Genetic and ecological constraints to the evolution of virulence and reproduction in a plant pathogen

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DEDICATION

This thesis is dedicated to my dad who inspired me to go into science, and to my mom who put up with both of us.

ABSTRACT

Hosts and pathogens are engaged in an ongoing evolutionary struggle. In human-managed systems, rapid evolution of pathogen populations can reduce the effectiveness of important control methods such as antibiotics and genetic resistance in crop species. My thesis research investigates potential constraints to pathogen evolution by examining genetic and ecological factors affecting the evolution of infection and reproduction in the plant pathogen *Puccinia coronata.* I first investigate genetic variation underlying three pathogen life history stages within the host and show that variation in pathogen life-history stages within the host is affected by both the pathogen and host genotype. Next, I evaluate the relationship between pathogen infection and reproduction and show evidence of a trade-off between the number of resistant host genotypes infected and two key pathogen life history traits. Finally, I quantify the variation in infection and reproduction among eight different agricultural populations of *P. coronata* and ask whether the genetic diversity of the host population affects the evolution of pathogen infection and reproduction. While I do not find conclusive evidence that host genetic diversity affects the evolution of these traits, I do I find significant variation among populations that is not explained by pathogen population structure, indicating that selection structures pathogen populations.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1

Supplemental Tables

Chapter 2

Supplemental Tables

Chapter 3

Supplemental Tables

LIST OF FIGURES

Chapter 1

Chapter 2

Supplemental Figures

Chapter 3

Supplemental Figures

INTRODUCTION

Hosts and pathogens are engaged in an ongoing evolutionary struggle. The rapid evolution of antibiotic resistance in human pathogens (Maple et al. 1980, Baquero and Blazquez 1997, Davies and Davies 2010) and the recent eruption and spread of Ug99, a super-virulent strain of the stem rust pathogen, *Puccinia coronata*, threatening global wheat production (Pretorius et al. 2000, Singh 2010) suggest few limits to pathogen evolution. However work in natural host-pathogen systems demonstrates that coevolutionary dynamics are more complicated than the evolutionary arms race observed in human-managed systems (Thrall and Burdon 2003, Thrall et al. 2012). For example, Thrall and Burdon (2003) found that super-virulent strains of flax rust were restricted to resistant host populations, suggesting potential limits to the evolution of pathogen virulence. In addition, Stahl et al. (1999) and Bakker et al. (2006) found evidence that polymorphisms for host resistance have been maintained over long periods of time, again suggesting constraints to evolutionary arms-races. The above examples raise the central question of this thesis: what genetic and ecological factors limit pathogen adaptive evolution?

Trade-offs are deeply embedded in our understanding of evolutionary constraints and are predicted to constrain the evolution of several important pathogen traits (May and Anderson 1983, Leonard 1994, Alizon et al. 2009). A trade-off between the ability to infect a greater range of host genotypes and reproduction within the host has the potential to constrain the evolution of increasing host breadth (Leonard 1977, Tellier and Brown 2007). A trade-off may also constrain the evolution of reproductive rate if increased reproduction results in increased damage to the host, and thus reduces the total number of new infections (May and Anderson 1983). Empirical support for trade-offs varies considerably among systems, and the ubiquity of trade-offs has been questioned (Antonovics and Alexander 1989, Ebert and Bull 2003, Froissart et al. 2010)

Pathogen evolution may also be constrained without invoking trade-offs if particular ecological contexts provide conflicting selection on different pathogen traits (Damgaard 1999, Laine and Tellier 2008), or if demographic processes limit the adaptive potential of pathogen populations (Kaltz and Shykoff 1998, McDonald and Linde 2002, Gandon and Michalakis 2002). Indeed, ecological variation is likely

important to understanding pathogen evolution even if genetic trade-offs are present, since such variation can reduce the power of selection on trade-offs (Asplen et al. 2012).

I focus on genetic and ecological factors constraining the evolution of two important pathogen life-history stages, infection and reproduction in the oat crown rust pathogen, *Puccinia coronata* f.sp. *avenae*. Infection is defined as the qualitative ability of the pathogen to infect the host while reproduction is defined as the quantitative reproductive output of a pathogen following successful infection. These two stages are an integral part of the lifecycle of all pathogen species, since completion of the life-cycle depends on successful infection, followed by reproduction within the host. While there is an extensive body of work on the evolution of both pathogen infection and reproduction, research on these two pathogen life history stages has been largely separate (Dybdahl and Storfer 2003). Pathogen infection has been the focal trait in many host-pathogen coevolution studies in plant and invertebrate systems where genetic resistance in the host organisms provides defense by limiting pathogen infection (Krist et al. 2000, Thrall and Burdon 2003, Lively et al. 2004, Thrall et al. 2012). In contrast, much of the research on pathogen reproduction has focused on the trade-off relationship between pathogen transmission to new hosts and the amount of damage inflicted on the host (May and Anderson 1983, Mackinnon and Read 1999, Bell et al. 2006, de Roode et al. 2008, Alizon et al. 2009). Since both infection and reproduction contribute to pathogen fitness, understanding the relationship between these two traits is critical to predicting evolutionary trajectories.

Most studies of plant-pathogen coevolution have focused on the evolution of pathogen infection ability, leaving the evolution of pathogen reproductive rate relatively unexplored. Yet reproducitve rate is critical to predicting the rate and direction of pathogen evolution. Moreover, predicting the outcome of selection on pathogen reproduction requires an understanding of the genetic contributions of both the pathogen and the host. In chapter one, I investigate the effects of different pathogen and host genotypes on three distinct stages of pathogen life history that contribute to pathogen reproduction within the host. For each pathogen life history stage, I ask whether the pathogen or host genotype explains the most variation.

A trade-off between infection and reproduction has the potential to constrain the evolution of either trait, and I explore this hypothesis in chapter two. In *P.*

coronata and many other plant-pathogen systems infection is determined by gene for gene interactions (Flor 1956, Thompson and Burdon 1992), such that a host carrying a resistance gene can only be infected by pathogen genotypes that carry a corresponding virulence gene. Thus, virulence confers a clear benefit to pathogens by allowing access to otherwise resistant hosts. Yet low-virulence pathogens are still frequently found in nature (Clarke 1997, Thrall and Burdon 2003, Leonard et al. 2004). A trade-off between virulence and reproduction is *the* most widely cited hypothesis in the literature to explain the maintenance of these low-virulence strains (van der Plank 1968, Leonard 1977, Thrall and Burdon 2002, Tellier and Brown 2007), yet few studies have explicitly tested it. In chapter two I quantify within-host reproduction and life history stages of *P. coronata* genotypes that varied greatly in their levels of virulence, and analyze the relationship between virulence level and life history stages.

The contribution of life history stages to pathogen fitness may depend on ecological context. For example, the fitness benefit to a pathogen with the ability to infect a resistant host will depend on the relative abundance of the resistant host in the population. In chapter three I quantify the average virulence level and fecundity of pathogen populations collected from mixed-genotype and single genotype host populations and ask whether there is evidence that genetic diversity of the host population limits evolution of pathogen reproduction.

Since pathogen adaptive evolution can also be constrained if other evolutionary processes such as drift or migration are more powerful than selection, chapter three also includes an analysis of the genetic structure of the pathogen populations. I first ask whether there is evidence that local adaptation is limited by a lack of genetic variation. Then I ask whether there is evidence that selection structures the variation at pathogen virulence loci.

In summary, my thesis examines genetic and ecological factors affecting the evolution of both infection and pathogen reproduction. I find evidence in support of a trade-off between pathogen infection and the onset of reproduction that has the potential to limit the evolution of increasing pathogen infection in susceptible host populations. Yet, I also find that infection and reproduction are affected by multiple ecological factors. I show that variation in pathogen life-history stages within the host is affected by both the pahtogen and host genotype. I also find that infection and reproduction vary substantailly within and among pathogen populations, but that this variation is not driven by differences in host genetic diversity. Thus, my work builds

on other studies of pathogen evolution that focus on genetic trade-offs between infection and reproduction, by considering the ecological mechanisms of evolutionary constraint.

CHAPTER 1

Pathogen and host genotype differently affect pathogen fitness through their effects on different life-history stages

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SUMMARY

Adaptation of pathogens to their hosts depends critically on factors affecting pathogen reproductive rate. While pathogen reproduction is the end result of an intricate interaction between host and pathogen, the relative contributions of host and pathogen genotype to variation in pathogen life history within the host are not well understood. Untangling these contributions allows us to identify traits with sufficient genetic variation for selection to act and to identify mechanisms of coevolution between pathogens and their hosts. We investigated the effects of pathogen and host genotype on three life-history components of pathogen fitness; infection efficiency, latent period, and sporulation capacity, in the oat crown rust fungus, *Puccinia coronata* f.sp. *avenae*, as it infects oats (*Avena sativa*). We show that both pathogen and host genotype significantly affect total spore production but do so through their effects on different life-history stages. Pathogen genotype has the strongest effect on the early stage of infection efficiency, while host genotype most strongly affects the later life-history stages of latent period and sporulation capacity. In addition, host genotype affected the relationship between pathogen density and the later life-history traits of latent period and sporulation capacity. We did not find evidence of pathogen-by-host genotypic (GxG) interactions. Our results illustrate mechanisms by which variation in host populations will affect the evolution of pathogen life history. Results show that different pathogen life-history stages have the potential to respond differently to selection by host or pathogen genotype and suggest mechanisms of antagonistic coevolution. Pathogen populations may adapt to host genotype through increased infection efficiency while their plant hosts may adapt by limiting the later stages of pathogen growth and spore production within the host.

INTRODUCTION

Evolution of differing investment in life-history stages is a powerful mechanism of organismal adaptation to ecological challenges (Roff 2002), including the challenges imposed on host populations by ever-evolving pathogens (Agnew et al. 2000, Gandon et al. 2002, Bonds 2006). While the evolution of host life-history strategies such as earlier maturation and reproduction in response to greater pathogen prevalence has been explored (Chadwick and Little 2005, Fredensborg and Poulin 2006, Ohlberger et al. 2011), the evolution of pathogen life histories in response to host genotypic variation is less well understood. Pathogen life histories have been shown to evolve in response to changes in the abiotic environment (Woodhams et al. 2008), shortened host life spans (Nidelet et al. 2009), and multiple infections (Ben-Ami et al. 2008). Although fewer in number, the above studies are important because they demonstrate the potential role of pathogen life history in adaptation to varying ecological factors. What remains unclear is the extent to which varying host genotype might affect the evolution of pathogen life history. Here we examine the effects of host and pathogen genotype on variation in critical stages of a pathogen's life history within the host, spanning infection to reproduction.

Pathogen growth and reproduction within the host are critical processes in all predictive models because they directly contribute to lifetime fitness. While overall reproductive rate is determined by both transmission between hosts and growth and reproduction within the host (May and Anderson 1983), within-host processes directly affect the evolution of virulence (Leonard 1977, Frank 1994, Tellier and Brown 2007) and of aggressiveness (May and Anderson 1983). Throughout, we use the term virulence to denote the qualitative ability of the pathogen to infect specific host genotypes, and aggressiveness to denote the damage to the host as a result of pathogen infection and growth. Much of the focus of coevolutionary studies has been on qualitative virulence and resistance systems that result in "arms race" evolution (Thompson and Burdon 1992, Bergelson et al. 2001, Agrawal and Lively 2002, Thrall and Burdon 2002, Tellier and Brown 2007). For example, the ability to infect is determined by the interaction of pathogen virulence gene products and host resistance proteins (Jones and Dangl 2006) in many plant-pathogen systems. Such qualitative systems have provided a wealth of information on the classic gene-for-gene coevolutionary processes (Thompson and Burdon 1992, Gandon et al. 1996, Thrall

and Burdon 2002, Laine 2005, Burdon and Thrall 2009, Thrall et al. 2012), but the effects of pathogen life-history stages following infection on the coevolutionary process remain understudied. Some authors have hypothesized that qualitative host resistance genes may contribute to quantitative resistance after infection during pathogen growth and reproduction in the host (Young 1996, Poland et al. 2009) and host genotype may not affect all pathogen life-history stages in the same way. For example, Duneau et al. (2011) found that two distinct stages of infection; spore activation and attachment, differed dramatically in host-genotype specificity; activation was not affected by host genotype while spore attachment was strongly affected by the interaction between host and pathogen genotype. For most systems, we have little information on the relative effects of pathogen and host genotype on the quantitative expression of pathogen life-history stages from infection to the production of propagules.

Recent work demonstrates that both pathogen and host genotype affect pathogen reproductive rate in several quantitative systems; murine malaria (Grech et al. 2006), a protozoan butterfly pathogen (de Roode and Altizer 2010), an oomycete plant pathogen (Salvaudon et al. 2007b), and two different microsporidium parasites of Daphnia (Decaestecker et al. 2003). Host and pathogen genotypic interaction effects (GxG interactions) can play a key role in pathogen adaptation to sympatric host genotypes (Kaltz and Shykoff 1998, Sicard et al. 2007, Greischar and Koskella 2007, Lambrechts et al. 2009a) as well. Local adaptation is expected in pathogens with dispersal ranges greater than their hosts (Thrall and Burdon 2002) and there, GxG interactions of pathogen and host genotype may account for a large portion of the variation in pathogen reproductive rate. Explicitly accounting for GxG interactions improves the accuracy of predictive evolutionary models (Restif and Koella 2003, Lambrechts et al. 2006, Alizon et al. 2009, Asplen et al. 2012). Given the importance of pathogen reproductive rate to disease dynamics in both natural (May and Anderson 1983, Jarosz and Davelos 1995, Alexander et al. 1996) and agricultural (Gilligan 2008, Pariaud et al. 2009b) systems, untangling the effects of pathogen and host genotype on pathogen life history within the host is an important step towards making accurate predictions of pathogen evolutionary trajectories. Three pathogen life-history stages are essential to within-host reproduction; infection, the latent period of growth within the host, and finally, propagule production (Pariaud et al. 2009b). Together these life-history stages determine a pathogen's overall

reproductive success within the host, however, the contribution of individual life-history stages to overall reproduction can vary among pathogen genotypes (Pariaud et al. 2009) and across ecological contexts (Woodhams et al. 2008). Pariaud et al. (2009b) found that the relative contribution of pathogen latent period and sporulation capacity to overall reproductive rate varied among different genotypes of wheat leaf rust. Woodhams et al. (2008) found that the contribution of specific life-history stages to the overall reproductive rate of the amphibian fungal pathogen *Batrachochyrium dendrobatidis* varied with temperature. At low temperatures, the fungus took longer to mature and developed fewer reproductive structures (zoosporangia) but ultimately produced a large number of zoospores per zoosporangium. At high temperatures, the fungus matured rapidly and developed many more zoosporangia, but produced fewer zoospores per zoosporangium. While there is a growing realization that ecological context will affect the evolution of pathogen life histories (Rigaud et al. 2010), and that pathogen life histories strongly affect disease dynamics and coevolution (Barrett et al. 2008b), the relative importance of pathogen and host genotype in determining contributions of different life-history stages to pathogen fitness is not well understood.

Here, we investigate the effects of pathogen and host genotype on three life-history stages affecting pathogen reproduction within the host; infection efficiency (the proportion of spores that produce infections), latent period (the lag time between inoculation and spore production that occurs while the fungus grows within the host), and sporulation capacity (the number of spores produced per infection) in the agriculturally important pathogen, Puccinia coronata f.sp. avenae, the causal agent of oat crown rust. Importantly, to evaluate quantitative genetic effects on within-host pathogen reproduction, we utilized susceptible host genotypes carrying no specific resistance genes preventing infection by the pathogen. We first tested the null hypothesis that only pathogen genotype affects pathogen reproductive rate. Next, we tested the null hypothesis that after infection, qualitative host resistance genes have no effect on pathogen life history and reproductive rate. Finally, we tested the null hypothesis that the relative effects of pathogen and host genotype do not differ across different pathogen life-history stages.

Our results show that pathogen reproductive rate is affected by both the pathogen and the host genotype. We do not find evidence to suggest that a qualitative host resistance gene affects quantitative levels of within-host pathogen reproductive rate. Finally, we show that the relative effects of pathogen and host genotypes vary

across pathogen life-history stages with pathogen genotype having the greatest effect in the early life-history stage of infection efficiency, while host genotype has the greatest effect in the later life-history stages of latent period and sporulation capacity.

METHODS

Study system

In this study, we used the rust fungus, *Puccinia coronata* f.sp. *avenae* (Erik.) as it infects oat (*Avena sativa*) plants and causes crown rust disease. Because the qualitative gene-for-gene interactions are well described for this system (Fleischmann and Baker 1971, Chong et al. 2000) we could examine the quantitative effects of host and pathogen genotype on pathogen life history and reproduction. Like many rust fungi, *P. coronata* has a complex life cycle in which asexual reproduction occurs on one host (*A. sativa*) and sexual reproduction occurs on a second host (*Rhamnus cathartica*; common buckthorn). Aeciospores produced on the buckthorn host infect oats in early spring and produce pustules on leaves that are filled with bright orange asexual urediniospores, giving the rusts their name. Because urediniospores readily re-infect oats and generation times are rapid (10–14 days), reproductive rate at this spore producing stage is a critical factor in the large epidemics caused by this and many other rust fungi. Consequently, we evaluated the effects of host and pathogen genotype on life-history stages for the urediniospore stage.

We sampled from an experimental nursery for oats and oat crown rust that was established in 1953 in St. Paul, MN and maintained since to test resistance in oat agronomic lines to oat crown rust. The nursery consists of eight 560m^2 plots in which over 1,000 different warm and cool season oat lines are planted every year. The oat plots are interspersed with mature hedgerows of buckthorn. Buckthorn plants are inoculated each spring using oat straw from the previous year and the resulting infections on buckthorn produce aeciospores that infect the oats. Thus, we assume that the P. coronata population we sampled is representative of a long evolutionary history with oats because of the length of time that the nursery has been in place.

Pathogen strains

Five different pathogen genotypes (strains) were isolated from collections made in 2008 at the University of Minnesota St. Paul buckthorn nursery. Previous studies have shown that the P. coronata population in the nursery harbors high levels of genetic diversity (Leonard et al. 2004, Carson 2008) and results below. We collected aeciospores from five different buckthorn plants in the nursery and used

standard isolation techniques (Carson 2008) to insure that each of the five resulting strains represents a single genotype. Briefly, aeciospores were used to infect one-week old seedling mixtures of the oat lines Marvelous and Starter because these very susceptible hosts should provide little selection on the pathogen genotype. We used a low inoculum level ($1x10^5$ spores/mL) to insure that each resulting infection focus (pustule) produces a single genotype of urediniospores. The leaves of infected oat seedlings were trimmed so that each plant harbored a single pustule. The urediniospores originating from a single pustule represent a single genotype because they are produced by asexual reproduction. The urediniospores produced in each individual pustule were collected and inoculated onto new, uninfected seedlings to produce sufficient quantities of the single-genotype urediniospores for the inoculation experiments described below. These urediniospores were collected, desiccated, and stored at −80°C until used for experiments.

We conducted further evaluations to insure that all five strains were genetically distinct by determining the gene-for-gene virulence alleles carried by each strain. In the gene-for-gene system, when the host plant carries a resistance gene that recognizes a specific pathogen product encoded by the virulence allele, it mounts a resistance response. If the pathogen carries a virulence allele that escapes detection by the host resistance factor, it will successfully infect the host. We tallied the presence or absence of specific virulence alleles in the five pathogen strains by inoculating each strain against a set of 21 oat 'differential' lines that each differ at single resistance gene (Fleischmann and Baker 1971). We assessed infection 10 days later based on the presence or absence of sporulating pustules.

Host lines

To examine the effect of host genotype on pathogen reproduction, we chose five susceptible agronomic oat varieties, Ogle, Otana, Pendek38, Pendek, and Portage (Table 1.1), that had similar heights and times to maturity. All these oat varieties (host lines) have been grown in the buckthorn nursery except Pendek, but many oat lines in the nursery, including Pendek38, carry the Pendek genetic background. Pendek and Pendek38 are reported as isolines that differ only at a single resistance gene, and were included in the experiments to evaluate whether qualitative resistance genes contribute to quantitative host resistance. Pendek38 contains a crown rust resistance

gene, Pc38, derived from the wild hexaploid oat, *A. sterilis* (Fleischmann and Mckenzie 1968). We used the highly susceptible oat lines Marvelous and Starter, which are also routinely grown in the buckthorn nursery, to propagate the strains as described above. We did not use them in experimental treatments to avoid confounding effects of inadvertent selection for strains that grew well on these varieties. All seeds were obtained from the USDA Cereal Disease Lab with the exception of the Pendek host line, which was obtained from USDA-ARS National Small Grains Collection in Aberdeen, ID (Table 1.1). Preliminary inoculation trials confirmed that all host genotypes were susceptible to all pathogen genotypes used in this experiment.

Experimental design

To determine the effects of pathogen and host genotype on pathogen reproduction, each of the five pathogen strains described above was inoculated onto single plants representing each of the five host genotypes. A balanced incomplete split-plot design was used to assign pathogen and host treatments: 50 pots were planted with two different host genotypes per pot and both plants in the pot were inoculated with the same pathogen genotype. Each pathogen genotype was inoculated onto each combination of two hosts. Each pathogen by host genotype combination was replicated four times, for a total of 100 individual plants. Pots were arranged in a completely randomized design in the growth chamber. Thus, pathogen genotype was randomized at the whole-pot level, while host genotype was randomized at the split-pot level. Because of the labor-intensive effort required in making inoculations, half the plants were inoculated on each of two consecutive days, at the same time each day. Two replicates of each host by pathogen treatment were placed in each inoculation day block.

Experimental procedures

To prepare inoculum for the above experimental design, spores of each pathogen genotype were retrieved from −80 °C storage, heat shocked at 40°C for 10 minutes (Carson 2008) and then inoculated onto a mixture of one-week old susceptible seedlings (Marvelous and Starter). Urediniospores from each strain were collected 13 days later, spores were mixed with mineral oil (Soltrol 170, Philips-

Conoco, Houston, TX), and concentrations were estimated from an average of four haemocytometer counts.

To grow plants for the above experimental design, seeds were planted on June 16, 2009 in 14 cm pots filled with pasteurized soil. Two host genotypes were planted 6.5 cm apart per pot. Pots were randomized and placed in a growth cabinet under controlled conditions of 16 h, 22°C day, and 8 h, 18°C night. Pots were arranged in 10 trays, with 5 pots in a tray and watered from the bottom. Three weeks after planting, pots were amended with one tablespoon of 'Osmocote' (14 N-14P-14 K) slow-release fertilizer (Scotts Miracle-Gro Co.). An additional supplement of water-soluble 20 N-20P-20 K fertilizer was applied once a week starting 4 weeks after planting.

