear model. This model directly tested the contribution of plant genotype, plant domestication status, and pathogen genotype (isolate) to variation in lesion size. The final model explained 60% of the variance for lesion size, and showed that genetic variation within both the host plant and the pathogen had significant effects on lesion growth, but pathogen isolate diversity explained 3.5x more of the variance than plant genotype (10.2% of total variance under isolate vs. 2.9% under plant, Table R1, Figure R4A). Interestingly, tomato domestication status significantly impacted *B. cinerea* virulence, as shown by the small but significant effects of genetic variation between domesticated and wild tomatoes (<1% of total variance, Table R1). There was no evidence for significant interaction effects between pathogen isolate and plant genotype, but this term contributed the largest proportion of the plant-related variance in lesion size (7.5% of total variance, Table R1). This lack of significance may be due to the vast number of degrees of freedom in this term (Table R1). Thus, the interaction between tomato and *B. cinerea* was significantly controlled by genetic diversity within the host plant and the pathogen, including a slight effect of domestication status.

**Domestication and Lesion Size Variation**

Existing literature predominantly reports that crop domestication decreases plant resistance to pathogens {Stukenbrock 2008; Smale 1996; Couch 2005; Rosenthal 1997; Dwivedi 2008}. In our analysis, we identified a significant difference in the resistance of wild and domesticated tomato to the population of *B. cinerea* isolates (p <2e-16, Table R1). This agrees with the theory that domestication decreases resistance, as the average lesion size was slightly greater (18% increase) on domesticated than on wild tomato genotypes (Table R1, Figure R2). However, this domestication effect was not the dominant source of variation, as genetic variation within the domesticated and wild genotypes contributed 3.8 fold more variation in resistance than domestication alone (2.9% vs. 0.8% of total variance, Table R1). So while we did observe the expected increase of susceptibility in domesticated tomato, domestication did not explain the major effects of tomato genotype on lesion size variation and there was significant genetic variation within both wild and domestic tomato species for *B. cinerea* resistance.

In addition to altering trait means, domestication commonly decreases genetic variation in comparison to wild germplasm due to bottlenecks during domestication, including for tomato {Tanksley 1997; Doebley 2006; Bai 2007}. We would expect this decreased genetic variation to restrict phenotypic variation, including disease phenotypes. Interestingly in this tomato population, the domesticated tomato genotypes had a wider range of average lesion size than wild genotypes; the 90th percentile range (95th percentile to 5th percentile) was 2.03 cm2 lesion size variation on domesticated tomato versus 1.76 cm2 variation on wild tomato. Additionally, the wild and domesticated tomato genotypes showed statistically similar variation in resistance (F-test, F=1.39, 96 df, p=0.11)(Figure R3). Overall, we found evidence for a slight domestication impact on average resistance to *B. cinerea* that depended on the host genotype, but no evidence of a phenotypic bottleneck due to domestication.

**Pathogen Specialization to Source Host**

One evolutionary model of generalist pathogens suggests that isolates within generalist pathogen species may specialize on specific hosts. Alternatively, isolates may also be generalists, with specialization absent or occurring only at the gene level. Our collection includes five pathogen isolates which may be adapted to tomato, as they were collected from *S. lycopersicum*. To test if there is evidence for specialization to the source host, we compared the virulence of the *B. cinerea* isolates obtained from tomato to the broader pathogen population. For *B. cinerea* genotypes isolated from tomato tissue vs. other hosts, there was no significant difference in lesion size across all hosts on domesticated tomato (t-test; t=1.10, 4.3 df, p=0.33), wild tomato (t-test; t=1.09, 4.2 df, p=0.33) or across all tomato genotypes (t-test; t=1.60, 9.7 df, p=0.14) (Figure R4E). In fact, one isolate collected from tomato tissue (KGB1) was within the 10 least-virulent isolates in this study (Figure R4E), and one was within the 10 most-virulent isolates (Triple3). This demonstrated significant genetic variation in virulence across the *B. cinerea* isolates, and that *B. cinerea* isolates are not strongly host-specific {Rowe 2007; Ma 2005; Stylianos 2012; Martinez 2003}.