Plant inoculations were carried out on July 30th and 31st, 2009, when plants were beginning to flower. Spores obtained above were diluted to a standard concentration of 1 x 10^5 spores/mL, an inoculum dose shown to be well below the carrying capacity of the leaf in preliminary experiments. The penultimate leaf of each host was fixed to a board 30 cm from the spray nozzle and covered with a clean paper frame so that only 15 cm of leaf was exposed. Spores were sprayed on to the adaxial leaf surface with a pump sprayer moving at a speed of 7.5 cm/second from the bottom to the tip of the leaf (Scientific apparatus shop, University of Minnesota). A clean spray nozzle was used for each strain. Because not all sprayers deliver exactly the same dose, we estimated the actual inoculation dose for each leaf using a glass microscope slide covered with double-sided sticky tape and fixed to the board such that it was sprayed in line with the leaf. After allowing the inoculum to dry for one hour, plants were placed in a dew chamber with 30 seconds of mist every 2 minutes for 12 hours to stimulate spore germination.

To assess levels of spore viability of each P. coronata strain, 500µL of 1x10^6 spores/mL solution was inoculated onto each of two petri plates with 1.5% water agar (15 g DifcoBacto agar per 1000 mL distilled water), on the same day as plant inoculations. Plates were incubated at room temperature for 12 hours and the numbers of spores that did or did not germinate were counted.

Life-history measurements

We investigated the effects of pathogen and host genotype on three life-history stages affecting pathogen reproduction within the host: infection efficiency (the

proportion of inoculated spores that produce sporulating pustules), latent period (the lag time between inoculation and spore production), and sporulation capacity (the number of spores produced per pustule). The development of pustules was monitored daily beginning seven days after inoculation. From that time, inoculated leaves were examined with a magnifying glass at the same time each day for successive five days and the number of sporulating pustules was recorded.

To estimate total spore production, the inoculated leaf was placed inside horizontally suspended glassine bags adjusted so that spores could not escape, but so that airflow was maintained. No condensation accumulated in the bags during the two weeks that the bags were in place. Spores were collected at 3 weeks after inoculation when pustule development was complete, by tapping the leaves to shake spores into the bags. Any remaining spores were collected from the leaf with a small vacuum and added to those collected in the bag. Spores were desiccated in a 20% relative humidity chamber for one week and weighed. Leaves were harvested, scanned, and total leaf area of the inoculated region was determined using ImageJ (Abramoff et al. 2004).

Total spore production was measured as the total dry mass of spores produced and normalized per square centimeter of leaf tissue. Infection efficiency was measured as the number of sporulating pustules per square centimeter of leaf tissue. Latent period was quantified by determining the number of days required for 50 percent of the pustules to reach sporulation. Sporulation capacity was calculated as the average spore mass per pustule. Plant heights from the soil surface to flowering head and from the soil surface to inoculated leaf were measured as potential covariates.

Statistical analysis

Separate statistical analyses were performed on total spore production and on the three life-history stages contributing to total spore production: infection efficiency, latent period, and spore production per pustule. The effects of pathogen and host genotype on total spore production were determined with a split-plot analysis of variance using inoculation day as a blocking factor. We accounted for variation due to inoculation dose by using the spore counts on the sticky slides (see experimental procedures) as a covariate. The effects of pathogen genotype and of block (inoculation day) were tested using the whole-plot error term, while the effects of host

genotype and of inoculum dose were tested using the split-plot error term. We examined all pairwise differences among pathogen and host genotypes using conservative Bonferroni corrections for multiple comparisons, since this method did not change the error structure of the split-plot design. Since two of the host lines, Pendek and Pendek38, were reported as isolines and therefore not truly independent, we ran three analyses; with all five host lines, with the Pendek line excluded, and with the Pendek38 line excluded.

Infection efficiency is defined as the proportion of inoculated spores that form sporulating pustules. We measured infection efficiency as the number of pustules per square centimeter of leaf tissue, and accounted for the variation in inoculum dose (spores deposited per cm^2) using a split-plot ANCOVA model. We used covariate modeling to estimate infection efficiency rather than direct calculation of the ratio of pustules to the inoculum dose because the analysis of covariance explicitly allows a test for density dependent effects. Pathogen genotype effect was tested using the whole-plot error term, while the effects of host genotype, and inoculum dose were tested using the split-plot error term. Results for one leaf were excluded because the sticky slide was missing.

Latent period, the time from inoculation until spore production, was calculated by plotting the percentage of sporulating pustules over time, fitting a smoothing line, and graphically determining the time at which 50 percent of the pustules were producing spores (JMP 5.01.a). The percentage of sporulating pustules for each day was calculated by dividing the number of sporulating pustules by the final number of pustules observed 12 days after inoculation (x 100%) when pustule development was complete. Latent period was analyzed with a split-plot ANCOVA, with log transformed pustule density included as a covariate in the model. One leaf was excluded from the analysis since no pustules developed on it.

Sporulation capacity is defined as the number of spores produced per pustule. We used a covariate approach to analyze sporulation capacity because our method of collecting spores pooled across all the pustules on a given leaf. We analyzed the log transformed dry spore weight normalized to leaf area (mg of spores per cm^2 of leaf tissue) in a split-plot ANCOVA with pustule density (pustules per cm^2 of leaf tissue) as a covariate. Three leaves had fewer than five pustules and were omitted from the analysis as outliers.

We also directly calculated spore production per pustule by dividing the total spore mass by the number of pustules and then determined if spore production per pustule declined with increasing pustule density using regression analysis. Spore production per pustule was transformed to the ¼ power to fit a linear model and pustule density was log transformed. Fourteen replicates produced no measurable spore mass, and were not included in the regression analysis. All analyses were performed in R 2.10.1 (The R Foundation for Statistical Computing 2009) using the aov function and type II sums of squares.

RESULTS

Variation in the number of virulence alleles

We found that the five pathogen strains each exhibited a unique infection profile against the 21 differential oat lines tested (Table S1.1). Individual strains carried between 11 and 16 virulence alleles and, because each carried a unique set of virulence alleles, we conclude that the five strains we used in these experiments represent unique genotypes. We found no significant correlations between the number of virulence alleles carried by a given strain and the quantitative measures of infection efficiency (Slope = 0.0962, $t_{98} = 1.636$, p = 0.105) or total spore production (slope = 0.0522, $t_{98} = 1.693$, p =0.0937). There was a slight, but significant, negative correlation between number of virulence alleles and latent period (Slope = −0.06694, standard error = 0.03263, $t_{97} = -2.051$, p = 0.0429).

Variation in total spore production

We found significant main effects of both pathogen and host genotype on total spore production, but no significant pathogen by host genotypic interaction (Table 1.2). Inoculum dose demonstrated a significant positive correlation with total spore production and was used as a covariate in the analysis. Inoculation day (as block) and variation among pots within the growth chamber also had a significant effect on total spore production (Table 1.2). We did not find significant interactions between inoculum dose and pathogen genotype, or between inoculum dose and host genotype and these interaction terms were dropped from the final model. Excluding one or the other of the Pendek isolines did not change the significance of the results (Table S1.2).

Total spore production varied significantly among pathogen genotypes. Pairwise comparisons revealed that strain 2 produced significantly more spores than strain 40 across all hosts (Figure 1.**1** A). Spore production also varied significantly among host genotypes (Table 1.2) with significantly more spores produced on the Otana host genotype than on any other host genotype (Figure 1.**1**B). Host genotypes differed significantly in total plant height ($F_{4,95} = 12.165$, p < 0.0001) and height from the ground to the inoculated leaf ($F_{4,94} = 16.017$, p < 0.0001) but we did not find a significant correlation between plant height and total spore production or any of the

individual life-history stages. Thus, finding that host genotype had a significant impact on total spore production does not support the first null hypothesis that only pathogen genotype affects pathogen reproductive rate, and we turn to evaluating the effects of host and pathogen genotype on separate life-history stages.

Effect of the host resistance gene Pc38

The Pendek38 host line carries one additional resistance factor, Pc38, compared to the Pendek line. All pathogen strains were able to infect Pendek38, making Pc38 a "defeated" resistance gene. We did not detect a significant difference in mean total spore production between the host lines Pendek and Pendek38, nor we did find a significant difference in any of the measured individual pathogen life-history stages between these two host lines. Thus, we accept the second null hypothesis that the qualitative host resistance gene Pc38 has no effect on pathogen life-history stages or on reproductive rate. Since this is a somewhat limited test of the effects of qualitative resistance genes on quantitative pathogen reproduction, we do not pursue this result further.

Variation in pathogen life-history stages

We evaluated the contributions of three life-history stages to total spore production: infection efficiency, latent period, and sporulation capacity. For each, we asked whether pathogen genotype, host genotype, or the interaction of pathogen and host genotype explained variation in that life-history stage.

Infection efficiency

We found that pathogen genotype and inoculation day had significant effects on infection efficiency but that host genotype and the interaction term for pathogen and host genotypic effects were not significant (Table 1.3). Inoculum dose was a significant predictor of infection efficiency, but the slope of relationship between inoculum dose and infection efficiency did not vary with pathogen genotype. Pathogen strains 2 and 22 produced the most pustules across hosts while strains 21 and 40 produced the fewest across hosts (Figure 1.**1** C). When corrected for multiple comparisons, only the difference between strains 2 and 40 was significant.

Differences in infection efficiency among pathogen genotypes could be a result of differences in spore viability at the time of inoculation. To test this, we estimated the germination rate as described above. There was significant variation among pathogen genotypes, despite the fact that all inoculum sources were grown in very similar conditions and that germination rate was above 80% for all genotypes. Surprisingly, strains 21 and 40 had the highest germination rates (97% and 93%, respectively) despite having the lowest infection efficiency. Consequently, spore viability does not explain variation in infection frequency. Inoculation day also had a significant effect, indicating that infection efficiency is sensitive to variation in abiotic environmental conditions.

Latent period

We found that both pathogen and host genotype had significant effects on latent period, but that the interaction term for pathogen and host genotypic effects was not significant (Table 1.**4**). Pustule density had a significant negative effect on latent period, such that sporulating pustules developed more quickly on more crowded leaves. Inoculation day and pot also had significant effects, indicating that latent period is sensitive to environmental variation (Table 1.**4**). Excluding one or the other of the Pendek isolines did not significantly change outcomes of the analysis (Table S1.3).

Latent period varied significantly among pathogen genotypes with strains 2 and 22 having the shortest latent periods on most hosts, and strain 8 taking the longest to develop pustules on most hosts (Figure 1.**1**E). Although significant, the difference in latent period among pathogen genotypes was short, with an average of only 15 hours separating the fastest and slowest genotypes under the growth chamber conditions used here. We did not find a significant interaction effect between pathogen genotype and pustule density because all strains demonstrated shorter latent periods on leaves with more pustules (Table 1.**4**).

Host genotype had a strong effect on latent period, a result mostly due to the significantly longer latent period of all pathogen genotypes on the Ogle host line (Figure 1.**1**F). In addition, latent period for most pathogen genotypes was shorter on the Otana host line compared to the other three hosts, but this was not statistically significant. Interestingly, the longer latent period on the Ogle host line did not

correspond to significantly less spore production. Indeed, spore production on the Ogle host line was the second highest. Latent period decreased with increasing pustule density on all host genotypes and there was a marginally significant ($p = 0.06$) interaction effect between pustule density and host genotype on latent period (Table 1.**4**). The slope of the relationship between pustule density and latent period was steepest for the host line Portage (slope = 0.8874, se $t_{19} = -3.017$, $p = 0.006$). In contrast, there was no significant relationship between pustule density and latent period on the Otana host (slope = −0.1588, $t_{19} = -0.662$, $p = 0.51$) where latent period was consistently short even at low pustule densities (Figure 1.**2**).

Sporulation capacity

We evaluated sporulation capacity as the relationship between the total mass of spores produced and the number of pustules, normalized to the leaf area (cm^2). Both pathogen and host genotype had significant effects on sporulation capacity, but the interaction term for pathogen and host genotypes was not significant (Table 1.**5**). Pathogen genotype had a significant effect on sporulation capacity but did not affect the slope of the relationship between pustule density and spore production and the interaction term was dropped from the final model. In contrast, host genotype affected both the slope and intercept of the relationship between pustule density and spore production. The slope was steepest on the Otana host genotype (slope = 1.02x10, $t_{19} = 2.803$, $p = 0.012$) compared to all other hosts (Figure 1.**3**), although no statistical difference in slope was detected between the Otana host genotype and the Pendek host genotype (Table S1.**4**). Interestingly, the relationship between spore production and pustule density is strikingly similar among the remaining host genotypes, suggesting a similar underlying physiology in those interactions. Inoculation day and pot also had significant effects (Table 1.**5**), indicating that sporulation capacity, like infection efficiency and latent period, is sensitive to environmental variation. Excluding one or the other of the Pendek isolines did not significantly change the outcome of these analyses (**Table S1.5**).

We did not see any evidence that spore production leveled off at high pustule densities on any host, indicating that pustule densities were well below carrying capacity of the leaves. When sporulation capacity was directly calculated by dividing the total spore mass by the number of pustules, there was a significant negative

correlation between spore mass per pustule and pustule density (slope = −0.0107, $t_{81} = -2.801$, $p = 0.0064$) suggesting that crowding might limit sporulation capacity at high densities. One highly influential replicate that only produced a single pustule but had high spore production was removed.

Pathogen and host genotype effect size

Overall, we found that the amount of variation explained by pathogen and host genotypes varied considerably among the three life-history stages evaluated (Table 1. 6). Pathogen genotype explained more of the variation in infection efficiency (effect size η^2 x 100% = 24%) than in the later two life-history stages, latent period (14%) and sporulation capacity (17%). Differences in infection efficiency also accounted for most of the among-strain variation in total spore production because pathogen genotypes with the greatest infection efficiency (Figure 1.**1**,C) produced the greatest total number of spores (Figure 1.**1**,A). Likewise, pathogen genotypes with the lowest infection efficiency experienced the lowest total spore production (Figure 1.**1**,A and 1.1C). In contrast, host genotype had no significant effect on infection efficiency, but explained a large portion of the variation in later stages, latent period (49.1%) and sporulation capacity (41.7%). Differences in sporulation capacity accounted for most of the among-host variation in total spore production (compare Figure 1.**1**,B and 1.1,H). Thus, we reject the third null hypothesis that the relative effects of pathogen and host genotype do not differ across different pathogen life-history stages. Pathogen genotype more strongly affected the early stage of infection efficiency while host genotype more strongly affected the later stages of latent period and sporulation capacity.

DISCUSSION

Fitness is the currency of natural selection and together with heritable variation, is the basis of phenotypic evolution. Yet measuring fitness is rarely straight forward, particularly for pathogens where fitness cannot be measured outside of the host. In this research, we measured total spore production as an estimate of within-host fitness of the plant pathogen P. coronata. Our results are consistent with a growing body of work demonstrating significant effects of both host and pathogen genotype on pathogen fitness (Carius et al. 2001a, Grech et al. 2006, Salvaudon et al. 2007a, Lambrechts et al. 2009a, de Roode and Altizer 2010) and provide new evidence that the effects of host and pathogen genotype vary across the pathogen's life history within the host.

Host genotype significantly affects total spore production allowing us to reject the null hypothesis that reproductive rate is affected only by pathogen genotype. Instead, our results show that quantitative variation in pathogen reproduction depends on the host genotype and that the magnitude of these host effects varies over the life history of the pathogen in the host. Host genotype had little effect on infection efficiency but strongly affected later life-history stages of latent period and sporulation capacity. Latent periods were significantly longer on the Ogle host line while sporulation capacity was significantly greater on the Otana host line, suggesting differences among these hosts in levels of host defense (Niks and Rubiales 2002) or resource availability (Staples 2001, Robert et al. 2004).

Several life-history stages showed apparent density-dependent effects; greater pustule density was correlated with shorter latent periods and lower sporulation capacity. These results are consistent with density-dependent effects observed in other systems where increased pathogen numbers within the host are correlated with lower reproduction per individual (Newton et al. 1997, Bell et al. 2006). Interestingly, we found significant interaction effects between host genotype and density on latent period and sporulation capacity. The interaction effect for latent period arises because pathogen genotypes grown on the Otana host line did not demonstrate decreased latent period with increased pustule density as they did other host lines. In contrast, the interaction effect for sporulation capacity arises because the relationship of sporulation capacity and pustule density for the Otana host genotype is much steeper

than for other host genotypes such that many more spores were produced at high pustule densities. The differences among hosts in density-dependence suggest that the intensity of competition among pustules varies across these host genotypes. We know of no other study demonstrating host genotype effects on the relationship between pathogen density and reproduction. The result is important because competition is thought to select for greater pathogen aggressiveness (May and Nowak 1995). If the intensity of pathogen competition varies across a host population, a diverse host population will modulate and slow the evolution of increased pathogen aggressiveness. Further work is needed to determine whether results we show here for single strain infections can be extended to host-mediated effects in multiple strain infections.

Pathogen genotype had significant effects on total spore production and on all three of the life-history stages we measured. The magnitude of these pathogen effects varied over life-history stages, with the strongest effects on variation in infection efficiency and lesser effects on the variation in latent period and sporulation capacity. The differences in infection efficiency among P. coronata genotypes could not be easily attributed to spore viability or to a cost of virulence (Leach et al. 2001, Sacristan and Garcia-Arenal 2008, Pariaud et al. 2009a), as the number of virulence alleles carried by a pathogen genotype did not correlate with total spore production. The results do suggest that virulence alleles evolve independently from those affecting quantitative variation in life-history stages. Instead, the differences in infection efficiency among pathogen genotypes may be due to differing abilities to recognize and penetrate the host stomata or to evade basal host defenses (Niks and Rubiales 2002, Jones and Dangl 2006). Interestingly, although pathogen genotype affects all three life-history stages, we did not observe negative correlations among these traits, thus life-history trade-offs do not obviously limit the evolution of more aggressive genotypes.

Together, the results for host and pathogen genotype show an interesting pattern of decreasing pathogen genotype effects and increasing host genotype effects over the life history of the pathogen. Yet, we found little evidence that pathogen by host genotypic (GxG) interactions affect variation in pathogen life-history stages or total spore production. This result is surprising given the strong main effects of both pathogen and host genotype on latent period and on sporulation capacity. While GxG interactions explain a large portion of the variation in pathogen reproduction in some

systems (Carius et al. 2001, Salvaudon et al. 2007, Lambrechts et al. 2009, de Roode and Altizer 2010), they may have little explanatory value in other systems (Van Ginkel and Scharen 1988, Grech et al. 2006). One explanation for an apparent lack of GxG interactions in our study is that the genetically diverse host populations planted at the sampled location have generated selection for a generalist host-use strategy where most pathogen genotypes infect, grow, and reproduce in most hosts, as has been observed in other studies (Villaréal and Lannou 2000, Legros and Koella 2010). An alternative explanation is that the small sample of P. coronata genotypes and hosts that we used in these experiments lack sufficient variation to detect GxG interactions, although sufficient for detecting main effects. Neither explanation can be excluded but results do suggest that considerable variation in life-history traits has been maintained in this pathogen population.

Variation in pathogen life-history traits is likely maintained through annual sexual recombination (Groth and Roelfs 1982) and by ecological factors such as the abiotic environment (Wolinska and King 2009), density-dependent effects, and spatial and genetic variation in the host population. Variable abiotic conditions such as occurred over different inoculation days can be a factor maintaining pathogen diversity because environmental effects will limit the effectiveness of directional selection for increased pathogen reproduction rate (Gilbert 2002). We observed varied density-dependent effects across host genotypes and conclude that variation in biotic conditions, especially those represented by varied host genotypes, may constrain evolution of life-history traits and maintain variation.

Our results suggest the potential for coevolutionary processes to shape pathogen life-history stages within the host from infection to reproduction. While coevolutionary models have largely focused on qualitative virulence and resistance loci controlling infection (Leonard 1977, Agrawal and Lively 2002, Thrall and Burdon 2002, Tellier and Brown 2007) we demonstrate that host genotype continues to affect quantitative pathogen fitness following infection. Interestingly, our results indicate that the capacity for pathogen and hosts to respond to selection is different at different life-history stages. Consequently, evolution of increased infection efficiency in the pathogen population might be countered by evolution of host mechanisms limiting sporulation capacity, if lower sporulation increases host fitness. Certainly, different pathogens differ greatly in the mechanisms of reproduction within the host and thus, their detrimental effects on host fitness (Bushnell 1984, Jarosz and Davelos

1995, Gilbert 2002). Oat crown rust and other obligate or foliar pathogens that use host resources for their own reproduction (Bushnell 1984, Staples 2001) likely induce selection on the host to reduce their growth and reproduction within the host. In contrast, pathogens that greatly increase juvenile mortality (Bushnell 1984, Jarosz and Davelos 1995) or necrotrophs that use toxins to kill the host (Staves and Knell 2010) should induce selection for host mechanisms that reduce the pathogen's infection efficiency or effects of toxins. In any case, examination of the life-history stages most strongly affected by host and pathogen genotype will inform coevolutionary models and improve predictions for the evolution of pathogen aggressiveness.

CONCLUSIONS

Here, we provide evidence for mechanisms by which genetic variation in host populations may drive the evolution of pathogen life-history traits. Complimenting the better-known effects of pathogens on host life histories, our results show that host genotype most strongly affects pathogen life-history stages of growth and reproduction within the host. Moreover, we show that the effects of competition among infections vary with host genotype, suggesting that host population structure has the potential to modulate the evolution of pathogen aggressiveness. We conclude that the capacity of the pathogen or host genotype to respond to selection will be different for different pathogen life-history stages, suggesting an important mechanism of antagonistic coevolution.