**Pathogen Specialization to Host Variation**

Though we did not find evidence for *B. cinerea* adaptation to tomato based on isolate source host, the *B. cinerea* isolates may contain genetic variation that allow them to better attack subsets of the tomato genotypes. A visual analysis of the data suggested an interaction between the genomes of *B. cinerea* and tomato (Figure R4). However, when using the full model, we found no significant interaction between isolate and individual host genotype, even though there was a large fraction of variance within each term (Table R1). This may indicate a lack of interaction between genetic variation in the host and pathogen. However, this negative result may also be because F-tests in factors with high degrees of freedom can be underpowered, as in the case of the isolate x plant genotype interaction term (df: 940). To assess these two possibilities, we used an additional statistical approach to test for an interaction between *B. cinerea* and host genotype. We performed a linear model analysis individually on each isolate to directly test the fixed effects of domestication, plant genotype nested within domestication, and experiment. Through this single-isolate GLM analysis, none of the isolates show a significant (p < 0.05, FDR corrected) interaction with host genotype. However, when looking across the full isolate population, performance does vary between host genotypes. When comparing mean lesion size between paired plant genotypes, 58% of host pairs significantly affected the distribution of lesion sizes across all isolates (Wilcoxon signed-rank test, Table R2). This pattern was consistent, irrelevant of whether we compared only domesticated host pairs, wild host pairs, or pairs across species (Wilcoxon signed-rank test, Table R2). As such, we do not find evidence that any subset of isolates show sensitivity to tomato genetic variation, but variation between tomato genotypes does change the distribution of lesion sizes across our *B. cinerea* population.

While none of the individual isolates showed differential sensitivity to genetic variation between tomato genotypes, we used the same approach to test if isolates show sensitivity to genetic variation associated with tomato domestication. Under the single-isolate ANOVAs including the fixed effects of plant, domestication, and experiment, two isolates showed a significant effect of domestication on lesion size (p < 0.05, FDR corrected) (Figure R4F). These included one of the highly virulent isolates (Fd2), and one of the saprophytic isolates (Rose), suggesting that *B. cinerea* adaptation to tomato domestication is not dependent on isolate virulence. Both of these isolates were more virulent on domesticated than on wild tomato. Further, isolate ranking by mean lesion size differed between domesticated and wild hosts (Wilcoxon signed-rank test, W = 5946, p-value = 0.002) (Figure R3), suggesting a broader pattern of *B. cinerea* specialization to domestication, among a subset of isolates. These domestication-sensitive isolates may be adapted to domesticated tomato, or more broadly to domesticated plants.

**Quantitative Genetics of Pathogen Virulence on Tomato**

Genetic variation within *B. cinerea* had a large effect on virulence on tomato and responded to domestication within tomato. This suggests that there is genetic variation within the pathogen in which some alleles enhance and other alleles decrease virulence. To potentially identify these pathogen genes controlling differential virulence, we proceeded to conduct a genome wide association mapping analysis within the pathogen. Due to the large effect of plant genotype on resistance to *B. cinerea*, we performed GWA using the model-corrected least-squared mean virulence measured on each tomato genotype as a separate trait. We used a ridge-regression approach in combination with 272,672 SNPs from *B. cinerea* to estimate the phenotypic effects across the genome {Shen 2013}. To determine significance of SNP effects, we permuted phenotypes 1000 times to calculate 95, 99, and 99.9% effect size thresholds within each plant host.

This GWA analysis showed that the genetic basis of *B. cinerea* virulence on tomato is highly polygenic. We identified from 1,284 to 25,421 SNPs within *B. cinerea* that were significantly associated with altered virulence on the 12 different host genotypes (SNP effect size estimate exceeded the 99% threshold). Interestingly, some of these SNPs were found for virulence on all of the different tomato genotypes with 5 SNPs significant on all 12 tomato hosts (Figure R5B). By chance, we would expect only 2.72e-19 SNPs to overlap for 12 traits over the 99% effect threshold in a set of 272,672 SNPs. 215 SNPs were called in at least 10 hosts, and 3.3k SNPs were called in at least half of the hosts while 27% (46,000) of the significant SNPs were linked to virulence on only a single host tomato genotype. Focusing on only the top 1000 SNPs (largest estimated effects) per phenotype, the overlap across phenotypes is reduced, with 1 SNP called in 10 of the hosts, 68 SNPs in at least 6 hosts, and 76% of significant SNPs unique to a single phenotype (Figure R7B). However, this overlap is increased when we annotate candidate genes within 2kb of each of the top 1000 SNPs per phenotype. We find 1 gene linked to all 12 phenotypes, 41 genes linked in at least 6 hosts, and only 56% of genes uniquely linked to a single phenotype. This is indicative of multiple haplotypes contributing to virulence at the candidate genes, with individual SNPs sampling unique haplotypes within a region. These findings suggest that there is significant genetic variation in *B. cinerea* virulence that is dependent upon the hosts genetic background, which is in agreement with the fraction of variation attributed to this term in the linear model. Thus, the pathogen appears to rely on polygenic small effect loci to customize virulence on the different tomato hosts.

**Quantitative Genetics of Pathogen Response to Tomato Domestication**

To directly map the *B. cinerea* genes that control differential virulence on wild and domestic tomatoes, we used the least-squared mean virulence of each isolate on all wild and all domesticated tomato genotypes as two traits. We also calculated a domestication sensitivity trait; the difference in lesion size for each isolate between domesticated vs. wild hosts. Using these three traits, we conducted GWA within *B. cinerea* to map genes in the pathogen that respond to domestication shifts in the plant. Many SNPs exceeded the 99% threshold for domestication phenotypes as well as the phenotype of lesion size on individual plant genotypes (Figure R6). *B. cinerea* response to tomato domestication appears to be polygenic, with many loci of small effect sizes which are trait dependent. Domestication sensitivity often identified unique SNPs from domesticated or wild tomato alone (Figure R8).

We annotated genes from *B. cinerea* T4 gene models within 2kb of significant SNPs. At the gene level, 8 genes were associated with domesticated, wild, and domestication sensitivity phenotypes, and 1040 genes were uniquely identified by a single domestication phenotype (Figure R8C). A total of 1935 genes contained significant SNPs (>99% SNP within 2kb) when studied for one or more of the domestication phenotypes (Table S1). These 1935 genes represent a total of 723 functional categories as annotated. Of these, only 17 functions are significantly overrepresented (Fisher exact test, p=0.05; Table S1) when compared to the whole-genome annotation of 14539 genes and 2539 functions. These functional categories include enzymes, metal ion binding, transport, catalysis, signaling, gene silencing and mRNA splicing. None of the overrepresented functions include classical virulence or pathogenicity. This suggests that most variation in *B. cinerea* genetic control of virulence acts to change biochemistry in the pathogen.

**DISCUSSION**

The genetics of plant resistance to generalist pathogens are mostly quantitative, depend upon pathogen genotype, and include many direct defense genes. Previous studies found a quantitative genetic basis of tomato {Finkers 2007} and Arabidopsis {Rowe 2008; Corwin 2016} resistance to *B. cinerea*. Domestication is expected to reduce the genetic variation available for plant resistance to pathogens. Resistance to *B. cinerea* varies between domesticated and wild tomato species {Egashira 2000; Nicot 2002; Guimaraes 2004; Ten Have 2007; Finkers 2008}, but it was previously unclear how *B. cinerea* virulence responds to tomato domestication. In this study we addressed the pathogen side of the contributions of genetic variation to virulence, in response to plant genotype and host domestication. *B. cinerea* virulence on tomato, as measured by lesion size, is a product of pathogen genotype, host genotype, and host domestication status. Tomato domestication affects virulence less than expected, we do not see evidence of a domestication bottleneck. We find little evidence for specialization of isolates to tomato, supporting the hypothesis that *B. cinerea* is a generalist at the isolate level. The genetics underlying *B. cinerea* virulence on tomato are highly quantitative, and vary with tomato genotype and domestication status. Some genes contribute to virulence on most of the hosts tested, and we find some evidence for domestication-sensitive genes within *B. cinerea*.