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TABLES & FIGURES

Table 1.1. Oat genotype accessions, year of release, and location

Host genotype	Accession	Year released	Location developed
Otana	CIav-1976	1976	Montana/Idaho
Ogle	CIav-9401	1980	Illinois
Pendek	CIav-7801	1960	Netherlands
Pendek38	CN 32995	1968	Manitoba, Canada
Portage	CIav-7107	1960	Wisconsin

Accession numbers are for the USDA Germplasm Resource Information Network (GRIN) except for Pendek38, for which we have listed the accession number for Agriculture and Agri-Food Canada

Table 1.2. Summary of ANCOVA results for total spore production

Source	DF	Type II SS	F	p
Block	1	3.456	9.714	0.0032
Pathogen	4	4.745	3.334	0.0181
Whole-plot error	44	15.656	3.198	0.0014
Inoculum dose	1	0.460	4.138	0.0527
Host	4	3.800	8.539	0.0002
Pathogen x Host	16	2.145	1.205	0.3290
Split-plot error	25	2.781		

The response variable is total spore production (mg spores per cm^2 of leaf tissue) and it was log transformed before analysis. Log transformed inoculum dose (spores deposited per cm^2) was used as a covariate. The effects of block (inoculation day) and pathogen genotype were analyzed at the whole-plot level, while the effects of inoculum dose, host genotype, and the interaction term, pathogen x host genotype, were analyzed at the split-plot level

Table 1.3. Summary of ANCOVA results for infection efficiency

Source	DF	Type II SS	F	p
Block	1	15.785	131.67	<0.0001
Pathogen	4	6.503	13.560	<0.0001
Whole-plot error	44	5.275	0.568	0.9504
Inoculum dose	1	1.156	5.478	0.0275
Host	14	1.262	0.427	0.9500
Pathogen x Host	16	2.272	0.673	0.7928
Split-plot error	25	5.275		

The response variable is pustule density (pustules per cm^2 of leaf tissue) and was used as the response variable and it was log transformed before analysis. Log transformed inoculum dose (spores deposited per cm^2) was used as a covariate. The interaction effect of pathogen genotype by inoculum dose was not significant and was removed from the model

Table 1.4. Summary of ANCOVA results for latent period

Source	DF	Type II SS	F	p
Block	1	8.238	26.612	<0.0001
Pathogen	4	3.595	2.903	0.0323
Whole-plot error	44	13.621	4.495	0.0002
Pustule density	1	2.301	33.407	<0.0001
Host	4	5.783	20.993	<0.0001
Host x Pustule density	4	0.757	2.746	0.0556
Pathogen x Host	16	1.292	1.173	0.3604
Split-plot error	21	1.446		

The response variable is latent period, and it was measured as days until 50% of the pustules were producing spores. Log transformed pustule density (pustules per cm^2 of leaf tissue) was used as a covariate. The interaction effect of pathogen genotype by pustule density was not significant and was removed from the model

Table 1.5. Summary of ANCOVA results for sporulation capacity

Source	DF	Type II SS	F	p
Block	1	3.616	11.05	0.0018
Pathogen	4	4.396	3.36	0.0175
Whole-plot error	44	14.404	6.48	<0.0001
Pustule density	1	2.189	43.34	<0.0001
Host	4	3.438	17.01	<0.0001
Host x Pustule density	4	0.618	3.06	0.0393
Pathogen x Host	16	0.944	1.17	0.3636
Split-plot error	21	1.061		

The response variable is total spore production (mg spores per cm^2 of leaf tissue) and it was log transformed before analysis. Log transformed pustule density (pustules per $cm^2$of leaf tissue) was used as the covariate. The interaction effect of pathogen genotype x pustule density was not significant and was removed from the model

Table 1. 6. Effect size (η^2) of pathogen and host genotypes

Life-history stage	SS_{WP}	SS_P	$P\eta^2$	SS_{SP}	SS_H	$H\eta^2$
Total spore production	23.86	4.74	0.199	9.19	3.80	0.414
Infection efficiency	27.56	6.50	0.236	ns	ns	ns
Latent period	25.45	3.59	0.141	11.58	5.78	0.499
Sporulation capacity	22.42	4.40	0.196	8.25	3.44	0.417

Pathogen effect sizes ($P\eta^2$) were calculated by dividing the sum of squares explained by the pathogen (SS_P) by the total sum of squares at the whole-plot level (SS_{WP}). Host effect sizes (Hη2) were calculated by dividing the sum of squares explained by the host (SS_H) by the total sum of squares at the split-plot level (SS_{SP}). We did not calculate the effect size for host genotype on infection efficiency because the term was not significant (ns)

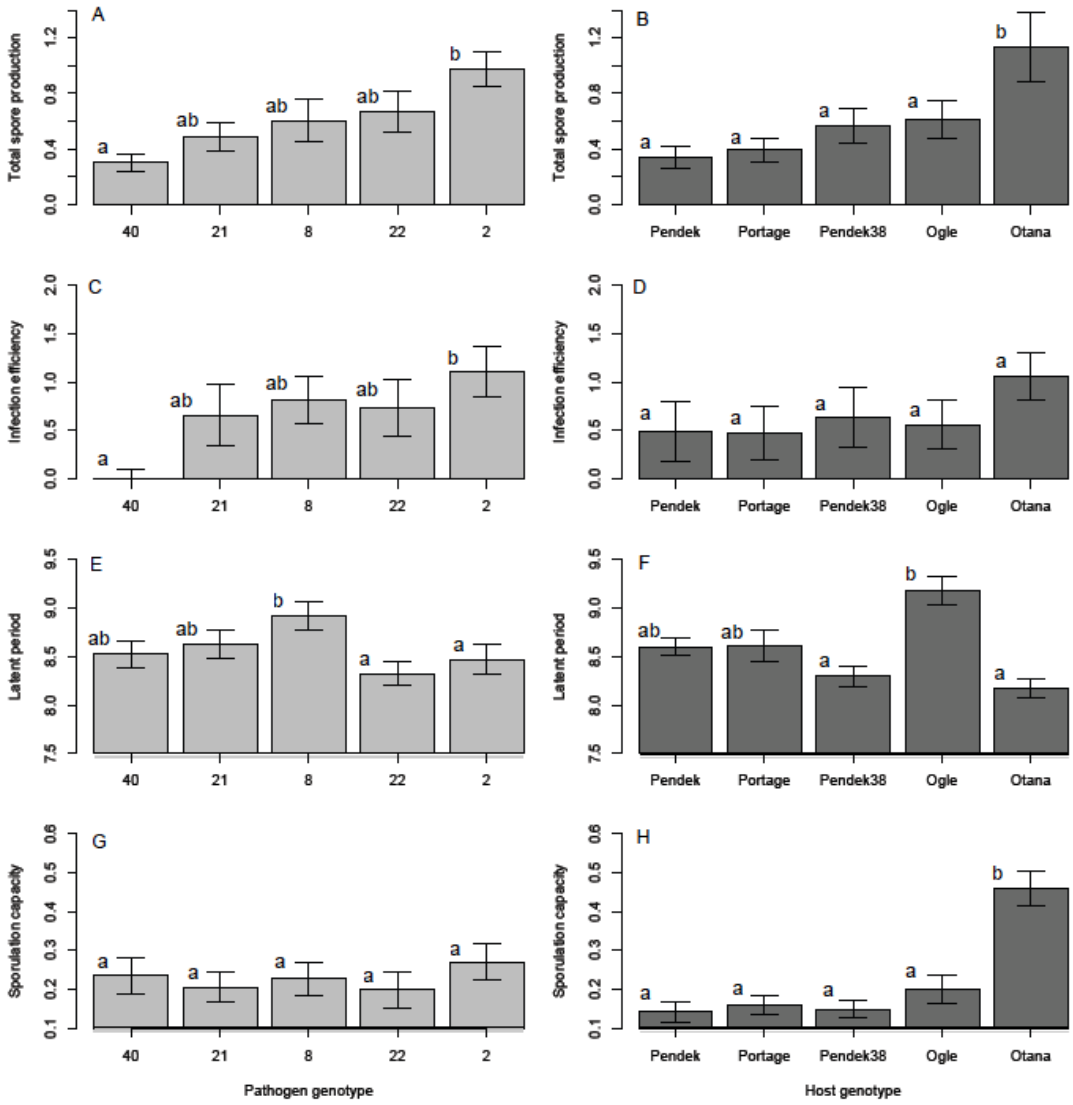

Figure 1.1. Main effects of pathogen and host genotype.

A, B) total spore production (mg spores per cm2of leaf tissue), C, D) infection efficiency (log of number of pustules per cm2of leaf tissue/log of number of spores deposited per cm2), E, F) latent period (days until 50% of pustules reached sporulation), and G, H) sporulation capacity (mg spores per pustule). Different lower case letters indicate significant differences among mean values (at $p = 0.05$ level, with Bonferroni correction for multiple comparisons). Error bars represent ± 1 S.E.

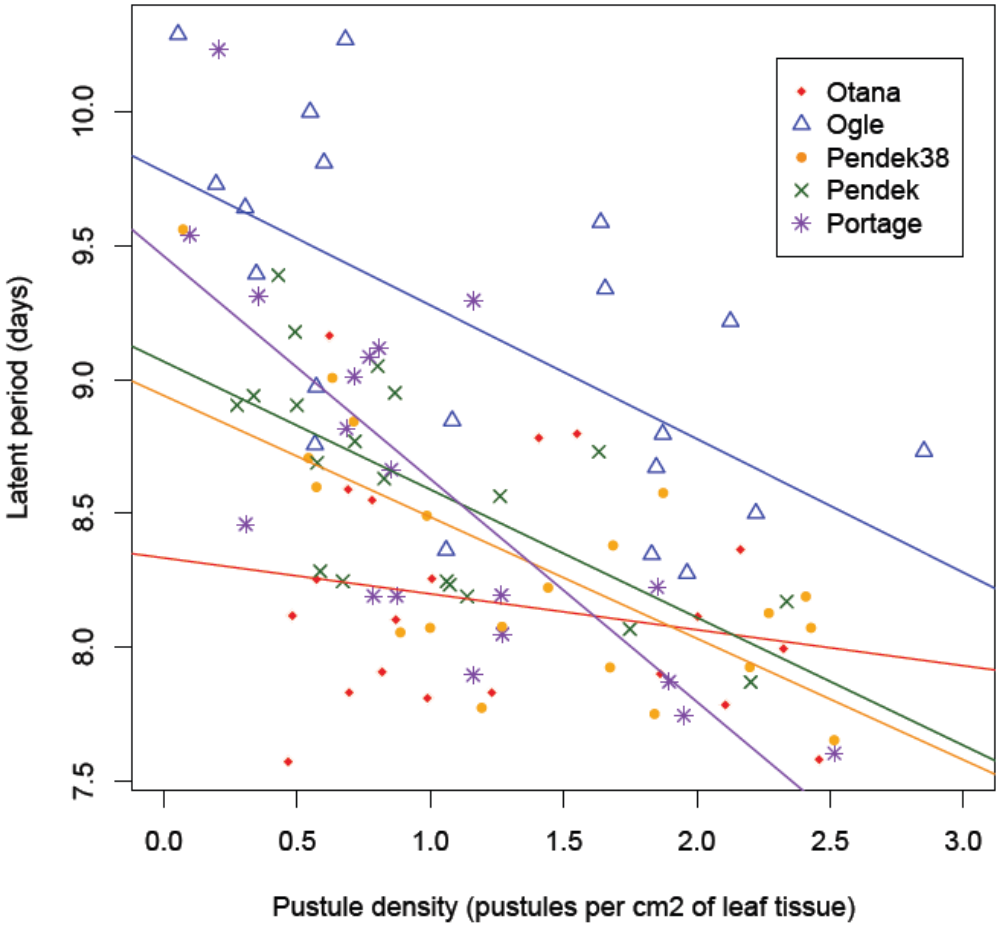

Figure 1.2. Effect of host genotype on the relationship between pustule density and latent period.

The slope of the regression line for the Portage host genotype is significantly different from the slope of the regression line for the Otana host genotype ($t_{19} = -3.017$, $p = 0.007$)

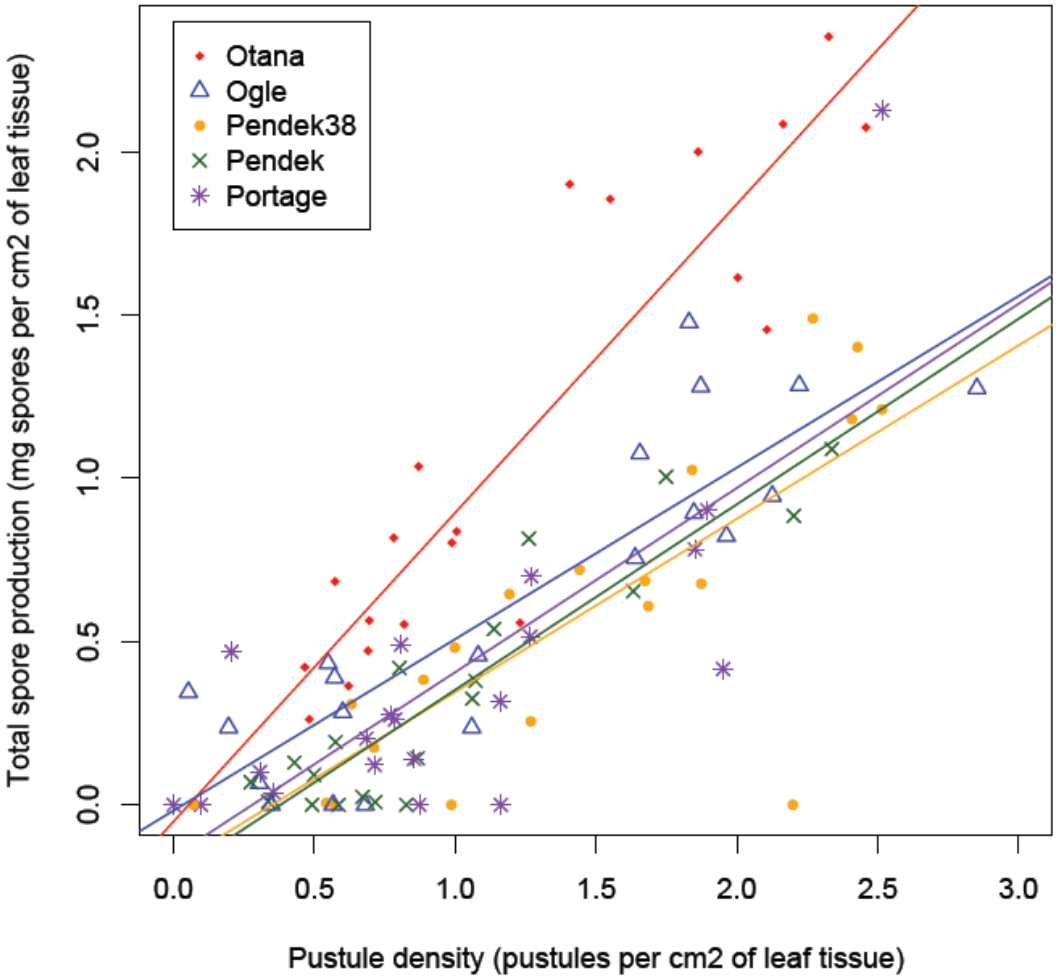

Figure 1.3. Effect of host genotype on the relationship between pustule density and total spore production.

The slope of the regression line for the Otana host genotype is significantly different than the regression slopes for all of the other host genotypes except Pendek (Table S1.4)

SUPPLEMENTAL TABLES & FIGURES

Table S1.1**.** Virulence profile of five pathogen genotypes against 21 differential oat lines.

Host line	**21**	**8**	**40**	**22**	**2**
Pc36		■	■	■	■
Pc51	■		■	■	■
Pc56		■	■	■	■
Pc59		■	■	■	■
Pc35	■	■			■
Pc40	■	■			■
Pc48			■	■	■
Pc52			■	■	■
Pc68		■	■	■	
Pc14	■	■			
Pc45				■	■
Pc46			■	■	
Saia				■	■
Pc61	■				
Pc67			■		
Total	5	7	9	10	10

Pathogen genotypes are in columns, while oat resistance genes and cultivars are shown in rows. Dark bars indicate successful infection, while white bars indicate a resistant reaction and no infection. The bottom row gives the total number of virulence alleles carried by each pathogen strain, as determined by the number of different host lines infected. 21 host lines were tested but only the 15 host lines that had differential infection of the five strains are shown. All five pathogen strains were virulent on Pc38, Pc39, Pc70, Trisperma, and Bondic, and all five were avirulent on Pc60.

Table S1.2. Summary of ANCOVA results for total spore production for datasets with either of the Pendek or Pc38 host genotypes excluded.

		Pendek38 excluded				**Pendek excluded**		
Source	**DF**	**Type II SS**	**F**		**DF**	**Type II SS**	**F**	
Block	1	2.747	7.733	***	1	2.882	7.890	***
Pathogen	4	3.965	2.790	*	4	4.026	2.755	*
Whole plot error	44	15.630	2.664	*	44	15.343	4.951	*
Inoculum dose	1	0.538	4.032		1	0.304	4.120	
Host	3	2.700	6.748	***	3	2.695	12.173	***
Pathogen * Host	12	1.332	0.832		12	2.676	3.022	*
Split plot error	12	1.600			13	0.959		

Both the response variable, total spore production (mg per cm^2 of leaf tissue) and the covariate, inoculum dose (spores deposited per cm^2), were log transformed. Asterisks indicate significance at $p < 0.05$, 0.01, and 0.001, respectively. Significance of model effects do not differ from the full model with all 5 host lines included (Table 3).

Table S1.3. Summary of ANCOVA results for latent period for datasets with either of the Pendek or Pendek38 host genotypes excluded.

	Pendek 38 excluded				Pendek excluded			
Source	**DF**	**Type II SS**	**F**		**DF**	**Type II SS**	**F**	
Block	1	6.7669	23.46	***	1	6.8069	19.28	***
Pathogen	4	4.0439	3.51	*	4	3.2353	2.29	
Whole plot error	44	12.6894	4.02	*	44	15.5334	3.69	*
Pustule density	1	0.1164	1.62		1	0.9878	10.31	**
Host	3	7.2406	33.68	***	3	5.0688	17.64	***
Host * density	3	0.7613	3.54	*	3	1.1238	3.91	*
Pathogen * Host	12	0.9853	1.15		12	1.2898	1.12	
Split plot error	10	0.7166			10	0.9577		

Latent period was measured as days until 50% of the pustules were sporulating and log transformed pustule density (pustules per cm^2 of leaf tissue) was used as a covariate. Asterisks indicate significance at $p < 0.05$, 0.01, and 0.001, respectively. Significance of pathogen and host effects do not change from the full model with all 5 cultivars (Table 5). The effect of pathogen genotype is not significant ($p=0.075$) when Pendek is excluded from the model.

Table S1.4. Pairwise comparison of slopes due to host genotype for the regression of spore production on pustule density

Pairwise comparison	**Slope**	**Standard error**	**t**	**p**
Otana[a]	0.644	0.230	2.803	**0.012**
Otana vs Ogle[b]	-0.587	0.174	-3.373	**0.004**
Otana vs Pendek38	-0.437	0.186	-2.358	**0.031**
Otana vs Pendek	-0.152	0.177	-0.859	0.403
Otanta vs Portage	-0.413	0.165	-2.510	**0.022**

[a] Estimated slope of the regression between pustule density (pustules per cm^2 of leaf tissue) and total spore production (mg spores per cm^2 of leaf tissue) for the Otana host line.

[b] Difference and standard error of the difference between the estimated slopes of the regression of for the Ogle host line and the regression for the Otana host line. Pairwise comparisons of slope are only shown for the Otana host line since this is the only host line that had differed significantly in slope. All estimates and standard errors are from the full ANCOVA model used to analyze sporulation capacity (Table 5).

Table S1.5. Summary of ANCOVA results for sporulation capacity for datasets with either of the Pendek or Pendek38 host genotypes excluded.

	Pendek 38 excluded				Pendek excluded			
Source	**DF**	**Type II SS**	**F**		**DF**	**Type II SS**	**F**	
Block	1	3.026	8.15	**	1	3.294	9.13	**
Pathogen	4	3.924	2.64	*	4	3.728	2.58	*
Whole plot error	44	16.327	14.98	**	42	15.156	8.70	**
Pustule density	1	1.456	58.77	***	1	0.464	11.18	***
Host	3	1.959	26.36	***	3	1.991	15.99	**
Host * density	3	0.475	6.39	*	3	0.401	3.22	
Pathogen * Host	12	0.803	2.70		12	1.308	2.63	
Split plot error	8	0.198			9	0.373		

Log transformed total spore production (mg spores per cm^2 of leaf tissue) was used as the response variable and log transformed pustule density (pustules per cm^2 of leaf tissue) was used as the covariate. Asterisks indicate significance at $p < 0.05$, 0.01, and 0.001, respectively. Excluding Pendek38 or Pendek does not change the significance of pathogen, host, or interaction effects detected in the full model (Table 6). Excluding Pendek does decrease the significance of the host*density effect ($p=0.076$).

CHAPTER 2

Increased virulence level comes at a cost of delayed reproduction and smaller pustule size in a plant pathogen

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SUMMARY

Understanding the mechanisms that maintain genetic diversity is a central goal in evolutionary biology. Nowhere is this goal more important than in the rapidly evolving interactions of pathogens and hosts. Fitness costs associated with host resistance and pathogen virulence are hypothesized to play an important role in the maintenance of genetic diversity, yet empirical support for such costs is limited. Here we test the hypothesis that pathogen virulence alleles carry a cost of reduced reproduction within the host. We used strains of the oat crown rust fungus, *Puccinia coronata*, which varied widely in virulence level, defined here as the number of virulence alleles. For each strain, we quantified total within-host reproduction, and the expression of four pathogen life history stages: infection efficiency, latent period, pustule size, and spore production per pustule. We then examined the relationship between virulence level and each quantitative trait to determine whether there was evidence in support of trade-offs. We found that the onset of reproduction was significantly delayed and the size of sporulating pustules decreased with increasing virulence level. We conclude that trade-offs associated with reproductive timing and pustule size have the potential to constrain the evolution of increasing pathogen virulence level.

INTRODUCTION

A half century of research on interactions of plant hosts with their pathogens has led to tremendous advances in our understanding of the molecular basis of these interactions (Chisholm et al. 2006, Jones and Dangl 2006, Bent and Mackey 2007), yet considerable debate surrounds the mechanisms limiting the evolution of pathogen virulence (Laine and Tellier 2008, Stukenbrock and McDonald 2009, Thrall et al. 2012). Pathogen populations harbor significant genetic variation at virulence loci that determine the qualitative infection success on particular host genotypes (Dinoor and Eshed 1987; Clarke 1997; Thrall and Burdon 2003; Leonard et al. 2004), raising the question of the mechanisms that maintain such variation. Central to the debate is the issue of costs of pathogen virulence. While fitness costs are embedded in conceptual models for coevolution of pathogens and their hosts (Leonard 1977, Sasaki 2000, Thrall and Burdon 2002, Tellier and Brown 2011), few studies have directly evaluated costs of virulence to the reproductive success of pathogens. Here we use a well-studied host-pathogen system to evaluate the relationship of pathogen virulence and reproduction within the host and to assess the potential for trade-off between these two traits to limit the evolution of increasing virulence.