**Domestication and altered pathogen virulence genetics**

Our results provide evidence of a mild host domestication effect on resistance to the generalist pathogen, *Botrytis cinerea.* However, domestication status alone is a poor predictor of a specific tomato host’s resistance to infection by *B. cinerea*. This suggests that while plant domestication does affect this plant-pathogen interaction, it is not the primary evolutionary force in defining this interaction. We measured an 18% increase in susceptibility across domesticated varieties, but this contributes less than 1% of the total variance of *B. cinerea* lesion size on tomato. This effect of host domestication varies across the *B. cinerea* genotypes, and we were able to identify specific loci in the pathogen that control domestication sensitive virulence. This supports the approach of studying natural variation within *B. cinerea* to understand the factors contributing to *B. cinerea* virulence and how this is altered by crop domestication. Studies of few isolates could miss the host domestication effect entirely, or provide a false positive signature of uniformly elevated virulence on domesticated hosts.

Host domestication is theoretically expected to decrease resistance to pathogens as alleles are lost in the domestication bottleneck as found for specialist pathogens [GIVE EXAMPLES]. Surprisingly, we did not find evidence for a domestication bottleneck in the phenotypic resistance to *B. cinerea*. This is in contrast to previous studies that explicitly show that there is a genotypic bottleneck within tomato domestication {Miller 1990; Koenig 2013}. This suggests that at least for this generalist pathogen, the genetic bottleneck has not imparted a phenotypic bottleneck. One possible explanation is that resistance to this pathogen is so polygenic in the plant that our experiment is not sufficiently large to pick up this effect using phenotypic variance. These patterns, of mild increase in resistance to *B. cinerea* due to plant domestication, and within-species plant variation exceeding the contribution of domestication itself, may be unique to interactions between *B. cinerea* and tomato, or more general. It remains to be seen if these patterns hold for *B. cinerea* on its other host plants. It is unclear whether domestication has a universal effect on plant resistance to *B. cinerea*, or if each domestication event is unique.

**Polygenic quantitative virulence and breeding complications**

Our results indicate a highly polygenic basis of quantitative virulence of the generalist *B. cinerea* on tomato. The effect size of individual SNPs is very small (on the scale of 0.01 mm2), and many SNPs, approximately 1200 – 25,000 on each plant genotype, are associated with *B. cinerea* virulence. This SNP effect estimate may be deflated, and number of contributing SNPs inflated, if individual SNPs are sampling several different haplotypes in the regions associated with *B. cinerea* lesion size. This genetic architecture of virulence is distinctly different from specialist pathogens that often have one or a few large effect genes that control virulence {De Feyter 1992; Keen 1992; Abramovitch 2004; Vleeshouwers 2014; Boyd 2013} but see {Lannou 2012}. Further studies are needed to test the relationships between SNP and haplotype effect size estimates in *B. cinerea* and to compare how the host plant species may affect this image of genetic variation in virulence.

Our results indicate some particular challenges for breeding durable resistance to generalist pathogens. The highly quantitative nature of *B. cinerea* virulence, and the variation between isolates, suggests that we cannot clone or introgress single genes to breed durable resistance against this pathogen. In contrast, we will likely need to work on breeding resistance through targeting entire pathways or mechanisms. To breed resistance to *B. cinerea* or other generalist pathogens, it is likely necessary to use a genetically variable pathogen population to properly phenotype the plant germplasm. Our study indicates the genetics of the specific host, the general domestication status, and the genetics of the pathogen will all combine to affect the estimated breeding value inferred from any experiment. As such, utilizing a single or even a few pathogen genotypes to guide resistance breeding in plants is unlikely to translate to durable resistance against *B. cinerea* as a species. However, the lack of a domestication bottleneck on tomato resistance to B*. cinerea* suggests that, at least for tomato, the domesticated germplasm has sufficient resistance alleles and it is not necessary to introgress genes or alleles from wild relatives to improve resistance.

**Molecular mechanisms and polygenic virulence**

The genetics of *B. cinerea* virulence do not conform to our expectations based on the genetics of virulence in specialist pathogen studies. The mechanisms of quantitative virulence identified in this study are in contrast to previously-described qualitative virulence loci. Major mechanisms we identified include enzymatic pathways and protein degradation and transport. The classic qualitative resistance pathways of pathogen sensing (receptors) and signaling (immune response pathways) are not the major contributors to quantitative resistance. We did not identify any MAMPs or PAMPs as major loci contributing to virulence across tomato varieties, nor any chitins, glycans, or glycolipids which are often recognized by plant receptors for qualitative resistance. Nor did we identify any mannans as top contributors to *B. cinerea* virulence {Corwin 2017}. Further, our identified loci did not include any known virulence loci, such as NEPs, or PGs.