In gene-for-gene interactions common to many plant-pathogen systems (Thompson and Burdon 1992), plant resistance gene products recognize and trigger strong defense responses that limit infection upon recognition of specific pathogen effector molecules (Ellis et al. 2000, Dodds et al. 2006, Jones and Dangl 2006). Pathogens that carry a 'virulence' allele of the effector gene avoid recognition by host resistance proteins and successfully infect the host (Chisholm et al. 2006, Jones and Dangl 2006, Bent and Mackey 2007). Since pathogen effector molecules that are the targets of host resistance gene-products carry out a diverse array of functions within the pathogen (Ellis et al. 2007; Kamoun 2007) many authors hypothesize that mutations that confer virulence may come at cost of decreased function (Leach et al. 2001, Jones and Dangl 2006, Bent and Mackey 2007). Note that here and throughout we use the term virulence to refer to the specific alleles involved in gene-for-gene interactions with host resistance rather than the level of damage incurred by the host.

Since a virulence allele enables a pathogen to infect all host genotypes whether or not they carry the corresponding resistance gene, new virulence alleles entering a pathogen population are predicted to increase in frequency and potentially sweep to fixation. Yet many empirical studies of natural populations demonstrate considerable polymorphism for virulence and avirulence (Oates et al. 1983, Jarosz and Burdon 1991, Clarke 1997, Thrall and Burdon 2003). Early theoretical studies hypothesized that a trade-off between pathogen virulence and within-host reproduction, or a 'cost of virulence', is crucial to the maintenance of polymorphisms (Leonard 1977, Leonard and Czochor 1978, Leonard 1994). While more recent studies have demonstrated that spatial structure (Thrall and Burdon 2002, Tellier and Brown 2011), multi-locus dynamics (Sasaki 2000), and genetic drift (Salathe et al. 2005) also play important roles in the maintenance of virulence polymorphism, a cost of virulence remains an important parameter in all these models (Sasaki 2000; Thrall and Burdon 2002; Salathe et al. 2005; Tellier and Brown 2011, but see Damgaard 1999).

Empirical tests of the cost of virulence in plant pathogens are relatively few in comparison to the number of theoretical models, and support for virulence costs is mixed (Leach et al. 2001, Sacristan and Garcia-Arenal 2008). In the plant pathology literature, a cost of virulence has been invoked to explain an observed decrease in the frequency of virulence following the removal of resistant host genotypes (Grant and Archer 1983). However, inference of costs from these kinds of observations is difficult because the frequency of virulence can also be affected by environmental variation and genetic drift (Antonovics and Alexander 1989). Results of controlled selection experiments offer better insight into the role of costs in the evolution of pathogen virulence level. Leonard (1969) provided evidence in support of virulence costs as he found that the frequency of virulence alleles in a heterogeneous population of oat stem rust declined after eight generations of selection on a susceptible host. However, Kolmer's (1993) results suggest few costs to virulence as he found that the frequency of virulence alleles in wheat leaf rust increased after 12 generations of selection on a susceptible host. Studies where the pathogen's genetic background is held constant provide the clearest test of virulence costs, but results vary across these studies as well. For example, Vera Cruz (2000) found that the virulence allele at the *AvrXa7* locus in the rice blight bacterium, *Xanthomonas*

oryze, was associated with a significant reduction in lesion size, but that virulence at a different locus, *AvrXa10*, had no effect on lesion size. The authors infer that that the observed fitness cost of virulence at *AvrXa7* is the result of a less functional virulence gene product. Bahri et al. (2009) show a significant decrease in the competitive ability of *Puccinia striiformis* (wheat stripe rust) strains associated with virulence at *Avir4* and *Avir6*, but an apparent competitive advantage for strains with *Avir9*. While results from the above studies can inform predictions about the evolution of virulence at individual loci, pathogens can carry virulence alleles at more than one locus, and an assessment of the cumulative cost of virulence at multiple loci is needed to inform predictions about the evolution of virulence level, defined here as the number of virulence alleles carried by a single pathogen strain.

Pathogens that carry virulence alleles at more than one locus will be able to infect a broader range of host genotypes. Consequently, if fitness costs of virulence at individual loci are cumulative, then there will be a trade-off between virulence level and pathogen reproduction. Few studies have directly evaluated the correlation between virulence level and quantitative pathogen fitness traits (Thrall and Burdon 2003; Montarry et al. 2010). Thrall and Burdon (2003) found a trend of decreasing spore production with increasing virulence level in wild populations of *Melampsora lini* (flax rust) while Montarry et al. (2010) found a trade-off between virulence level and lesion size in *Phytophthora infestans* (potato late blight). To our knowledge no study has estimated the proportion of genetic variation for pathogen reproduction that is due to virulence level, yet such an estimate is required to predict the response to selection.

Pathogen reproduction within the host is the product of several life history stages and these stages may be differently affected by virulence level. For example, Montarry et al. (2010) found that lesion size of *P. infestans* decreased with increasing virulence level, as predicted by the cost of virulence hypothesis, but found that latent period, the lag time between infection and reproduction, decreased with increasing virulence level. Moreover, reproduction within the host is only one component of a pathogen's lifetime fitness, measured as the number of new infections generated over the lifetime of one infected host (May and Anderson 1983). Individual life history traits such as infection efficiency and latent period that affect transmission and time of reproduction may have larger

impacts on a pathogen's lifetime fitness than total within host reproduction (Leonard and Mundt 1984, Antonovics and Alexander 1989). Thus, understanding the contribution of individual life history stages to pathogen fitness is necessary to evaluating evolutionary costs of virulence.

Here we ask whether increased virulence level has costs to pathogen fitness for the agricultural pathogen *Puccinia coronata* f.sp. *avenae*, the causal agent of oat crown rust. The average virulence level of *P. coronata* strains in the United States has increased significantly over the last decade (Carson 2008, 2011), demonstrating that virulence level is rapidly evolving in this system. Our first objective was to test the hypothesis predicting a trade-off between pathogen virulence level and reproduction within the host. A cost of virulence will be supported by a negative correlation between the number of virulence alleles and total spore production within the host. Our second objective was to test the hypothesis predicting a trade-off between virulence level and the quantitative expression of four pathogen life history traits within the host: *infection efficiency*, the proportion of deposited spores that successfully infect the host*, latent period,* the lag time between spore deposition and reproduction, *pustule size,* the size of infection foci, and *spore production per pustule*, the number of spores produced per infection focus. We then estimate the amount of genetic variation in each quantitative trait that is explained by virulence level and estimate the contribution of each life history trait to total reproduction within the host.

Our results provide evidence of trade-offs between virulence level and latent period, and between virulence level and pustule size but show no evidence for a trade-off between virulence level and total spore production within the host. We find that pathogen strains with lower virulence levels reproduce earlier and form larger sporulating pustules than strains with higher virulence levels. We conclude that trade-offs between virulence level and the quantitative expression of these key life history traits have the potential to constrain the evolution of virulence level, since strains with lower virulence level will have a fitness advantage on susceptible hosts.

METHODS

Our goal was to test hypotheses for a cost of virulence to total spore production and to individual life-history traits. To test these hypotheses we quantified total spore production and the life history stages of infection efficiency, latent period, pustule size, and spore production per pustule on a susceptible host of 29 strains of *P. coronata* that ranged widely in virulence level. We then used multiple-regression modeling to determine the effect of strain and virulence level on total spore production and the quantitative expression of individual life history traits. Lastly, we used analysis of covariance to determine the contribution of each life history stage to total spore production.

Study system

Puccinia coronata f.sp. *avenae* is an obligate fungal pathogen of oats (*Avena sativa*), where it causes the disease oat crown rust. Like many rust fungi, *P. coronata* has a complex lifecycle that incorporates two distinct hosts and five spore stages. In early spring spores that overwintered on oat straw germinate, go through meiosis and produce haploid spores that infect the leaves of common buckthorn (*Rhamnus cathartica*). There, mating occurs to produce dikaryotic (N+N) spores that infect oats (*Avena sativa*). These spores germinate and penetrate the leaves of oat plants to produce infection foci (pustules) on the surface of the leaves that are filled with the bright orange urediniospores that give rusts their name. All of the urediniospores produced in a singe pustule are the same genotype because they are produced asexually by mitotic cell divisions. Urediniospores readily re-infect oats, and, with rapid generation times of 8 to 12 days, large-scale disease epidemics result. All of the experiments described here utilize the urediniospore stage because the gene-for-gene system governing infection on oats is well described and oat tester lines (differentials) can be used to detect specific virulence alleles in each *P. coronata* strain (Fleischmann and Baker 1971).

Pathogen strains

We used 29 *P. coronata* strains (Table S2.**1**) from the USDA Cereal Disease Lab's (CDL) long-term rust collection that were obtained in annual crown rust surveys across the oat-growing region of the USA. Prior to preservation in liquid nitrogen, CDL staff ensured that each strain represented a single genotype and then determined virulence level. We used stratified-random sampling to select *P. coronata* strains that varied widely in virulence level as follows. We pooled data for all *P. coronata* strains collected by the CDL in 2007 (134 strains) and 2008 (138 strains) and an additional 41 strains sampled from St. Paul, MN in 2008 into a single database. Then we sorted the records into five classes based on estimated virulence level (0-5, 6-10, 11-15, 16-20, 20+ virulence alleles). This process yielded a small number of potential strains for the lowest virulence class (0-5 virulence alleles) and for the higher virulence class (20+), thus we chose three additional strains in each of these virulence classes from the 2006 collection. With at least eight strains per class, we selected six strains from within each class. Where possible, we selected three strains from Northern and three strains from Southern regions of the USA.

To prepare large quantities of spores for each of the 30 strains chosen above, we "increased" individual genotypes as follows. Spore vials were retrieved from cryo-storage and spores were heat shocked in a 40°C water bath for 10 minutes, and then mixed with 500μL mineral oil (Soltrol 170, Philips-Conoco, Houston, TX). Spores were sprayed onto 10 cm square pots containing one-week old seedlings of the susceptible oat varieties Marvelous and Starter. Inoculated seedlings were placed in a dew-chamber with 100% relative humidity overnight and then maintained in isolated, individual cubicles in the greenhouse. The soil was amended with a water-soluble 20N-20P-20K fertilizer after inoculation and every seven days thereafter. To insure that each strain represented a single genotype, plants were trimmed 10 days after inoculation so that each plant only supported a single pustule. Spores from these single pustules were collected seven days later and used to inoculate a second set of one-week old Marvelous and Starter oat seedlings. Newly inoculated plants were maintained in isolated cubicles as above, and allowed to produce multiple pustules and large amounts of inoculum. Of the 30 strains chosen above, 29 produced sufficient inoculum for use in the experiments.

Quantification of virulence level

To verify the specific virulence alleles carried by each strain and estimate virulence level, we inoculated each pathogen strain onto a set of 30 differential oat lines used in annual crown rust virulence surveys (Carson 2008). Each differential oat line carries a unique crown rust resistance gene (Fleischmann and Baker 1971) and therefore strains that can infect a particular differential line must carry the corresponding virulence allele. We counted the number of virulence alleles per strain to determine the virulence level.

The 30 oat differential lines and the susceptible control line, Marvelous, were planted into two 30.5 x 20.3cm trays filled with vermiculite. Six to eight seeds of each host line were planted in a single marked location in the tray. Seedlings were inoculated 9 days after planting. Each tray was inoculated with fresh spores from a single pathogen strain using the inoculation methods described above. We assessed infection 15 days after inoculation using a qualitative scale as follows: Host lines that were covered in large pustules with little to no chlorosis were scored as susceptible, while host lines that had no pustules, or very small pustules with a strong chlorosis reaction, were scored as resistant. Intermediate reactions that produced moderate sized-pustules but with a strong chlorosis reaction were noted. We used the infection reaction on the control line Marvelous to gauge the expected size and density of pustules in a susceptible reaction. We repeated the entire experiment in four separate trials between November 2009 and May 2010.

Quantification of total spore production and life history stages

To assess pathogen reproduction within the host and quantify the expression of life history stages, we inoculated each of the 29 different *P. coronata* strains onto adult oat plants of the susceptible cultivar Otana. Previous work has demonstrated that Otana is highly susceptible to infection from *P. coronata* (Bruns et al. 2012). Each strain was inoculated onto six replicate pots, and each pot contained two plants. Within a pot, one plant was inoculated with a low dose of 10^4 spores/mL while the other plant was inoculated with a high dose of 10^5 spores/mL. Six additional pots were mock inoculated with mineral oil, and served as contamination controls. Thus we had a total of 180 pots and 360 individual plants. Plants were arranged in a completely randomized design on

two greenhouse benches. Inoculations were carried out on three separate days due to time constraints and limited dew chamber space. We used inoculation day as a blocking factor such that two replicate pots of each pathogen strain were inoculated on each of three successive days. Inoculation order was randomized each day, but all plants receiving the same pathogen strain were inoculated in succession to reduce the risk of cross contamination.

Host plants

Seeds of the susceptible oat cultivar Otana were planted in 15.2cm diameter pots filled with pasteurized soil amended with 5mL of 'Osmocote' (14N-14P-14K) slow-release fertilizer (Scotts Miracle-Gro Co.) in September 2009. Seeds were planted in two positions within the pot, 6.5 cm apart, with three seeds per position to insure germination. Plants were thinned to two plants per pot positioned 6.5 cm apart one week after germination. Plants were raised in a greenhouse with 22°C day and 18°C night. Supplemental lighting maintained a 16-hour day.

Inoculation procedures

Fresh spores were collected from infected seedlings maintained in greenhouse cubicles on each of three subsequent inoculation days and mixed with 200μL of mineral oil. Spore concentration was determined as the average of two hemacytometer counts, and diluted to obtain suspensions containing a low dose of 10^4 spores/mL and a high dose of 10^5 spores/mL.

Experimental plant inoculations were carried out when oat plants were beginning to flower, approximately 2 months after planting at the CDL greenhouses. The penultimate leaf of each plant was fixed to a vertical board and a clean paper frame was fixed over the leaf so that only a 15cm section of the adaxial side of the leaf was exposed. Spores were applied to the leaf with a quantitative spray inoculator (Scientific apparatus shop, University of Minnesota). To prevent cross-contamination, clean spray nozzles, inoculation frames and board covers were used for each pathogen strain. To estimate the variation in inoculation dose among sprayers a glass slide mounted with double-sided tape was fixed next to the leaf receiving the high spore dose. Following inoculation,

plants were placed into a dew chamber with 30 seconds of mist every 2 minutes for a period of 12 hours and then returned to their randomly assigned position in the greenhouse.

To determine whether strains differed in spore viability, we assessed spore germination rate *in vitro*, on the same day that inoculations were performed. Spore viability was estimated for 200μL of the 10^5spores/mL suspension by spreading these over to two 1.5% water agar plates (1.5% Difco Bacto® Agar), incubating overnight at 22°C, and counting the number of spores that did or did not germinate. Spore germination was only measured for the 2nd and 3rd inoculation blocks because a problem with incubation conditions in the first inoculation block resulted in no germination.

Measurements of life history traits

We quantified total spore production as the dry spore mass per square centimeter of leaf tissue. Spores were collected by horizontally suspending inoculated leaves in glassine bags and adjusted to maintain airflow and avoid condensation. The leaf tips beyond the 15cm inoculated region were trimmed so that leaves were not folded. Spore bags and leaves were harvested 23 days after inoculation. Total dry spore mass was measured by using a small vacuum to collect spores from each bag into a gel capsule which was then desiccated, and weighed (Bruns et al. 2012).

We quantified the life history stages of infection efficiency, latent period, pustule size, and spore production per pustule by monitoring disease progress. We counted the number of sporulating pustules on each leaf beginning 8 days after inoculation. Pustules were counted daily until no new pustules were detected, a period that ranged from 4-7 days across individual plants. Leaves were harvested and scanned 23 days after inoculation and ImageJ (NIH) was used to determine total pustule area. We also recorded plant height, distance from the leaf base to the inoculated portion of leaf, and leaf mortality at harvest. Leaves were recorded as dead if less than 50% of the tissue was green.

The number of pustules produced per square centimeter of leaf tissue was used as a proxy for infection efficiency since we did not have estimates of spore deposition for the 10^4 spores/mL dose treatment. Since not all pustules within a leaf sporulated at the

same time, we estimated latent period by calculating the weighted average of the number of days until sporulation for all pustules within a leaf. We find this to be a reasonable measure of latent period since latent periods appear to be normally distributed within a leaf, and we found no evidence to suggest that the standard deviation of the latent period estimates varied among pathogen strains ($F_{28,309}$= 1.0514, p= 0.3982). We estimated average pustule size by dividing the total pustule area of an inoculated leaf by the number of pustules. We used covariate analysis (described below) to estimate spore production per pustule rather than direct calculation because this trait is known to be strongly density-dependent (Newton et al. 1997, Bruns et al. 2012).

Statistical analysis

We used a two-step analysis to assess the relationship between virulence level and quantitative values of total spore production and each life history trait. First, we asked whether there was significant genetic variation for the trait at the level of the strain. Then, if a significant strain effect was detected, we replaced the categorical explanatory variable of strain with the continuous variable of virulence level to determine whether some of the genetic variation in the trait was due to virulence level. If a significant effect of virulence level was detected, we used the partial regression of the trait on virulence level to estimate the direction and slope of the relationship. To test our first hypothesis that there is a trade-off between virulence level and within-host reproduction, we analyzed the effect of strain and then virulence level on total spore production. To test our second hypothesis that there are trade-offs between virulence level and the quantitative expression of pathogen life history traits we used the same two-step process described above to determine the effect of strain and virulence on each life history trait but included the variation in previous life history traits as covariates in the model (Figure 2.**1**).

We used separate mixed-effect models with restricted maximum likelihood (nlme package in R version 2.12.0, The R Foundation for Statistical Computing) to determine the effect of pathogen strain and virulence level on total spore production and on each measured life history stage. Pot was included as a random factor to account for potential correlation of response for two plants in each pot. Pathogen strain and virulence level,

inoculation day block, and environmental variables such as greenhouse bench and pot position were modeled as fixed effects that varied between pots. Inoculum dose treatment, and measurements of previous life history traits were nested within pot. To take into account the biological order of life history stages, we used type I sums of squares rather than type III, and fit each life history stage into the model in sequential order beginning with infection efficiency. We started by fitting a full model that included all blocking factors (i.e. inoculation day, greenhouse bench), potential covariates (plant height, leaf length), and interactions between strain and previous life history traits. We simplified models using AIC and likelihood ratio tests. We tested the assumption of linear relationships between life history traits against more complex polynomial models with likelihood ratio tests. Transformations were performed to meet assumptions of normality as follows: total spore production was square root transformed, and infection efficiency (pustules per cm^2 of leaf tissue) and pustule size (cm^2) were log transformed.

We performed t-tests on total spore production and each life history trait to determine whether there was a difference between pathogen strains collected from the Northern and Southern Great Plains.

Leaf mortality

Since over 20% percent of the infected leaves died prior to harvest, we used a logistic regression models with likelihood ratio tests to determine whether strain and virulence level had effects on leaf mortality (glm in R version 2.12.0). Similar to the above analysis, we first analyzed the effect of strain and virulence level on leaf mortality without explicitly accounting for the variation in life history stages. Then, we included infection efficiency and latent period as covariates to determine whether these life history stages affected leaf mortality. Since image analysis was not possible on dead leaves, we were unable to estimate the effect of pustule size on leaf mortality (Figure 2.**1**).

Effect size

We used a split-plot analysis of variance (aov in R version 2.12.0) to estimate the effect size of infection efficiency, latent period, and pustule size on total spore production. We also estimated the effect size of strain and virulence level on each life

history trait, and used these to estimate the proportion of the strain variation in each trait affected by virulence level.

RESULTS

Variation in virulence level

We defined a consensus virulence profile for each pathogen strain as the set of virulence alleles determined in at least 3 out of 4 replicate trials. Overall, most pathogen strains showed a high level of consistency in reaction to host differential lines across trials, however, some uncertainty arose in those pathogen by host combinations that gave intermediate reactions, or from trials where infection rates on the susceptible control were low. To account for this uncertainty in virulence level, we calculated the upper and lower bounds for the total number of virulence alleles carried by each strain. We ran all regression models three times using the consensus virulence level, and the lower and upper bounds for virulence level. We then adjusted the confidence intervals of the regression slope so that they spanned the confidence range of all three regression-models. Virulence level per strain ranged from 1 to 21 alleles (Table 2.**1**), providing a broad range of virulence levels. Each pathogen strain had a unique virulence profile (Table S2.**2**) indicating that all 29 strains are unique genotypes.

Effect of pathogen strain on spore production and life history traits

We found considerable variation in total spore production and all measured life history traits (Table 2.**1**). Pathogen strain had a significant effect on the total number of spores produced, demonstrating that there is genetic variation for spore production (Table 2.2A).

We did not find a significant effect of strain on infection efficiency (Table 2.3). For the high inoculum dose treatment, we analyzed the effect of strain on infection efficiency, using spore deposition (spores deposited per cm^2) estimated in high dose treatment, as a covariate. Spore deposition was indeed a significant predictor of infection efficiency ($F_{1,142}$=29.123, $p<0.0001$). However, even after accounting for the variation in spore deposition, there was still no significant effect of pathogen strain ($F_{28,142}$ = 1.180, p=0.2607). Thus, infection efficiency appears to be mainly an environmentally determined trait on this susceptible host.

We found significant effects of pathogen strain on the life history stages of latent period (Table 2.**4**A), pustule size (Table 2.**5**A), and spore production per pustule (Table 2.**6**A), demonstrating genetic variation for these traits. Pathogen strain also had significant effects on the proportion of spores that germinated on water agar plates ($F_{28,81}$= 2.178, p= 0.0036) and on leaf mortality (Table 2.**7**A), indicating that there is a genetic variation for germination rate and for pathogen aggressiveness, the amount of damage inflicted on the host by the pathogen. The likelihood of leaf mortality was not significantly affected by infection efficiency or latent period (Table 2.**7**A) suggesting that the observed effect of pathogen strain on leaf mortality may be due to causes other than greater pathogen growth in the host.

There was no significant difference between pathogen strains collected from the Northern and Southern United States in total spore production (t_{337}= 1.132, p = 0.258), infection efficiency (t_{337} = 0.691, p=0.490), latent period (t_{337}=-1.419, p=0.157), or pustule size (t_{270}=1.021, p=0.308).

Effect of virulence level on spore production and life history traits

Virulence level did not significantly affect total spore production (Table 2.2B), and thus, it appears that virulence level does not have an apparent cost to pathogen reproduction within the host.

We did not test for the effect of virulence level on infection efficiency because no strain effect was detected (Table 2.3). Among the life history traits with a significant strain effect, virulence level had a significant effect on latent period (Table 2.**4**B) and a marginally significant effect on pustule size (Table 2.**5**B). We found a significant, positive slope for the partial regression of virulence level on latent period (Slope =0.02987, SE=0.0077, t_{1169}=3.891, p<0.0001; Figure 2.**2**) even after accounting for the uncertainty in virulence level (adjusted 95% confidence interval for slope = 0.0122 to 0.040). We found a marginally significant negative slope for the partial regression of virulence level on pustule size (Slope= -0.0212, SE = 0.0119 t_{155}=-1.77, p = 0.078; Figure 2.3) although the slope was not significant after accounting for the uncertainty in virulence level (adjusted 95% confidence interval for slope = -0.0521 to 0.0024). Thus,

increased virulence level appears to come at a cost of a longer latent period and a somewhat smaller average pustule size.

Virulence level did not have a significant effect on germination rate *in vitro* (Slope=0.00018, t_{219}=0.069, p=0.945), spore production per pustule (under either model 1: Table 2.**6**B, or model 2: Table S2.**3**) or leaf mortality (Table 2.**7**B). Thus, while we observe significant strain variation for germination rate, spore production per pustule, and leaf mortality, variation in these traits is not explained by virulence level.

Virulence level explained varied amounts of the genetic variation in each life history trait due to strain. Virulence level had the greatest effects on latent period and pustule size where it explained 15-19% of the variation in latent period and 9-12% of the variation in pustule size due to strain. In contrast, virulence level explained less than 1% of the variation in spore production per pustule and total spore production due to strain. Thus, with the exception of infection efficiency, we conclude that there is a genetic basis to all the life history traits measured, and that the fraction of the genetic variation that is explained by virulence level varies greatly across life history stages.

The effect of life-history stages on total spore production

Total spore production was affected by infection efficiency, latent period, and pustule size (Table 2.**6**). Unsurprisingly, infection efficiency had a significant positive effect on total spore production (Slope = 0.1612, SE = 0.0071, t_{111}=22.82, p<0.0001), since leaves with more pustules produced more spores in total. Interestingly, total spore production increased non-linearly with increasing infection efficiency (Figure S2.**1**). Latent period had a negative effect on total spore production, such that longer latent periods resulted in lower spore production (Slope = -0.0186, SE = 0.0097, t_{111}=-1.916, p=0.0578). Pustule size had a significant positive effect on total spore production, such that larger pustules resulted in greater total spore production (Slope=0.0214, SE=0.0061, t = 3.489, p= 0.0007). Thus we conclude that greater infection efficiency, shorter latent periods, and larger pustule size all act to increase pathogen reproduction within the host.

We found negative correlations between infection efficiency and later life history stages of latent period, pustule size, and spore production per pustule. Latent period significantly decreased with increasing infection efficiency (Slope= -0.087724, SE=.0230

t_{164}=-3.816, p=0.0002) as did pustule size (Slope=-.01527, SE=0.0504,t_{112}=-3.028, p =0.0031). Spore production per pustule decreased non-linearly with increasing infection efficiency (Figure S2.**2**). Since infection efficiency was measured as pustule density, we conclude that the negative correlations between density and the life history traits described above are a response to crowding. Importantly, while crowding resulted in smaller pustules that produced fewer spores per pustule, the overall effect of increasing infection efficiency was a net increase in the total spore production.

We also found significant negative correlation between leaf mortality and total spore production (Slope=-0.0397, SE=0.0132, t_{133}=-3.001, p=0.0032; Pathogens on leaves that died prior to harvest produced fewer total spores than pathogens on leaves that were still alive at the end of the experiment. Thus, we conclude that early leaf mortality, a measure of pathogen aggressiveness, has a negative effect on within-host reproduction under the experimental conditions described here.

Overall, infection efficiency explained 90% of the variation in total spore production at the split-plot level while latent period explained less than 1% and pustule size explained just 1% of the variation (Table S2.**4**). Thus, infection efficiency, the life history stage that affects pustule density, appears to make the largest contribution to within-host reproduction while latent period and pustule size contribute much less. Latent period and pustule size likely contribute to other aspects of pathogen fitness besides within-host reproduction, and these effects are discussed below.

DISCUSSION

Do trade-offs limit the evolution of pathogen virulence? We addressed this question by first examining the relationship of virulence level to the pathogen's total within-host reproduction. We then evaluated the relationship of virulence level to the quantitative expression of life history stages contributing to pathogen fitness. Testing the first hypothesis, we found that virulence level did not significantly affect total spore production. In contrast, we found evidence in support of trade-offs between virulence level and key life history stages. Virulence comes at a cost of delayed reproduction because latent period, the lag time between spore deposition and reproduction, increased with increasing virulence level. Further, virulence comes at a cost of smaller pustules because pustule size decreased with increasing virulence level. To reconcile the seemingly incongruous results for effects of virulence level on within-host reproduction compared to effects on life-history stages, we evaluate the contribution of latent period and pustule size to pathogen fitness over several generations.

Total within-host spore production in this susceptible host is driven by the variation in infection efficiency due to environmental effects. Most of the variation in total spore production was explained by infection efficiency, a trait where no significant genetic effect due to strain was detected. Increased infection efficiency resulted in a greater number of infections and consequently, greater total spore production. While shorter latent periods and larger pustules also had positive effects on total spore production (after accounting for infection efficiency), the combined effect of these two stages had much less of an impact on total spore production than the number of infections. Moreover, the number of infections strongly affected the quantitative expression of all subsequent life history stages due to crowding. Pustule size and spore production per pustule decreased with increasing infection numbers, but the overall result was an increase in spore production. Thus, in this susceptible host, total within-host reproduction depends on spore load, which is highly subject to environmental influences but is insensitive to virulence level.

In contrast to the above environmental effects, the genetic effects of virulence level explained a significant portion of the variation in latent period and pustule size due to strain. After accounting for variation across strains, virulence level accounted for 15 - 19 % of the variation in latent period and 9-12% of the strain variation in pustule size. Consequently, while the trade-offs between virulence level and latent period, and between virulence level and pustule size explain only a small proportion of the variation in total within-host spore production, they are likely of consequence for the longer-term evolution of virulence level in this important agricultural pathogen.

The results reported here are important because they provide robust estimates of the slopes of trade-offs between virulence level and two important pathogen life history stages. Montarry et al. (2010) also report trade-offs between virulence level and the quantitative expression of life history stages in *Phytophthora infestans*, but interestingly, they found costs associated with different pathogen life history stages than those reported here. In their study, latent period decreased with increasing virulence level, whereas we found the opposite trend. Moreover, Montarry et al. (2010) found evidence in support of a cost of virulence when all life history traits were evaluated together, since they found that longer latent periods resulted in larger lesions, and ultimately, greater within-host reproduction. Thrall and Burdon (2003) found that virulence level in a wild flax rust pathogen was correlated with a trend of decreasing spore production per pustule, a result we did not obtain. However, we do find decreasing pustule size with increasing virulence level, a relationship that could drive similar results to Thrall and Burdon (2003) since smaller pustules produce few spores per pustule over the lifetime of the infection. The negative trend Thrall and Burdon (2003) observed may be strongly influenced by a few observations for individual strains with high or low virulence. Since we used strains with an even representation of virulence level, the results reported here are not influenced by single strains with extreme levels of virulence. Our results are also robust to variation in costs among individual virulence loci, as demonstrated by Vera Cruz et al. (2000) and Bahri et al. (2009), since we measured the cumulative cost of virulence summed over 30 different loci. Thus, we provide strong evidence in support of costs of virulence for life history traits that will affect pathogen fitness over several generations.

The evolutionary impact of virulence costs will depend on the effect of life-history traits critical to pathogen lifetime fitness. We found nearly a full day increase in latent period between pathogens with the lowest and highest virulence levels. Over an epidemic period of 100 days, pathogens with lower virulence level will go through nine asexual generations while the pathogens with higher virulence levels will go through eight. Thus, lower virulence strains potentially produce n^8 times more new infections than the higher virulence strain. Under the more realistic ecological condition of a limited carrying capacity, the earlier reproducing strains will reach that carrying capacity sooner during an epidemic. Interestingly, using a different approach, Leonard and Mundt (1984) predicted that shorter latent periods strongly increase pathogen lifetime fitness. They used published fecundity curves from six agricultural pathogens including *P. coronata* to show that latent period had a two to three-fold greater effect on the rate of epidemic increase than either infection efficiency or spore production per pustule. Thus, there is likely strong selection for shorter latent periods and against higher virulence levels in susceptible host populations.

Earlier reproduction conferred by a shorter latent period may also be important for pathogen competition. Pathogen strains that infect the same leaves compete for the same resources. Since resource uptake increases after the onset of sporulation in rust fungi (Baart et al. 1991), pathogen strains that reproduce earlier could acquire a larger share of host resources and outcompete other strains. Indeed, our finding of a shorter latent period with increasing pustule density certainly suggests that earlier reproduction occurs in response to crowding. Likewise, large pustule size may also be beneficial in resource competition, since larger pustules will use a larger share of resources and take up more space on the leaf. Thus, we predict that in susceptible host populations there will be selection for rust genotypes with lower virulence level and that reproduce more quickly and produce larger pustules.

The observed trade-offs between virulence level and latent period and virulence level and pustule size predict that in the absence of host resistance, there should be selection for decreasing virulence level in *P. coronata*. Yet annual monitoring of virulence level in the North American population of *P. coronata* reveals that virulence level has been steadily increasing for over a decade (Carson 2008, 2011). We infer that

the continued introduction and widespread use of resistance genes in agronomic oat varieties has created a selective environment where the benefits of increased virulence level to the pathogen outweigh the costs imposed by the trade-off with latent period and pustule size. The evolution of increasing virulence level in many agricultural pathogens is cause for serious concern, because resistance genes are a limited genetic resource. Pyramiding multiple resistance genes into a single host cultivar has become a common agricultural practice (Huang et al. 1997, Hoffman et al. 2006, Barloy et al. 2006), and such cultivars likely provide strong selection in favor of increased virulence level. Thus, while costs of virulence level may constrain the evolution of increasing virulence level in susceptible host populations, they are apparently unable to constrain evolution of increasing virulence in the face of strong selection imposed by resistant host populations.

CONCLUSIONS

Trade-offs that limit the evolution of host resistance or corresponding pathogen virulence may have considerable effects on coevolutionary dynamics. Without sizable trade-offs, new resistance or virulence alleles entering populations should be quickly swept to fixation, resulting in an evolutionary 'arms-race.' With trade-offs, polymorphism at resistance and virulence loci can be maintained through frequency dependent selection (Leonard 1994, Tellier and Brown 2007, 2011). Here we provide empirical support for trade-offs between virulence level and the quantitative expression of two key life history stages within the host that likely affect pathogen lifetime fitness. Our results suggest that in susceptible host populations, selection will favor pathogen strains with fewer virulence alleles, since these strains reproduce earlier and form larger pustules.

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TABLES & FIGURES

Table 2.1. Mean trait values and standard errors for 29 *P. coronata* strains

Strain	VL[a]	Germination rate[b]	LD Pust[c]	HD Pust[d]	Lat. Period (days)	Pustule size (cm^2)	Mort.[e]	Spore prod. (mg/cm^2 leaf)
07FL066	2 - 3	0.77 ± 0.10	15.8 ± 7.8	48.5 ± 18.8	11.21 ± 0.25	0.0102 ± 0.0019	6	0.168 ± 0.043
06WI045	3	0.84 ± 0.03	27.5 ± 8.4	22.0 ± 14.1	10.93 ± 0.13	0.0081 ± 0.0012	0	0.220 ± 0.057
07FL065	6	0.60 ± 0.10	16.0 ± 7.1	35.8 ± 14.4	10.78 ± 0.13	0.0110 ± 0.0029	6	0.146 ± 0.051
08TX004	8 -9	0.68 ± 0.16	25.0 ± 17.9	39.8 ± 18.4	11.49 ± 0.14	0.0105 ± 0.0016	2	0.250 ± 0.074
08SD080	8	0.86 ± 0.03	13.2 ± 5.0	38.7 ± 7.5	11.69 ± 0.16	0.0093 ± 0.0015	2	0.153 ± 0.029
07TX008	9	0.72 ± 0.14	25.5 ± 11.4	41.7 ± 20.8	11.38 ± 0.12	0.0097 ± 0.0017	2	0.292 ± 0.081
07AL058	9	0.60 ± 0.19	16.0 ± 4.8	52.5 ± 15.6	11.19 ± 0.17	0.0091 ± 0.0017	3	0.155 ± 0.046
07MN141	9	0.59 ± 0.19	14.8 ± 8.6	82.8 ± 37.8	11.05 ± 0.13	0.0065 ± 0.0005	0	0.228 ± 0.068
08AL052	9 - 11	0.97 ± 0.01	25.2 ± 10.2	79.8 ± 27.2	11.01 ± 0.09	0.0117 ± 0.0012	2	0.348 ± 0.106
07TX023	5 - 10	0.80 ± 0.07	13.5 ± 6.9	33.4 ± 13.0	11.57 ± 0.21	0.0087 ± 0.0009	3	0.178 ± 0.049
08TX028	10 - 13	0.65 ± 0.14	20.0 ± 9.8	59.8 ± 17.3	11.32 ± 0.18	0.0126 ± 0.0035	1	0.286 ± 0.083
08TX024	12	0.82 ± 0.09	31.8 ± 9.8	40.2 ± 14.9	11.10 ± 0.08	0.0076 ± 0.0010	2	0.217 ± 0.060
08TX002	13	0.85 ± 0.01	8.8 ± 1.9	66.0 ± 19.5	11.46 ± 0.13	0.0121 ± 0.0043	2	0.334 ± 0.107
08MN20	13 - 14	0.70 ± 0.17	20.5 ± 6.9	75.3 ± 35.0	11.66 ± 0.15	0.0098 ± 0.0016	1	0.185 ± 0.053
07MN143	13 - 15	0.70 ± 0.14	12.7 ± 4.4	62.0 ± 21.3	11.50 ± 0.17	0.0076 ± 0.0013	0	0.116 ± 0.034
08MN036	15 - 15	0.75 ± 0.06	19.8 ± 5.3	82.5 ± 30.5	11.79 ± 0.17	0.0065 ± 0.0009	2	0.208 ± 0.051
08WI083	13 - 15	0.83 ± 0.04	9.2 ± 4.1	25.7 ± 7.5	11.16 ± 0.18	0.0075 ± 0.0023	5	0.095 ± 0.027
06IA035	16	0.65 ± 0.12	11.3 ± 4.2	64.0 ± 14.5	11.44 ± 0.16	0.0095 ± 0.0014	2	0.271 ± 0.066

07TX006	16 - 17	0.82 ± 0.04	13.5 ± 4.0	63.5 ± 17.9	11.05 ± 0.09	0.0089 ± 0.0016	2	0.216 ± 0.052
06TX007	17 - 18	0.81 ± 0.09	19.0 ± 7.3	76.3 ± 17.8	11.92 ± 0.21	0.0112 ± 0.0044	4	0.303 ± 0.080
06MN100	12 - 17	0.44 ± 0.14	7.0 ± 3.2	23.3 ± 9.2	11.06 ± 0.13	0.0072 ± 0.0012	1	0.146 ± 0.054
07TX079	15 - 18	0.84 ± 0.05	5.2 ± 2.1	65.7 ± 30.6	11.32 ± 0.17	0.0161 ± 0.0011	6	0.205 ± 0.105
07TX077	18 - 19	0.38 ± 0.15	10.8 ± 4.6	29.3 ± 17.5	11.15 ± 0.15	0.0085 ± 0.0039	0	0.095 ± 0.022
08MN068	18	0.76 ± 0.10	21.5 ± 7.1	56.2 ± 23.7	11.30 ± 0.17	0.0080 ± 0.0010	3	0.193 ± 0.034
08IA116	18	0.75 ± 0.07	10.0 ± 4.1	41.3 ± 16.1	11.82 ± 0.19	0.0066 ± 0.0012	3	0.112 ± 0.036
08TX010	16 - 21	0.94 ± 0.02	17.2 ± 4.9	54.3 ± 13.5	12.03 ± 0.23	0.0068 ± 0.0008	2	0.205 ± 0.044
08MN072	12 - 20	0.82 ± 0.03	26.3 ± 15.8	61.8 ± 15.9	11.72 ± 0.19	0.0087 ± 0.0009	0	0.387 ± 0.104
07TX005	21 - 23	0.91 ± 0.03	37.0 ± 28.3	90.3 ± 35.3	11.02 ± 0.15	0.0043 ± 0.0004	2	0.302 ± 0.092
08FL077	21 - 22	0.61 ± 0.10	16.2 ± 10.3	31.4 ± 10.1	12.22 ± 0.18	0.0080 ± 0.0008	3	0.148 ± 0.042

[a] Upper and lower bounds of virulence level

[b] Proportion of spores that germinated *in vitro* in water-agar plates

[c,d] Average number of pustules at low dose (LD) and high dose (HD) inoculum treatments

[e] Number of inoculated leaves (out of 12) that died before harvest.

Table 2.2. Summary of fixed effects for total spore production

	numDF[a]	denDF[b]	F	p
A. *Strain model*				
Inoculation day	2	143	4.0339	0.0198
Inoculum Dose	1	173	55.090	<0.0001
Strain	28	143	1.6205	0.036
B. *Virulence model*				
Inoculation day	2	170	3.65	0.028
Inoculum Dose	1	173	55.090	<0.0001
Virulence level	1	170	0.4449	0.5057

Results of two mixed models showing the effect of pathogen strain and virulence level on total spore production (mg spores per cm^2 of leaf tissue). The response variable was cube-root transformed. [a] Numerator degrees of freedom, [b] Denominator degrees of freedom.

Table 2.3. Summary of fixed effects for infection efficiency

	numDF	denDF	F	p
Infection efficiency				
Inoculation day	2	143	4.0705	0.0191
Leaf distance	1	172	6.2402	0.0191
Inoculum dose	1	172	97.8372	<0.0001
Strain	28	143	0.8615	0.6678

Infection efficiency was measured as the number of pustules and log transformed. Spore germination preceded infection efficiency, but we did not find a significant correlation between average strain germination rate and infection efficiency ($F_{1,83} = 0.006$, $p=0.815$), and the term was dropped from the model. It may be that germination rate on water agar plates is not well correlated with germination on leaves, or that processes following germination are more limiting to infection.

Table 2.4. Summary of fixed effects for latent period

	numDF	denDF	F	p
A. *Strain model*				
GH Bench	1	142	1.77	0.1852
Pot position	1	142	3.64	0.0585
Infection efficiency	1	164	13.64	0.0003
Strain	28	142	3.93	<0.0001
B. *Virulence model*				
GH Bench	1	169	1.26	0.2638
Pot position	1	169	2.63	0.1068
Infection efficiency	1	164	14.31	0.0002
Virulence level	1	169	15.14	0.0001

Latent period was measured as the average number of days until sporulation. Inoculum dose treatment did not have a significant effect on latent period because infection efficiency better explained the variation in pathogen density.

Table 2.5. Summary of fixed effects for pustule size

	numDF	denDF	F	p
A. *Strain model*				
Infection efficiency	1	112	3.935	0.0497
Infection efficiency^ 2	1	112	4.779	0.0306
Latent period	1	112	0.340	0.5607
Strain	28	128	2.039	0.0041
B. *Virulence model*				
Infection efficiency	1	112	3.757	0.0551
Infection efficiency^ 2	1	112	3.887	0.0511
Latent period	1	112	0.422	0.5172
Virulence level	1	155	3.139	0.0784

Pustule size was measured as the average pustule size (cm^2) of all pustules on a leaf and was log transformed. Inoculum dose treatment did not have a significant effect on pustule size because infection efficiency better explained the variation in pathogen density.

Table 2.6. Summary of fixed effects for total spore production after accounting for variation in previous life history stages

	numDF	denDF	F	p
A. *Strain model*				
Inoculation day	2	124	32.311	<0.0001
Pot position	1	124	3.505	0.0635
Infection efficiency	1	81	2024.85	<0.0001
Infection efficiency $^{\wedge 2}$	1	81	184.169	<0.0001
Latent period	1	81	9.184	0.0003
Pustule size	1	81	12.641	0.0006
Strain	28	124	3.435	<0.0001
Strain*Infection efficiency	28	81	2.904	0.0001
B. *Virulence model*				
Inoculation day	2	151	209.914	<0.0001
Pot position	1	151	2.359	0.1267
Infection efficiency	1	109	1451.525	<0.0001
Infection efficiency$^{\wedge 2}$	1	109	131.927	<0.0001
Latent period	1	109	5.458	0.0213
Pustule size	1	109	8.725	0.0038
Virulence level	1	151	1.739	0.1893

The response variable, total spore production (mg spores per cm^2 of leaf tissue), was square-root transformed. Inoculum dose treatment did not have a significant effect on spore production per pustule because infection efficiency better explained the variation in pathogen density. In order to quantify the effect of pustule size on spore production, the analysis was carried out on the subset of leaves that were still alive at the end of the experiment. The analysis of spore production per pustule that included dead leaves is shows qualitatively similar results (Table S2.**4**)

Table 2.7. Effects of strain and virulence on leaf mortality

	DF	Deviance	p
A. *Strain model*			
Inoculation day	2	1.3223	0.5162
Pot position	1	9.9702	0.0016
Infection efficiency	1	1.8854	0.1697
Latent period	1	0.0463	0.8296
Strain	28	53.7790	0.0024
B. *Virulence model*			
Inoculation day	2	1.6452	0.4393
Pot position	1	11.3630	0.0007
Infection efficiency	1	1.5516	0.2129
Latent period	1	0.0415	0.8385
Virulence	1	0.5015	0.4788

Leaf mortality was modeled as a binomial response. Each effect listed above was tested by comparing the residual deviance of a null model lacking the effect to a full model with A) 304 degrees of freedom and a residual deviance of 268.29 and B) 331 degrees of freedom and a residual deviance of 321.22. Differences in deviance listed above were compared to a Chi-squared distribution.