**Conclusion**

This is one of the first studies examining the contributions of natural genetic variation to quantitative virulence in a plant pathogen, and the first explicit test comparing the effects of tomato domestication to tomato accession variation on pathogen virulence. We find that *B. cinerea* has a highly quantitative genetic basis of virulence on tomato, which is dominated by pathogen effects but also responds to tomato genotype and domestication. This study is only a single test of the *B. cinerea* response to host domestication; it remains to be seen how *B. cinerea* virulence responds to domestication more broadly. By extending future work to test additional domestication events, we may identify whether there is a consistent genetic basis of *B. cinerea* adaptation to plant domestication.

**FIGURES**

Table R1. Results of ANOVA from GLM of *B. cinerea* lesion area. Isolate is 91 *B. cinerea* genotypes, Domestication is *S. pimpinellifolium* or *S. lycopersicum*, Plant is 12 tomato genotypes nested within Domestication, Experiment is 2 replicate experiments, Block is 3 replicates nested within Experiment. Slash / indicates nesting, colon : indicates interactions between factors.

Table R2. Results of Wilcoxon signed-rank test for comparison of mean *B. cinerea* lesion area on pairs of plant genotypes. P-values are FDR corrected; bold text indicates significance at p<0.01 after correction, italicized text indicates non-significant.

Figure R1. *Botrytis cinerea* x tomato detached leaf assay and digital image analysis. Individual tomato leaflets of 6 *S. lycopersicum* genotypes and 6 *S. pimpinellifolium* genotypes are in randomized rows, spore droplets of individual *B. cinerea* isolates are in randomized columns. Digital images are collected 72 hours post inoculation (A). Digital masking of leaf and lesion (B) is followed by automated measurement of area for each lesion.

Figure R2. Relative susceptibility of tomato genotypes to *B. cinerea* infection. Violin plots are of lesion size due to *B. cinerea* growth on each tomato host genotype. Individual points are mean lesion size for each isolate-host pair.

Figure R3. *Botrytis cinerea* virulence responds to host domestication. The violin plots include each *B. cinerea* lesion on the host species. The interaction plot traces the average lesion size of a single *B. cinerea* isolate across the host species.

Figure R4. *Botrytis cinerea* virulence varies due to isolate-host interactions. Interaction plot of lesion size due to individual *B. cinerea* isolates on tomato host genotypes. The x-axis includes each tomato host genotype. Each line traces the average lesion size of a single *B. cinerea* isolate across hosts. A is a plot of all isolates, B-F highlight a subset of isolates. B is B05.10, C is the 10 most highly-virulent isolates, D is the 10 most saprophytic (low-virulence) isolates, E is 5 isolates collected from tomato tissue, F is 2 domestication-sensitive isolates.

Figure R5. *Botrytis cinerea* lesion size is a polygenic trait on tomato. A: Manhattan plot of *B. cinerea* lesion size GWA for a single accession, LA2706. B: Overlap in lesion size SNPs > 99% threshold across multiple host plant phenotypes. Chromosomes are differentiated by shading. Frequency is number of phenotypes in which the SNP exceeds the threshold. Vertical dotted lines indicate overlap between relatively large-effect SNPs for LA2706 and significance across the majority (≥6) of tomato genotypes tested.

Figure R7. Overlap in lesion size SNPs > 99% across individual hosts. A: Count of SNPS > 99% in common across individual plant hosts. B: Overlap in genes with SNPs >99% threshold within 2kb, across plant genotypes.

Figure R8. A: Top 50 SNPs for lesion size for each domestication phenotype. Domestication sensitivity is (domesticated – wild / domesticated). B: Venn diagram of SNPs identified >99.9% for each domestication phenotype. C: Venn diagram of genes with a significant SNP identified >99.9% for each domestication phenotype.

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