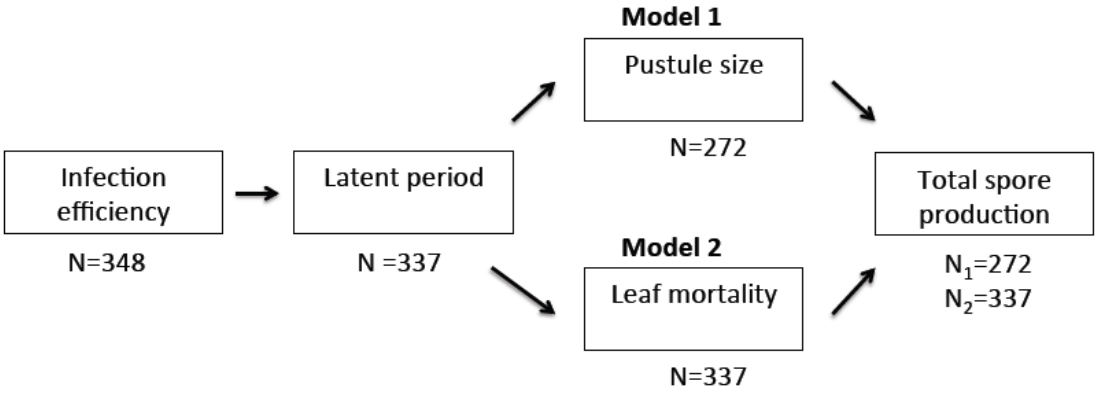

Figure 2.1. Sequential order of pathogen life history stages used in statistical analysis.

The effect of strain and virulence on each life history stage was analyzed after accounting for the effects of previous life history stages. Since it was not possible to quantify pustule size on leaves that had died, we used two different models to analyze total spore production; Model 1 accounts for the effects of infection efficiency, latent period, and pustule size on total spore production. Model 2 accounts for the effects of infection efficiency, latent period, and leaf mortality on total spore production. We used the full data set to analyze infection efficiency (N= 348), the subset of samples that produced pustules to analyze latent period (N=337), and the subset of these samples for which image analysis was possible to analyze pustule size (N=272). The analysis of total spore production given variation in previous life history stages depended on whether model 1 or model 2 was used.

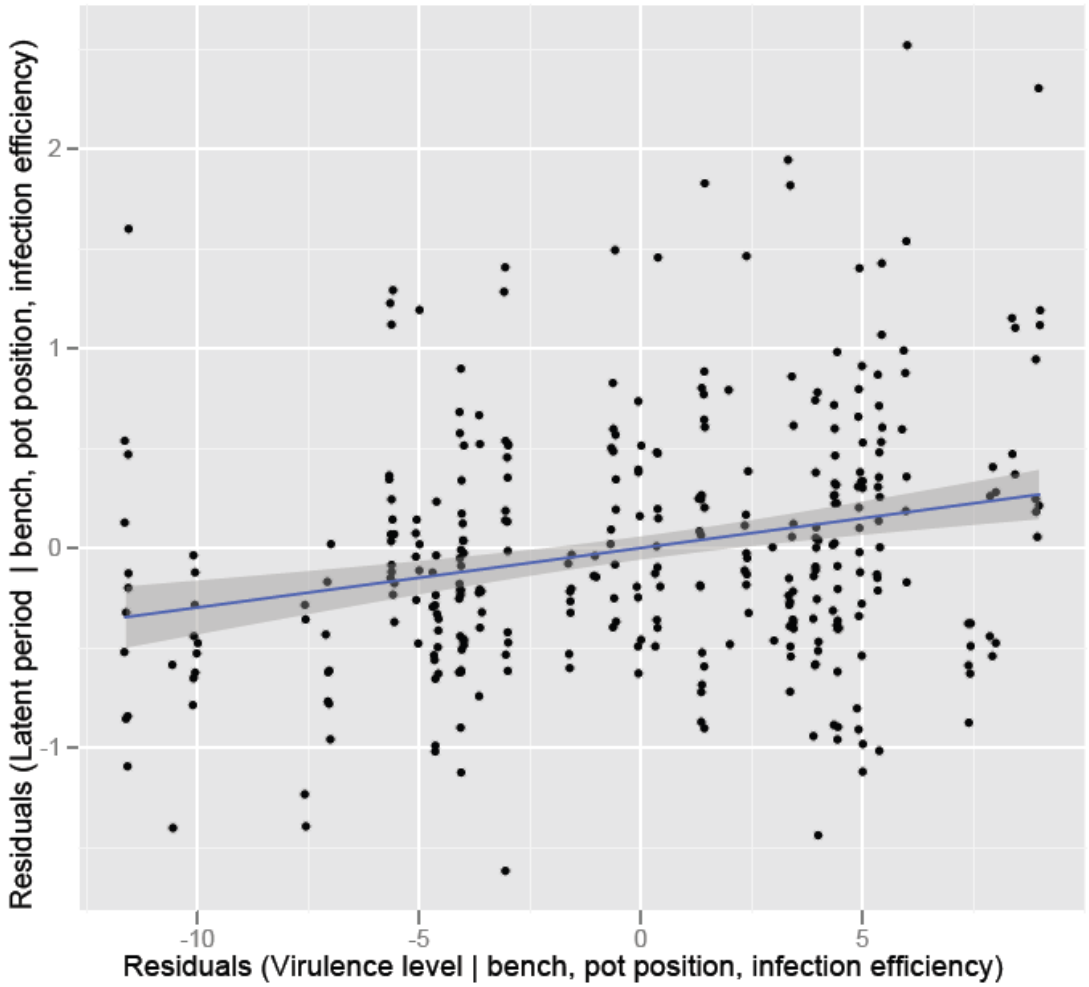

Figure 2.2. Partial regression of latent period on virulence level.

Residuals of the regression of pot position and infection efficiency on latent period are plotted against the residuals of the regression of pot position and infection efficiency on virulence level. The effects of pot position and infection efficiency are accounted for because these were included in the mixed model of latent period (Table 4). Each point on the graph represents a single replicate leaf. The random effect of pot is not shown here.

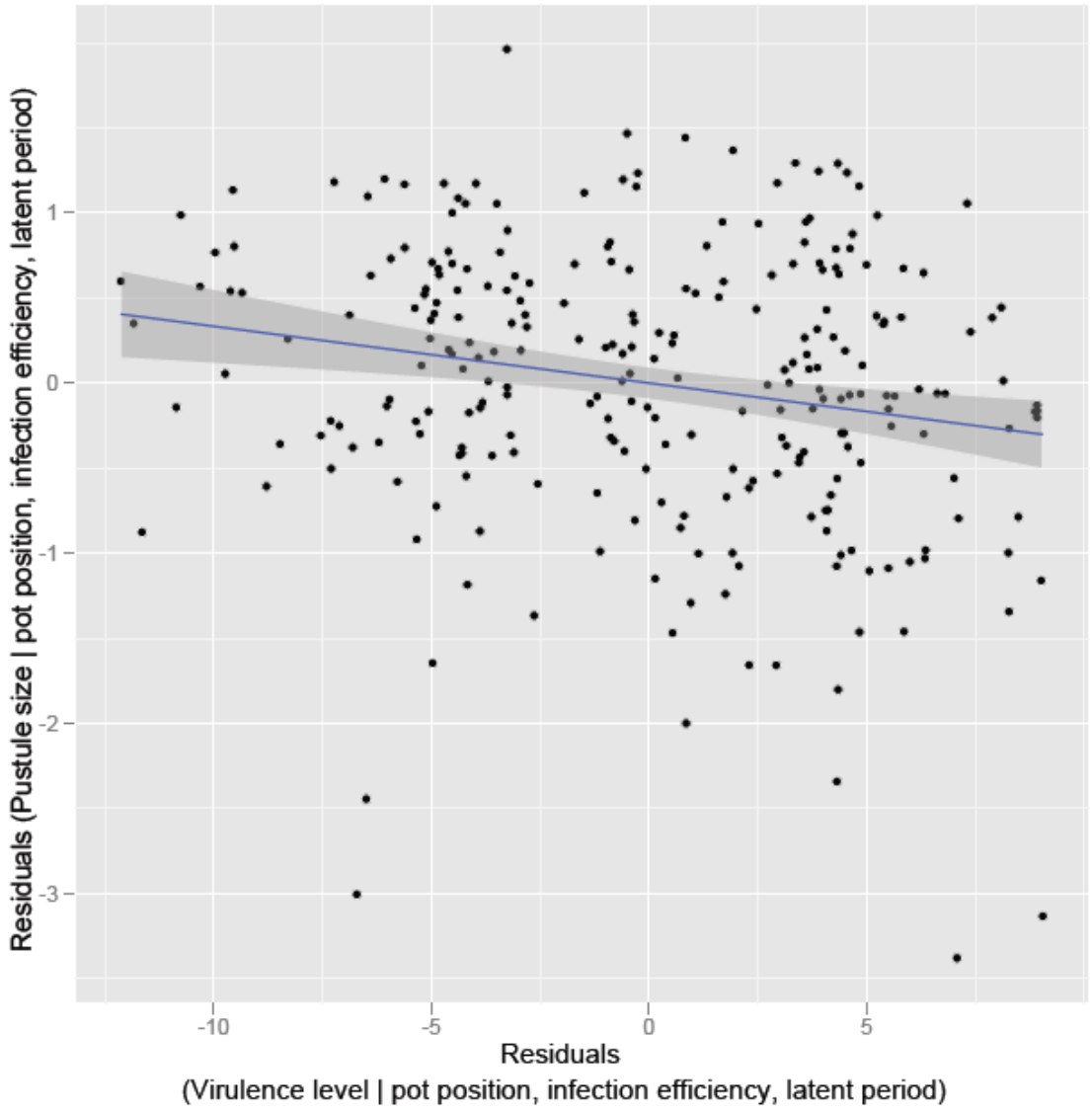

Figure 2.3. Partial regression of pustule size on virulence level.

Residuals of the regression of pot position, infection efficiency (pustules per cm^2 of leaf tissue), and latent period on pustule size are plotted against the residuals of the regression of pot, position, infection efficiency and latent period on virulence level. The effects of infection efficiency and latent period are accounted for because these were included in the mixed model of pustule size (Table 5). The random effect of pot is not shown here.

SUPPLEMENTAL TABLES & FIGURES

Table S2.1. *P. coronata* strains collection site information

Strain[1]	*Oat Type*[2]	*County*	*Date*	*Host species*[3]	*Source*[4]
07FL066	Winter	Gadsden	16-Apr	*A. sativa*	Nursery
06WI045	Spring	Pepin	29-Jun	*A. sativa*	Field
07FL065	Winter	Gadsden	16-Apr	*A. sativa*	Nursery
08SD080	Spring	Brookings	26-Jun	*A. sativa*	Nursery
08TX004	Winter	Bexar	22-Apr	*A. sativa*	Nursery
07LA058	Winter	Baton Rouge	1-May	*A. sativa*	Nursery
07MN141	Spring	Clay	8-Jul	*A. sativa*	Field
07TX008	Winter	Bexar	15-Apr	*A. sativa*	Nursery
08AL052	Winter	Baldwin	28-Mar	*A. sativa*	Nursery
07TX023	Winter	Guadalupe	16-Apr	*A. fatua*	Field
08TX028	Winter	Williamson	29-Apr	*A. fatua*	Field
08TX024	Winter	Bell	29-Apr	*A. fatua*	Field
08TX002	Winter	Bexar	21-Apr	*A. sativa*	Nursery
08BN020	Spring	Ramsey	May	*R. cathartica*	Nursery
07MN143	Spring	Kittson	9-Jul	*A. fatua*	Field
08WI083	Spring	Dane	25-Jun	*A. sativa*	Nursery
08BN036	Spring	Ramsey	May	*R. cathartica*	Nursery
06IA035	Spring	Bexar	11-Apr	*A. sativa*	Nursery
07TX006	Winter	Bexar	15-Apr	*A. sativa*	Nursery
08MN100	Spring	Renville	15-Jul	*A. sativa*	Field
07TX079	Winter	Burleson	5-Apr	*A. sativa*	Nursery
06TX007	Winter	Tama	22-Jun	*A. sativa*	Field
08IA116	Spring	Howard	unknown	*A. sativa*	Field
08MN068	Spring	Olmsted	23-Jul	*A. sativa*	Field
07TX077	Winter	Burleson	5-Apr	*A. sativa*	Nursery
08MN072	Spring	Waseca	22-Jul	*A. sativa*	Nursery
08TX010	Winter	Refuglo	23-Apr	*A. fatua*	Field
08FL077	Winter	Jackson	29-Apr	*A. fatua*	Field
07TX005	Winter	Burleson	28-Mar	*A. fatua*	Nursery

[1]Strain name. First two numbers refer to the collection year, the next two letters indicate the state collected in, and the last three numbers refer to the unique collection number.

[2] Strains were collected from spring oats in the Upper Midwest and winter oats in the Gulf Coast states.

[3] Strains were collected from either cultivated oats, *Avena sativa*, wild oats *Avena fatua* (often a weed in oat and wheat fields), or the alternate host *Rhamnus cathartica.*

[4]Collections were made from either oat fields or oat breeding nurseries.

Table S2.2. Virulence profile of *P. coronata* strains

	Host resistance gene																											
Strain	**91**	**50**	**53**	**68**	**54**	**96**	**58**	**64**	**67**	**45**	**46**	**48**	**52**	**35**	**59**	**55**	**63**	**40**	**60**	**36**	**39**	**56**	**71**	**38**	**61**	**14**	**51**	**57**	**VL**
07FL066	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0*	0	0	1
06WI045	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
07FL065	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	5
08TX004	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0**	1	0	0	0	0	0	1	1	1	1	7
08SD080	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	7
07TX008	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	1	1	8
07AL058	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	8
07MN141	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	1	0	8
08AL052	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0**	0	0	0	0**	1	1	1	1	8
07TX023	0	0	0	0	0	0	0	0	0	1*	0	0	0	0	0	0	1	0	0	0	1	1*	1*	1	1*	1*	1	0	9
07TX028	0	0	0	0	0	0	0	0	0**	0	1	0	0	1	0	0	0	1	1	1	0*	1	0	0	1	1	0**	1	9
08TX024	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	0	1	1	1	1	1	1	11
08TX002	0	0	0	0	0	0	1	1	1	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	0	1	1	0	12
08BN003	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	1	1	1	1	1	0*	0	1	1	12
07MN143	0	0	0	0	0	0	0	0	0**	1*	1	0	0	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	13
08BN036	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	1	1	0	0	1	1	1	1	1	1	0	1	1	14
08WI083	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1*	1	1	1	1	1	1*	0	1	1	14
06IA035	0	0	0	0	1	0	0	0	1	0	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	15
07TX006	0	0	0	0	1	0	0	0	1*	0	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	16
06TX007	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	0**	1	1	1	1	1	1	1	1	1	1	1	16
08MN100	0	0	0	0	0	0	0	0	0	0	1*	1	1	1	0	1*	1*	1	0	1	1	1	1*	1	0	1	1	1	16
07TX079	0	0	0	0	0	0	1	1*	1	0	0	1	1	1	1	1	0	1	1	1*	1	0	1	0	1	1	1	1*	17
07TX077	0	0	0	0	0	1	0	0	0	0	1	1	1	0**	1	1	1	1	1	1	1	1	1	1	1	1	0	1	17
08MN068	0	0	0	0	0	1	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	17
08IA116	0	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0*	1	1	17
08TX010	0	0*	0	0	0	0	0	0	0	1	1*	1	1	0*	1	1	1	1*	1	1	1	1	1	1	1	1*	1	1	18
07MN072	0	0	0	0	0	0	1	1*	1	1*	0	1	1	1	1	0**	1*	1	0	1	1*	1	1*	1*	0	1	1	0	18
07TX005	0**	0	1	0**	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20
08FL077	0	0	0	0	0	0	1	0	1	1*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21

Pathogen strains are listed by row in order of increasing virulence level. Host lines, listed by the individual crown rust resistance gene present (Pc genes), are listed by column, in order of increasing susceptibility. Zero indicates a resistant reaction, meaning the pathogen strain does not carry the corresponding virulence allele, while one indicates a susceptible reaction, meaning that pathogen strain must carry the corresponding virulence allele. VL indicates virulence level, the total number of virulence alleles carried. Asterisks denote uncertainty in the reaction. One asterisk indicates that one out of the four virulence trails was incongruous, while 2 asterisks indicate that two out of the four virulence trails were incongruous. The two host lines carrying genes *Pc62* and *Pc94* were resistant to all strains in the study and are not shown here.

Table S2.3. Spore production per pustule given leaf mortality

	numDF	denDF	F	p
A. *Strain model*				
Inoculation day	2	141	20.422	<0.0001
Pot position	1	141	26.695	<0.0001
Infection efficiency	1	133	1879.591	<0.0001
Infection efficiency^ 2	1	133	209.433	<0.0001
Latent period	1	133	7.417	0.0073
Leaf mortality	1	133	16.675	0.0001
Strain	28	141	3.434	<0.0001
Strain*Infection efficiency	28	133	2.359	0.0006
B. *Virulence model*				
Inoculation day	2	168	143.72	<0.0001
Pot position	1	168	18.789	<0.0001
Infection efficiency	1	161	1789.463	<0.0001
Infection efficiency^ 2	1	161	165.791	<0.0001
Latent period	1	161	5.353	0.029
Leaf mortality	1	161	11.616	0.0008
Virulence level	1	168	1.463	0.2282

The response variable, total spore production (mg spores per cm^2 of leaf tissue, was square-root transformed. The analysis was carried out on the subset of samples that produced pustules. The effect of pustule size was not estimated in this analysis because we were unable to measure pustule size on dead leaves.

Table S2.4. Sums of squares and effect sizes from split-plot ANOVA analysis of for total spore production given variation in previous life history stages

	DF	SS	Effect size
Whole pot level			
Inoculation day	2	0.3904	5.0%
Pot position	1	0.0346	0.4%
Strain	28	2.0389	26.3%
Error	125	5.2872	68.2%
Total		**7.7511**	100.0%
Split-pot level			
Infection efficiency	1	4.7555	82.6%
Infection efficiency $^{\wedge 2}$	1	0.409	7.1%
Latent period	1	0.0001	<0.01%
Pustule size	1	0.0307	0.5%
Infection efficiency * Strain	28	0.2396	4.2%
Error	83	0.3249	5.6%
Total		**5.7598**	100.0%

Effect size was calculated by dividing the SS of the effect by the total SS at either the whole or split-pot level.

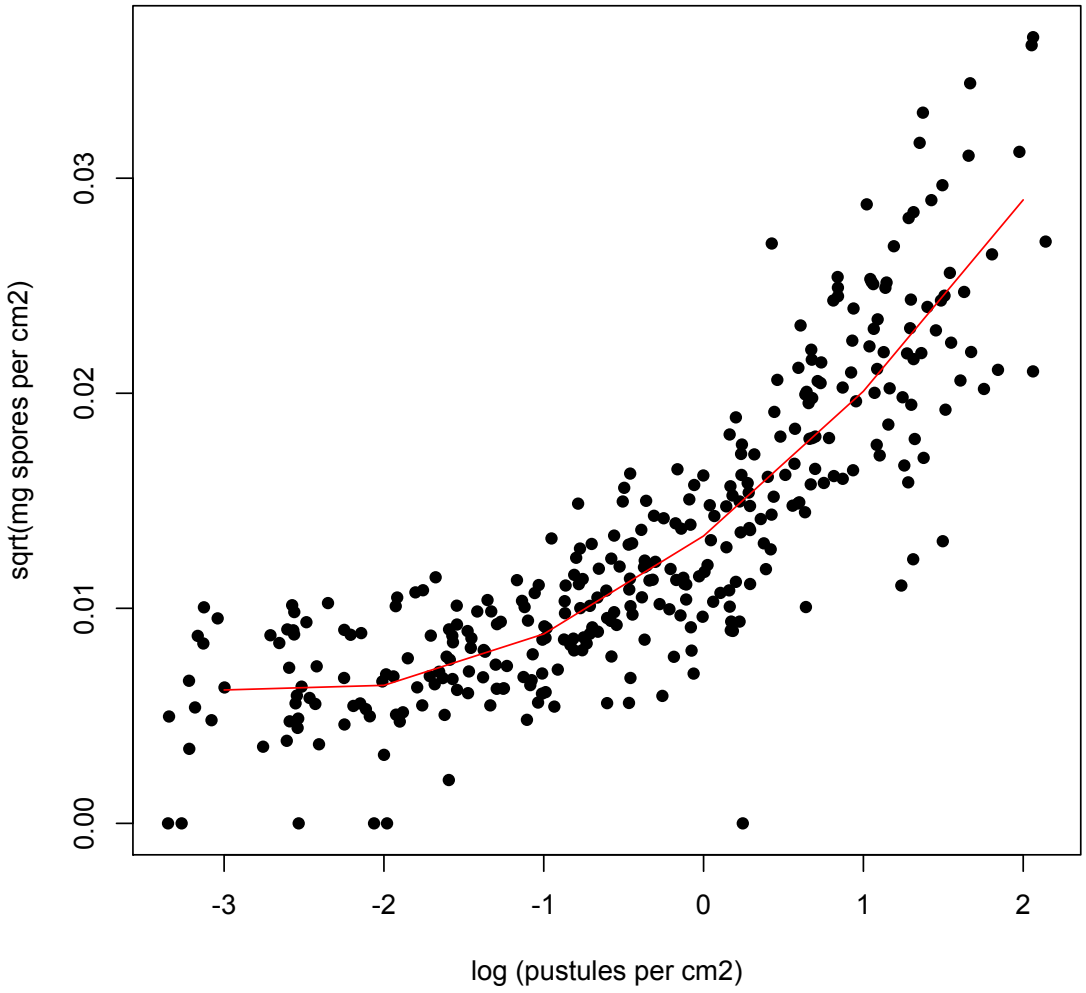

Figure S2.1. Relationship between infection efficiency and total spore production.

Total spore production (mg spores per cm^2 of leaf tissue) was square-root transformed and infection efficiency (pustules per cm^2 of leaf tissue) was log transformed. The red line shows the quadratic line of best fit.

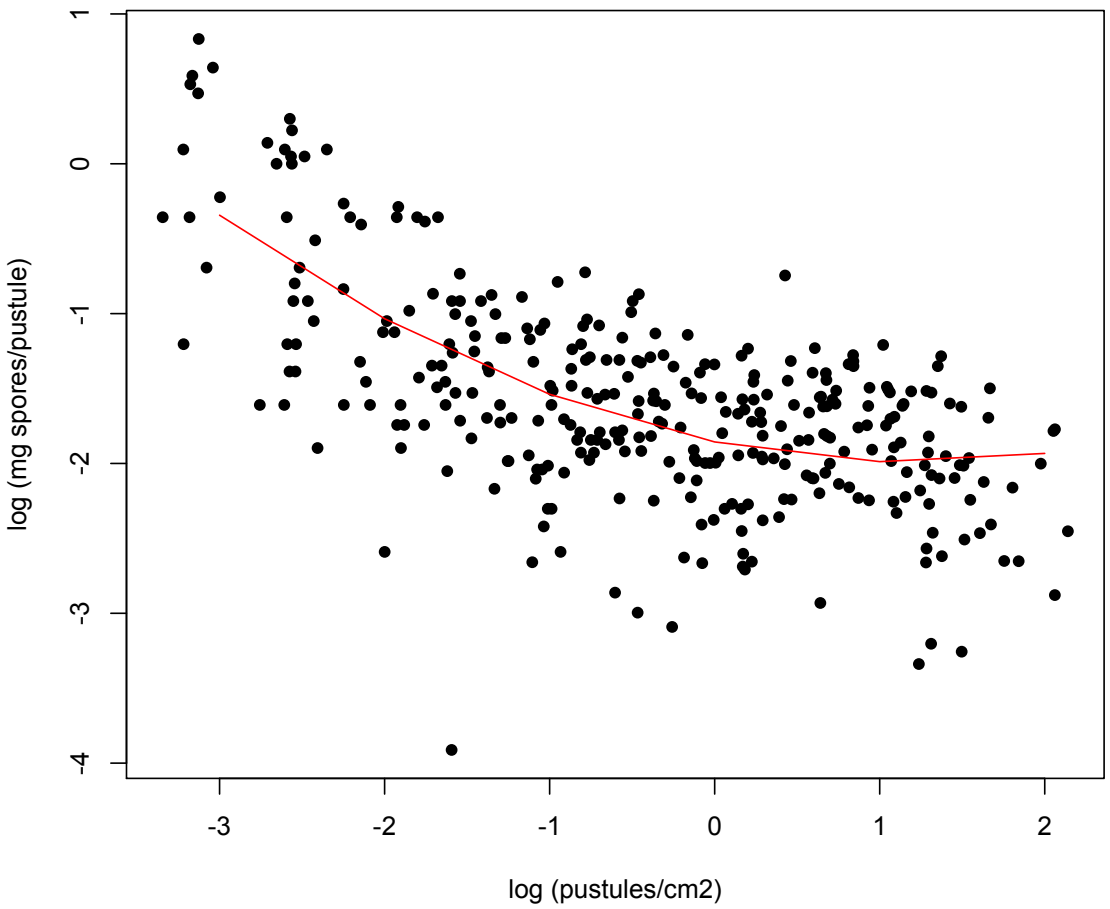

Figure S2.2. Relationship between infection efficiency and spore production per pustutle.

Spore production per pustule (calculated as total spore production/number of pustules) significantly decreased with increasing infection efficiency (pustules per cm^2 of leaf tissue). The line of best fit is y= -1.85644 -0.2247 * Infection efficiency + 0.0932 * Infection efficiency $^{\wedge 2}$.

CHAPTER 3

Constraints to local adaptation in agricultural populations of *Puccinia coronata*

SUMMARY

We investigated potential constraints to local adaptation in the agricultural pathogen, *Puccinia coronata*. We first asked whether there is evidence that the adaptive potential of *P. coronata* is constrained by low genetic variation. Next we asked whether genetically diverse host populations constrain the evolution of host breadth and pathogen fecundity. Lastly, we asked whether there is evidence of selection on virulence loci that underlie infection in *P. coronata*. In answer to the first question, we found no evidence of population genetic structure at microsatellite loci, and conclude that the adaptive potential of *P. coronata* is not constrained by low genetic variation. In answer to the second question we found considerable variation in host breadth and fecundity among and within pathogen populations, but did not find conclusive evidence that these differences were driven by the amount of genetic diversity in the host population. Finally, in answer the third question, we found evidence that pathogen populations were strongly structured with respect to virulence loci but not microsatellite loci, suggesting that selection is the underlying mechanism structuring pathogen virulence. We conclude that there is evidence of local adaptation in *P. coronata* at a regional scale.

INTRODUCTION

Pathogen local adaptation is an important consequence of host-pathogen coevolutionary dynamics (Nuismer et al. 1999, Thompson 1999) and reflects spatial structure of genetic diversity (Felsenstein 1976, Thrall and Burdon 2002, Kawecki and Ebert 2004, Tellier and Brown 2011). Pathogen local adaptation occurs when pathogens evolve increased fitness on the local host population. Evidence for local adaptation in pathogen populations is mixed, with some studies showing evidence of local adaptation and others showing no evidence of adaptation, or in some cases maladaptation (Kaltz and Shykoff 1998, Greischar and Koskella 2007). The essential question is one of limitations; what genetic ecological factors constrain pathogens' adaptation to their hosts? Here, we investigate potential constraints to local adaptation of *Puccinia coronata* (oat crown rust), a widespread agricultural pathogen. We first investigate demographic processes that might limit the adaptive potential of pathogen populations. Next, we look at ecological factors within populations that might constrain the evolution of specific pathogen traits. We focus on the effect of host genetic diversity and ask whether genetically diverse host populations constrain the evolution of increasing host breadth and pathogen fecundity. Lastly, we investigate the extent to which pathogen populations are structured by local selection.

Gene flow and adaptive potential

Gene flow, recombination, and mutation provide the genetic diversity within populations that is a prerequisite for local adaptation. On the timescale relevant to local adaptation in agricultural populations, gene flow is the primary source of new variation into populations. Wright's (1931) island model demonstrates that in the absence of gene flow, genetic variation within small populations will decrease over time due to drift. Thus, several authors have argued that dispersal distance is critical to a pathogen's adaptive potential (McDermott and McDonald 1993, McDonald and Linde 2002, Barrett et al. 2008b), and theoretical work predicts that pathogens with dispersal ranges greater than their hosts are more likely to be locally adapted (Gandon et al. 1996, Lively 1999, Gandon and Michalakis 2002, Gandon and Nuismer 2009). In support of this prediction,

Hoeksema and Forde (2008) found that dispersal range was the only pathogen life history trait that had a significant effect on the probability of local adaptation in a meta-analysis of 27 different pathogen local adaptation studies.

Wind dispersed pathogens are not typically thought of as having restricted gene flow, particularly in agricultural species of cereal rust, such as *P. coronata*, where long distance migration events are well documented (Brown and Hovmøller 2002). However, since persistence of pathogens of seasonal agricultural crops depends on recolonization of crop fields each year, founder effects might structure populations (Slatkin 1977). Thus, even if a large amount of genetic diversity is maintained at the regional level, repeated colonization and extinction events could limit genetic variation within individual fields (Thrall and Burdon 2002), thereby reducing the adaptive potential of a pathogen population.

Selection from host genetic diversity

Local adaptation occurs when pathogen populations evolve increased fitness on local host genotypes (Kawecki and Ebert 2004). While restricted gene flow can reduce the evolutionary potential of pathogen populations, ecological factors within populations may constrain the evolution of specific pathogen traits that are important to local adaptation. Here we ask whether the evolution of two important pathogen traits, host breadth and fecundity, are affected by selection from genetically diverse host populations. An increase in the amount of genetic diversity within host populations has been shown to strongly reduce pathogen prevalence in both agricultural (Chin and Wolfe 1984, Mcdonald et al. 1988, Mahmood et al. 1991, Zhu et al. 2000) and natural host-pathogen systems (Altermatt and Ebert 2008, Ganz and Ebert 2010), suggesting that genetically diverse host populations pose challenges to pathogen adaptation. However, few studies have investigated the effect of host genetic diversity on pathogen local adaptation.

Host breadth describes the number of different host genotypes a pathogen can infect. We hypothesize genetically diverse host populations will select for increased host-breadth since there is a high probability that offspring will be transmitted to a different host genotype. In contrast, there is likely little benefit to increased host breadth in agricultural fields planted with a single host genotype (monoculture) since previous work

indicates that increased virulence comes at a cost of delayed reproduction on a susceptible host (Bruns et al, *submitted*). In support of this hypothesis Chin and Wolfe (1984) and Carson (2009) found that pathogens from mixed-genotype crop fields had greater average host breadth than pathogens from monocultures. While these results suggest that increased host breadth may be an adaptive response to host genetic diversity, we do not know whether pathogens that evolve in mixed-genotype host populations have greater fitness in mixed host populations than pathogens that evolved in less diverse host populations.

Pathogen fecundity describes the reproductive output of a pathogen following successful infection. Genetic diversity of the host population is likely to affect the evolution of pathogen fecundity because the quantitative expression of pathogen fecundity is strongly affected by host genotype across a broad range of pathogen species (Grech et al. 2006, Salvaudon et al. 2007a, Lambrechts et al. 2009b, de Roode and Altizer 2010, Bruns et al. 2012). If pathogens face trade-offs in fecundity on different host genotypes (Rausher 1984, Futuyma and Moreno 1988, Joshi and Thompson 1995), then adaptation to any one host genotype may be constrained in genetically diverse host populations where pathogens are likely to encounter multiple host genotypes. In contrast, adaptation to a single host genotype is unlikely to be constrained in host monocultures. Villaréal and Lannou (2000) showed that wheat powdery mildew populations grown on single-genotype monocultures evolved higher infection efficiencies (a measure of fecundity) on their host genotype of origin over a single growing season, while mildew populations grown on a mixture of two host genotypes did not. Legros and Koella (2010) report a similar adaptive constraint on phage populations propagated on a mixture of bacterial clones compared to phage populations propagated on single clone monocultures.

Relative strength of selection and gene flow

While restriction of gene flow can reduce the adaptive potential of pathogen populations by reducing the amount of genetic variation within populations, high levels of gene flow can reduce the power of selection to structure populations if the influx of new, non-adapted genotypes swamps local selection (Holt and Gomulkiewicz 1997). Thus, to determine the power of selection to structure populations the relative strength of both gene flow and selection must be evaluated. One method of comparing the relative

effects of gene flow and selection is to compare patterns of variation in neutrally evolving loci to variation at loci that are potentially under selection (Ribeiro et al. 2011). Here we compare genetic structure at microsatellite loci to the structure at virulence loci and ask whether selection on virulence loci structures pathogen populations.

In many plant pathogens infection is determined by gene-for-gene interactions such that a host carrying a specific resistance gene can only be infected by pathogen strains carrying the corresponding virulence allele (Flor 1956, Thompson and Burdon 1992). Plant genomes contain multiple resistance loci (Michelmore and Meyers 1998, Meyers et al. 2003) and consequently, pathogen genomes contain multiple virulence loci that can either carry a virulent or avirulent allele (Jiang et al. 2008, Barrett et al. 2009). Since virulence can strongly affect a pathogen's ability to infect the host, changes in the frequency of virulence at specific loci are often interpreted as the result of selection (Kolmer 1989, Bousset et al. 2002, Leonard 2003) yet the alternative hypotheses of drift and gene flow are rarely evaluated (Barrett et al. 2008a). Here we ask whether there is evidence that selection structures the variation at virulence loci in oat crown rust populations by comparing variation at virulence loci and at microsatellite loci.

Study system and objectives

Here we investigate constraints to local adaptation in *Puccinia coronata*, the oat crown rust fungus. *P. coronata* is a sexually reproducing, obligate pathogen of cultivated oats (*Avena sativa*) worldwide. Agricultural plant pathogens are an excellent system for studying processes of local adaptation because host populations are well defined, and genetic diversity is easily manipulated. In addition, the year-to-year evolution of virulence alleles that underlie pathogen infection and host breadth are closely monitored in many agricultural pathogens, providing critical information on pathogen evolutionary patterns at the regional and continental scales. Like other rust fungi, *P. coronata* spores are wind-dispersed, often to long distances (Nagarajan and Singh 1990). The population in the United States is characterized by high levels of genetic diversity at both microsatellite (Dambroski and Carson 2008) and virulence loci (Leonard 2003, Carson 2008, 2011). However, the majority of our insight about the evolution of this highly successful pathogen comes from surveys of virulence alleles carried out at a regional and

continental scale and we know relatively little about evolutionary dynamics at the local scale.

Our first objective was to determine whether the adaptive potential of *P. coronata* is limited by restricted gene flow. We hypothesized that *P. coronata* is not limited by a lack of gene flow given the long distance spore dispersal and high levels of regional genetic diversity. We tested this hypothesis by examining the genetic structure at 12 microsatellite loci of pathogens collected from eight oat fields across Minnesota. If gene flow is high, most of the variation will be within rather than among populations, and there will be little population structure.

Our second objective was to determine whether host genetic diversity affects the evolution of host breadth and pathogen fecundity. We hypothesized that mixed-genotype host populations would select for greater pathogen host breadth than single-genotype host monocultures. In contrast, we hypothesized that mixed-genotype host populations would constrain the evolution of increased pathogen fecundity on any one host compared to single-genotype host monocultures. We tested these hypotheses by comparing average host breadth and spore production of pathogen populations collected from either mixed-genotype or single-genotype oat fields.

Finally, our third objective was to determine the extent to which selection on virulence structures pathogen populations. We compared the variation among populations at neutral microsatellite loci to the variation among populations at virulence loci. If the effects of selection are weaker than the effects of gene flow we expected to see few differences in the structure of microsatellite and virulence loci. If the effects of selection are greater than the effects of gene flow then we expected to see greater population structure in virulence loci than in microsatellite loci.

METHODS

Overview

To determine the population structure and evaluate local adaptation we collected samples of crown rust from eight different oat fields that varied in host genetic diversity. We cultured single genotype rust strains from each source population in the greenhouse and evaluated variation at 13 microsatellite loci. We used greenhouse inoculation trials to quantify host breadth and evaluate virulence level. Finally, we quantified the average spore production of each pathogen population on four different host populations.

Collection sites

We collected crown rust samples from eight oat fields in Minnesota in three geographic regions (north, central, and south; Figure 3.**1**). Collections were carried out in June and July of 2010, when oat plants were beginning to senesce. Three of the fields were mixed-genotype oat variety trials maintained by the University of Minnesota oat-breeding project, and the remaining five fields were single-genotype monocultures grown on private land (Table 3. **1**). Two of the mixed-genotype fields, CK-VT and RM-VT, were replicate variety trials of the same size, planted with the same 40 cultivars of oats. The remaining mixed-genotype field, TW-VT, was smaller and only contained 10 of the 40 cultivars that were present in the other two sites. In the two large variety trials, each cultivar was planted in three randomized blocks, measuring 5 x 12ft per block. At the TW–cultivars were planted in two randomized 10 x 12ft blocks. The single-genotype monoculture sites varied in size and were planted with either Colt, Morton, or Souris cultivars (Table 3. **1**). We used GPS coordinates to calculate all pairwise geographic distances among sampled populations (Table S3.**1**).

Sampling

We collected 60 diseased leaves from each field as follows. In single-genotype monoculture sites, we ran two 75m transects and collected two diseased leaves every five meters. We also recorded the number of diseased plants out of five plants every five meters to estimate disease incidence. In variety trial sites, we collected diseased leaves

from the following cultivars: Colt, Morton, Souris, Kame, Spurs, Excel, Esker, and Jerry. We collected a total of 12 diseased leaves from each cultivar, four from each of three replicate blocks within the variety trial. We also recorded the number of diseased plants out of five plants at one meter into the fourth row within each replicate cultivar block.

Diseased leaves were stored in glassine bags at room temperature until they were processed in the lab. No leaves were stored in the bags for more than 48 hours. We used a vacuum spore collector to transfer the spores into a gel capsule and then desiccated the capsule in a 4°C low humidity chamber for one week. Dried spore capsules were transferred to cryo-vials and stored at -70°C until use.

Genetic background of oat cultivars

The oat cultivars we sampled from were developed by several breeding programs in the upper Midwest (Table S3.2), but some are more closely related. We used the Pedigrees of Oat line (POOL) database supported by Agriculture Agri-Food Canada to obtain five-generation pedigrees for all oat lines in the study. We then calculated coefficients of co-ancestry among all host lines (Table S3.**3**; Falconer and Mackay 1996).

Strain isolation and culturing

Since the leaves we collected might have been infected with more than one rust strain, we used single-pustule isolations (Carson 2008, Bruns et al. 2012) in the greenhouse to generate single-genotype strains from each collection site. Briefly, spore capsules were heat-shocked at 40°C for 10 minutes and then mixed with mineral oil and inoculated onto one-week old seedlings of the susceptible oat cultivars Marvelous and Starter. Following inoculation, seedlings were placed in a dew chamber with 100% relative humidity overnight, and then transferred to individual cubicles in the greenhouse. Plants were trimmed down to a single sporulating pustule (uredinium) 8-9 days later. Since the urediniospore stage of *P. coronata* that infects oats is asexual, all of the urediniospores produced by a single uredinium should be genetically identical. To increase amount of inoculum for each of these single-pustule strains, spores from the single remaining pustule were collected two days later and used to inoculate healthy Marvelous and Starter seedlings. We allowed all pustules to develop on this second set of

infected seedlings, and collected three capsules of spores from each strain 12 to 14 days later. Spores were desiccated and stored at -80°C as described above. From here on we will refer to these single-pustule strains as simply 'strains.'

We only performed single-pustule isolations on a subset of the field collections due to the limited greenhouse space, and the considerable time required to isolate and increase single genotype strains (5 weeks per strain). From the CK-VT and RM-VT mixed-genotype variety trials, we isolated strains from collections on cultivars of Colt, Morton, Souris, and Excel. From the TW-VT variety trial, we isolated strains from collections on Colt, Excel, Kame, and Spurs because Morton and Souris cultivars were not present. Seven samples were isolated from each cultivar. In the CK-VT population, an additional seven strains were isolated from collections on cultivar Jerry. In each of the monoculture populations, 34 samples were randomly chosen for increase. In total, we generated 233 single pustule genotypes with 28 to 33 genotypes per population except for RM-SO, where we only generated 19 (Table 3. **1**).

I. POPULATION STRUCTURE

We used microsatellites to assess the genetic structure of sampled *P. coronata* populations. Thirteen microsatellite loci were chosen from a set of 35 loci developed for *P. coronata* by Dambroski and Carson (2008; Table S3. 4). All loci were reported as dinucleotide repeats.

DNA extraction

Genomic DNA was extracted from urediniospores of single-pustule *P. coronata* strains. Microcentrifuge tubes filled with10-15mg of spores were flash frozen in liquid nitrogen and then dried in a speed-vacuum overnight. To break open the hard cell walls, 25mg of diatomaceous earth (Sigma-Aldrich Co. LLC) and 20mg of 1mm glass beads (Bio Spec Products Inc.) were added and spores were pulverized in a Mini Beadbeater-8 (Bio Spec Products Inc.) for two 20-second bursts. Then DNA was extracted with an OmniPrep™ for Fungi (G-Biosciences, A Geno Technology, Inc.) kit was used according

to the manufacture's instructions. Genomic DNA was quantified with a Qubit fluorometer™ (Invitrogen) and diluted to 1ng/μL.

PCR

DNA was amplified in 12.5μL reactions containing 2.5ng of template DNA, 0.8μM fluorescent HEX/6FAM labeled forward primer (Integrated DNA Technologies), 0.8μM reverse primer (New England Biolabs) and final concentrations of the following reagents from the TaKaRa Taq™ kit (Takara Biotechnology Co., Ltd)10% 10X PCR, 8% dNTP mixture, and 0.8% R-Taq Polymerase. For most primer pairs, polymerase chain reaction was performed in a PTL-200 Pelteir thermocycler (MJ Research) with the following program: initial denaturing 94°C for 2 minutes, then 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, then a final extension step of 72°C for 10 minutes. For the primer pairs A18, A26, B78, and C33, the annealing temperature was increased to 62°C. PCR products were amplified separately for each microsatellite locus, except for the A26 and A18 loci where multiplexing worked well. PCR products were visualized on 1% agarose gel to evaluate quality.

Fragment analysis

Fragment size analysis was carried out on an ABI 3730xl Capillary Sequencer (Applied BioSystems) at the BioMedical Genomics center at the University of Minnesota. PCR products were diluted 30-fold and multiple products from a single individual strain were pooled such that all thirteen loci from a given strain were contained in 3 wells. ROX™-400HD (Applied BioSystems) was used as a size standard. All data were scored by hand using GeneMapper (Applied BioSystems).

Population structure analysis

We used the program Arelquin v3.5 (Excoffier et al. 2005) to estimate the number of alleles, size range of alleles at each locus, and observed and expected heterozygosity at each microsatellite locus. The population parameter, $\boldsymbol{\theta} = 4N_e\mu$, was estimated as the expected homozygosity $\boldsymbol{\theta}_H$ under a stepwise mutational model (Ohta and Kimura 1973). We also tested assumptions of Hardy-Weinberg equilibrium for each locus. We used the

program GENALEX version 6.0 (Peakall & Smouse 2005) to determine the number of unique multilocus genotypes in each population and calculate Shannon's diversity index.

To determine whether there was any evidence of geographic structure, we grouped populations into geographical regions (north, center, and south; Figure 3.**1**) and used AMOVA (Excoffier et al. 1992) to determine the proportion of the variation occurring among geographic regions, among populations within a region, and within populations. We also estimated pairwise R_{ST} values and determined whether they differed significantly from 0 by permuting individuals among populations 1000 times. Finally, we used a Mantel test to determine whether there was evidence of isolation by distance. Analyses were performed with Arelquin v3.5 (Excoffier et al. 2005).

We used the program STRUCTURE (Pritchard et al. 2000) to ask if there was detectable structure in the data without *a priori* assigning individuals to populations. The program uses a Bayesian clustering method to assign individual strains to genetic clusters and estimates the likelihood of the data given K, the number of clusters. We estimated K by comparing log probability of the data among replicate simulations that varied between values of K=1 to K=6. We used the admixture model that allowed individual strains to be assigned to more than one cluster. We allowed for linkage disequilibrium among loci and ran each simulation with a burn-in period of 30,000 and 100,000 iterations. We carried out four replicate simulations per value of K and ran the whole simulation twice; in the first run we assumed that alpha, the admixture parameter, was the same across all populations. In the second run we allowed alpha to vary among populations.

II. EFFECT OF HOST GENETIC DIVERSITY

A. Host Breadth

We quantified host breadth and virulence level to test the hypothesis that pathogens from mixed host populations can infect a greater number of host genotypes than pathogens from host monocultures. We define host breadth as the number of different host cultivars that a strain infects, and virulence level as the total number of virulence alleles per strain.

We assessed the host breadth of each pathogen strain by determining infection on the following 10 oat cultivars: Colt, Excel, Esker, Jerry, Kame, Morton, Rockford, Souris, Spurs, and Marvelous. Marvelous provided a positive control, since all strains were reared on Marvelous in the greenhouse. The other nine cultivars were present in the mixed genotype plots and pathogen strains were collected from these genotypes. We observed that Colt, Kame, Jerry, and Morton appeared generally more susceptible to crown rust while Excel, Esker, Rockford, Souris, and Spurs were generally more resistant (Table S3. **5**). The ten different oat cultivars were planted into two 10x10cm pots, with five cultivars planted in separate, labeled positions within the pots. Eight seeds of each cultivar were planted within a set. Seven days after planting we inoculated spores from a single strain onto both pots. Pots were transferred to a dew chamber overnight and then returned to the greenhouse. Infection was assessed 14 days later as a binary trait. On each host line, pathogen strains that did not produce pustules, or produced very small pustules with heavy chlorosis were scored as not-infecting, while strains that produced medium to large sporulating pustules on a given host line were scored as infecting. We calculated the host breadth for each strain as the total number of cultivars infected.

Next we assed infection on a set of 28 'oat differential lines'(Fleischmann and Baker 1971) that differ at a single known crown-rust resistance gene to determine virulence level, the number of different virulence alleles each strain carries. As with the host breadth experiment above, seeds were sown into a set of eight 10 x 10cm pots filled with vermiculite. Four different oat lines were planted in separate labeled locations within each pot. Marvelous was included as a positive control. Inoculation procedures and infection scoring were carried out as described above. The resistance genes in each differential line are known; we assumed that the strain must carry the corresponding virulence allele to obtain an infectious reaction. Because the entire set of differential lines represent a nested set of resistance genes, we can calculate the virulence level of each strain as the total number of differential lines successfully infected.

Statistical analysis

We used separate analyses of variance to ask whether pathogen populations differed in average host genotype breadth or virulence level (aov function in R. version

12.2.0). If a significant difference was found, we carried out pairwise comparisons using a Tukey HSD test to control for multiple comparisons. To determine whether the genetic diversity of the host population of origin could explain some of the among population variation, we used a likelihood ratio test to compare a model where pathogen population was a random factor nested within host diversity to a null model where host diversity was not included (lmer function in the nlme package in R). We examined the variation at individual virulence loci in a separate analysis that is explained in the final section of the methods (Page 98).

We also compared the proportion of each pathogen population that was able to infect each of the nine cultivars tested in the host breadth experiment to determine if there was evidence that pathogens were locally adapted to their host genotype of origin. We used the 'local vs. foreign' test that predicts that locally adapted pathogen strains will infect their host population of origin with greater frequency than 'foreign' pathogen strains from other sources (Kawecki and Ebert 2004). Within each tested host cultivar, we compared infection frequency of pathogen populations that were collected from monocultures of that same type of host cultivar, to the infection frequency of pathogen populations collected from different host populations.

B. Pathogen Fecundity

To determine whether the average spore production (a measure of fecundity) varied among pathogen populations from mixed-genotype variety trials and single-genotype monocultures, we inoculated pathogen populations onto oat seedling populations planted with single-cultivar monocultures or mixtures of eight cultivars. We allowed pustules to develop on the leaves and quantified total spore production after three weeks. The experiment was repeated twice (2010, 2012; Figure 3.**2**)

Host genotypes

In 2010, we set up four host population treatments: three monocultures and one mixture (Figure 3.**2**). In the monoculture treatments we used the cultivars Colt, Morton, and Souris because we had collected pathogen strains from these cultivars in monoculture fields. We used eight cultivars for the mixture treatment: Colt, Excel, Esker, Jerry,

Kame, Morton, Souris, and Spurs. These were the same cultivars used in the host breadth experiment above and all eight were present in the CK-VT and RM-VT host population. Thus one of the four host treatments was similar to the host population of origin for each of the eight pathogen populations (Figure 3.**2**). We used the same host treatments in 2012, but added the cultivar "Ogle" as an additional host monoculture treatment to help tease apart the effects of local adaptation from greater average spore production on susceptible hosts. Ogle is a highly susceptible cultivar that does not share the same genetic background as Morton and Colt (Table S3.**3**) and was not present in any of the fields. Thus, all of the pathogen strains used here likely had less opportunity to adapt to Ogle.

Experimental design

In both experiments we inoculated each pathogen population onto each host treatment, with three replicates per pathogen by host combination. In 2010, we paired each monoculture tray with a mixture tray on the bench (Figure 3.**2**), resulting in three replicates of each pathogen x monoculture treatment and nine replicates of each pathogen x mixture treatment. In 2012, the mixture treatment was replicated at the same level as all of the other monoculture treatments. In both experiments two additional host trays of each treatment were mock inoculated as a contamination control. Both experiments were set up as a randomized block design, with one full block of replicate pathogen by host treatments per greenhouse bench. Due to limited dew chamber space, inoculations in 2010 were carried out over three days, with one full replicate bench per day. The inoculation order of the pathogen populations was randomized each day, but all host treatments within a pathogen population were inoculated sequentially to reduce cross contamination.

Planting

Host treatments were planted into metal trays measuring 32 x 25cm and filled with sterilized soil. Soil was thoroughly watered and 400 oat seeds of the cultivar or mixture of host cultivars were evenly distributed on top. In the mixture treatment, 50 seeds of each cultivar were mixed together and then distributed on top of the soil. All

seeds were obtained from the Minnesota Oat Breeding project. Vermiculite and wet newsprint paper were placed on top to keep the seeds evenly moist until germination. To insure the growth of large primary leaves and restrict growth of secondary which makes spore collection difficult, trays were amended with 300mL of maleic acid hydrazide (5g per 30L H_2O) one day after germination (Rowell 1984).

Pathogen populations

To insure that experimental pathogen populations had the same diversity and abundance of strains we first reared 22 randomly selected strains from each of eight populations in isolation on seedlings of Marvelous and Starter. We generated experimental pathogen populations by combining 20mg of freshly collected spores from each of the 22 strains per population. Due to limited inoculum, the RM-SO population only contained 10 strains. Spores were thoroughly mixed and stored at 4°C and 0% relative humidity overnight. Since the 20 individual strains used were randomly selected from the pool of all strains in both experiments, the genetic make-up of the pathogen populations in 2010 differs from the populations in 2012 (Table S3. **7**, Table S3.8). In 2012, we used just 20 strains from each population and did not generate an experimental population from RM-SO strains, because fewer than 20 strains were available. Prior to inoculation, spores were suspended in mineral oil (Solitrol 170), and spore concentration was determined with two hemacytometer counts. Spore solutions were diluted to $1x10^5$ spores/mL in 2010 and $2.5x10^5$ spores/mL in 2012.

Inoculations

We inoculated 500μL of a 1 x 10^5 spores/mL suspension onto each host treatment with a spray inoculator. The inoculation chamber was rinsed with water and 70% ethanol between each pathogen population, and a clean inoculation nozzle was used. To insure that spore densities were even across treatments, two sticky tape spore traps were vertically suspended inside each tray during inoculation. In 2010, inoculated trays were transferred to a dew chamber with 100% RH for 12 hours and then returned to the greenhouse. In 2012, inoculated trays were returned to their position in the greenhouse and misted with DI water. Then, the entire greenhouse bench was sealed overnight with

plastic sheeting suspended approximately half a meter above the plants. Humidity was maintained overnight on each bench with a constant flow of distilled water mist piped in from a humidifier. Previous trials indicated that this method resulted in a high level of rust infection.

Spore collection

Spores were collected from the infected plants 17 days after inoculation in 2010 and 21 days after inoculation in 2012. We used a custom-made bulk spore collector (USDA CDL) attached to a shop vacuum to collect all the spores produced from a single tray. Spores were stored in glass vials, desiccated for one week, and then weighed. To determine infection incidence, we harvested all leaves in a 5 by 15cm strip at the center of each tray and counted the number of infected and non-infected leaves. Since infection was extremely low on the Souris monoculture treatment, we counted every diseased leaf in each of the Souris treatments. We also scanned all the harvested leaves and determined the total number of pustules, the average size, and the number of severely yellowed or dead leaves.

Statistical analysis:

We used analysis of variance (aov function in R, version 2.12.0) to estimate the main effects of host treatment, pathogen population, and the interaction of host treatment by pathogen population on infection incidence, total spore production, and total spore production given infection incidence. Inoculation day was used as a block. Inoculum dose, average leaf size, and the proportion of yellowed leaves were initially included as covariates in the model. However, only the average leaf area had a significant effect on spore production, and the other two covariates were dropped from the final model. In the 2010 experiment, we used type II sums of squares to account for the unbalanced number of mixture treatments. In 2012 we used type I sums of squares since the mixture treatment was replicated at the same level as all other host treatments.

To determine whether geographic region of origin (north, center, or south; Figure 3.**1)** had a significant effect on the pathogen spore production, we compared a mixed model where pathogen population was included as a random factor nested inside

geographic region to a null model where geographic region was not included. We carried out a similar analysis to determine if there was a difference in spore production between pathogen populations from mixtures and those from monocultures.

Finally, to determine whether there was evidence for local adaptation, we carried out pairwise comparisons using Tukey's honest significant difference test (and TukeyHSD function in R version 2.12.0) to correct for multiple comparisons. We first compared the average fecundity of each pathogen population on its host population of origin to average fecundity on other host populations (home vs. away). Then we looked within each host treatment and compared the average fecundity of 'local' pathogen populations that were originally collected from a similar host population to pathogen populations from different host sources (local vs. foreign).

III. VIRULENCE POPULATION STRUCTURE

We used the results of the oat differential infection test above to calculate the distribution of alleles at 28 virulence loci across pathogen populations. We assumed that a different pathogen virulence locus was associated with each tested host resistance gene. For example a pathogen strain able to infect the oat differential line carrying the *Pc14* resistance gene must carry a virulence allele at a corresponding '*v14'* virulence locus. Each locus was scored as a binary genotype of virulent or avirulent, and avirulence was assumed to be dominant. We used GENALEX version 6.0 (Peakall & Smouse 2005) to determine the number of unique virulence genotypes in each population and calculate Shannon's diversity index. Then, we estimated Nei's (1973) genetic distance and visualized differences among strains with principal coordinate analysis. To determine the amount of variation at virulence loci occurring among and within populations we used AMOVA in GENALEX version 6.0 (Peakall and Smouse 2005). We estimated ϕ_{ST}, a measure of F_{ST} for binary data (Michalakis and Excoffier 1996), and ran 999 permutations of the data to test the null hypothesis that $\phi_{ST} = 0$. Finally, we used a mantel test to determine whether there was a correlation between geographic distance and genetic distance.

We used the program STRUCTURE to ask if there was detectable structure at virulence loci without assigning populations *a priori.* We used the admixture model that allowed for linkage disequilibrium among loci with a burn-in period of 10,000 and 100,000 reps per run. To estimate *K*, the number of ancestral populations, we ran simulations for K =1 up to K=8, with the four replicate runs per value of K. We ran the whole simulation twice; in the first run we assumed that alpha, the admixture parameter, was the same across all populations. In the second run we allowed alpha to vary among populations. We then graphed the log likelihood of the data for both runs and found the value of K at which the greatest increase in log likelihood occurred (Pritchard et al. 2000).

RESULTS

Disease incidence in the field

Disease incidence was highest in the TJ-MO and AL-MO fields (Morton monocultures) and lowest in the TJ-SO and RM-SO fields (Souris monocultures; Table 3. **1**). Average disease incidence across the nine cultivars (Colt, Excel, Esker, Jerry, Kame, Morton, Rockford, Souris, and Spurs) we evaluated in the mixed-genotype host populations ranged from 6% to 37%, and reflected differences in host resistance across lines (Table S3. **5**). In monoculture fields planted with Souris (TJ-SO and RM-SO), we noted heavily infected plants among completely healthy plants across both suggesting that that Souris may not be a uniform genetic host line or that infrequent environmental factors strongly affect infection in this line.

Single pustule strains

We generated between 28 and 33 single-pustule strains from each host population except for RM-SO where we only managed to isolate 19 strains, for a total of 233 single-pustule strains (Table 3. **1**)

I. POPULATION STRUCTURE

Variation at microsatellite loci

We obtained microsatellite data for 182 rust strains at 13 loci (Table 3. **1**). The frequency of alleles at the A18, A26, B2, and C33 loci deviated significantly from Hardy-Weinberg expectations (Table 3.2). The deviation from expectations at the A18 and B2 loci was due to a single allele that occurred at high frequency (Figure S3. **1**), while the deviations at the A26 and C33 loci were due a dearth of heterozygotes. Analyses at the intra population level revealed that the C33 locus only deviated from expectations in a single population (TJ-MO), while the A26 locus had significantly fewer heterozygotes than expected in all populations. Since it is possible that these loci are linked to loci under selection, we ran all subsequent analyses with and without these four loci.

We examined allele frequencies at each locus to determine whether a step-wise mutational model could be assumed. Most loci appeared to have a normal distribution of allele sizes (Figure S3. **1**), indicating that the stepwise model is reasonable assumption. However, the C33, B25, and B54 loci were missing alleles expected under a step-wise model (Figure S3. **1**). Thus, we evaluated genetic distance and population structure under both the stepwise mutational model and the infinite allele model.

All pathogen populations had similar average number of alleles, allelic size range, and expected heterozygosity (Table 3.**3**). The population genetic parameter $\boldsymbol{\theta}$ (an estimate of $4N_e\mu$), ranged from 1.653 to 1.886 among populations. Genotypic diversity was extremely high. We found 174 unique multilocus microsatellite genotypes out of 184 pathogen strains, when all loci except for A26 were included. If the other three loci not in Hardy-Weinberg were included, then we found 171 genotypes. No multilocus genotype was found more than twice.

Population structure

AMOVA analysis (Arelquin; Excoffier et al. 2005), based on the sum of squared size differences under the step-wise mutational model revealed that all of the microsatellite size variation occurred within populations (Table 3.**4**). We estimated an overall R_{ST} value of -0.0021 (p= 0.591), demonstrating extremely low differentiation among populations. If we assumed an infinite allele model instead of a stepwise model, we found similarly low population structure (F_{ST} =0.001, p= 0.343). The only significant pairwise difference was between EG-CO and TW-VT (Table S3. **6**). A Mantel test found no significant correlation between geographic distance and genetic distance, under the stepwise model (r = -0.09700, p = 0.7690).

Results of the STRUCTURE analysis showed that the log likelihood of the data was greatest when K, the estimated number of genetic clusters, was equal to one. Thus, the results of the AMOVA and STRUCTURE analyses concur, and we conclude that all the samples in our study come from a single panmictic population.

Both AMOVA and STRUCTURE analyses were carried out with and without the four loci that deviated from Hardy-Weinberg expectations. Inclusion of the A18, B2, and C33 loci did not change the estimated values of R_{ST} or K, but inclusion of the A26 locus

did. When the A26 locus was included we found an R_{ST} value of 0.003, (p = 0.9189) and estimated K =3 genetic clusters.

Part I. Summary of genetic structure results

Our goal was to determine whether the adaptive potential of pathogen populations was constrained by limited genetic variation or low gene flow. We found high levels of genetic variation within populations and little evidence of population structure, suggesting high levels of gene flow. We conclude that our samples of *P. coronata* were obtained from a single, panmictic population with respect to the neutral microsatellite loci, and that adaptive potential is not constrained by a lack of genetic variation.

II. EFFECT OF HOST GENETIC DIVERSTIY

A. Host breadth

Variation in infection on different host cultivars

Not all strains were able to re-infect the host cultivar that they were originally collected from **(**Figure S3. **2).** Less than 60% of the strains collected from leaves of Excel, Spurs, or Souris cultivars could re-infect these cultivars. Since we collected rust strains from adult plants in the field but used seedlings to assess infection, one possible explanation is that seedling resistance and adult plant resistance differ (Kema and van Silfhout 1997, Jin et al. 2007). The low re-infection rates of Souris may due to genetic diversity for susceptibility within the Souris cultivar line (see discussion on page 100). There were eight seedlings of each cultivar in every infection trial, but we observed that only a small proportion of these eight Souris seedlings became infected. Indeed, heavily infected Souris seedlings grew side by side with completely healthy leaves, indicating genetic differences in resistance among Souris seedlings, rather than environmental. The other cultivars in the infection trial were much more consistent, and all seedlings within a pot showed the same infection reaction. Thus we excluded the Souris infection results from the analysis of host breadth below.

Effect of host genetic diversity on host breadth

We hypothesized that pathogen populations obtained from variety trials with mixed-genotype host populations could infect a greater number of cultivars (greater host breadth) compared to pathogen populations from single-genotype monocultures. We found a significant difference in average host breadth among pathogen populations ($F_{7,210}$= 2.2901, p= 0.0287; Figure 3.**3**) and a marginally significant effect of host genetic diversity on pathogen host breadth (Likelihood Ratio = 3.6013, p=0.0577). Pathogens from single-genotype monocultures did infect fewer host genotypes on average than pathogens from mixed-genotype variety trials (t_{212}=-2.298, p =0.0225), providing support for our hypothesis. However, this result was driven by the high average host breadth of strains from the RM-VT mixture and the low average host breadth of strains from the TJ-MO monoculture population. There were fewer differences in host breadth among the remaining mixture and monoculture populations Figure 3.**3**).

Effect of host genetic diversity on virulence level

We hypothesized that mixed-genotype host populations would select for pathogens with increased virulence level, the number of virulence alleles per strain, compared to monocultures. We found a significant difference in average virulence level among pathogen populations ($F_{7,124}$= 2.4023, p= 0.0244; Figure 3. **4**). Host diversity had a significant effect on the variation in virulence level (χ^2= 4.1, Df =1, p = 0.04288). In support of our hypothesis, pathogen strains from mixed-genotype host populations had higher average virulence levels than strains from single-genotype host monocultures. Not surprisingly, the results for virulence level paralleled those for host breadth above; and the finding of greater virulence level in mixed-genotype host populations was driven by the high average virulence level in the RM-VT host mixture population, and by the low average virulence level in the TJ-MO, Morton monoculture population. There was little difference in virulence level among the remaining pathogen populations (Figure 3. **4**).

Variation among and within populations

We found that 2.8% of the variation in host breadth occurred between diversity treatments, 2.9% of the variation occurred among populations within diversity treatments and 94.3% of the variation occurred within populations. Similarly, 5.8% of the variation

in virulence level occurred among populations within diversity treatments, 2.7% of the variation occurred among populations within a diversity group, and 91.7% of the variation occurred among strains within populations. Thus the majority of the variation in host breadth and virulence level occurs within populations.

Adaptation to the host cultivar of origin

We found significant variation among pathogen populations in the proportion of strains able to infect the different oat cultivars in the host-breadth inoculation trial. A large proportion of the strains in all pathogen populations were able to infect the more susceptible host cultivars of Kame, Colt, and Morton, while only a small proportion of strains in each population were able to infect the more resistant cultivars of Excel and Spurs (Figure 3.**5**). We predicted that on a given host cultivar, the proportion of infecting strains would be greater in populations that were collected from monocultures of the same host cultivar than in populations collected from different cultivar monocultures or mixed-genotype host populations. However, we did not find a consistent pattern of increased infection by such 'local' pathogen populations that would suggest local adaptation. For example, only one of the two populations collected from resistant Souris monocultures had greater infection on Souris than other pathogen populations (Figure 3.**5**). In addition, there was little evidence of local adaptation in pathogen populations from susceptible Colt and Morton monocultures since the majority of strains from all populations were able to infect these two cultivars.

B. Pathogen fecundity

We carried out two similar experiments in 2010 and 2012 to quantify average spore production of pathogen populations on different host population treatments that varied in host genetic diversity and host genotype. Results from the two experiments were very different: average spore production in the 2012 experiment was nearly 10 times that observed in the 2010 experiment as a result of a greater inoculum dose. The average inoculum dose in the 2010 experiment was 7.0 spores/cm^2 while the average inoculation dose in the 2012 experiment was 22.4 spores/cm^2. In addition, spores were

collected four days later in the 2012, which may also account for the greater 2012 spore production. Since the two experiments differed significantly in total spore production and in experimental design, we conducted separate ANOVAs and compare the results below.

Main effects of host treatment

While total spore production was significantly different in the 2010 and 2012 experiments, the main effects of host treatment were similar. Host treatment significantly affected infection incidence (the proportion of leaves infected) and total spore production in both 2010 (Table 3.5 A,B) and 2012 (Table 3.**6** A,B). In both experiments, the lowest incidence and spore production occurred on the resistant monoculture treatment of Souris, while the highest incidence and greatest spore production occurred on the two susceptible monoculture treatments (Colt and Morton; Figure 3.**6**). Infection incidence was a significant predictor of total spore production in both experiments, but in the 2010 experiment there was a significant effect of host treatment even after accounting for the variation in infection incidence (Table 3.5 C), suggesting differences among host treatments in the amount of spores produced per infected leaf. In 2012 we found little effect of host treatment after accounting for the variation due to infection incidence (Table 3.**6** C) suggesting that most of the variation in spore production among hosts is a result of variation in infection.

Main effects of pathogen population

We found a significant main effect of pathogen population on spore production in both 2010 (Table 3.5) and 2012 (Table 3.**6**), but the rank order of pathogen populations differed substantially between the two experiments. In the 2010 experiment, the TJ-MO population produced more spores than any other pathogen population (Figure 3.**7**B), while in 2012, this same population produced the fewest spores on average out of all the pathogen populations (Figure 3.**7**,D). Similarly spore production by the RM-VT population was comparatively lower in 2012 than in 2010. In both experiments, the effect of pathogen population on total spore production was significant even after accounting for infection incidence (Table 3.5C, Table 3.**6**,C), suggesting that pathogen

populations likely vary in reproductive traits that are expressed after infection, such as spore production.

The variation in results between the two experiments may reflect the variation in the strain composition of pathogen populations between the two experiments (Table S3. **7**, Table S3.8). For example there were four strains in the 2010 TJ-MO population that were not present in the 2012 population, and these may have accounted for the differences in spore production between the two experiments (Table S3.8.8). Although the strain composition differed among experiments, the average host breadth of the strains making up the populations did not differ appreciably (Table S3. **7**).

We did not find evidence to suggest that average spore production varied significantly between pathogen populations collected from mixed-genotype variety trials or single-genotype monocultures in either the 2010 experiment (Log likelihood=100.700, L. ratio =1.883, p=0.17) or the 2012 experiment (Log likelihood =138.069, L.ratio =2.4847, p =0.2887). Thus, we find no support for the hypothesis that spore production is greater on average in pathogen populations originating from host monocultures.

Local adaptation to host population of origin

We hypothesized that pathogen populations from single-genotype monocultures would show greater evidence of local adaptation to their host population of origin than pathogen populations from mixed-genotype variety trials. Below we evaluate evidence for local adaptation using two different tests, and then ask whether local adaptation is more common in pathogen populations from monocultures.

The home vs. away test of local adaptation predicts that pathogen populations that are locally adapted will have greater fecundity on their host population of origin than on other host populations. However, since all pathogen populations produced the fewest spores on the resistant host monoculture treatment (Souris) and the most spores on the susceptible monoculture treatments (Colt and Morton; Figure 3.**8**), this was not a useful test. To help separate local adaptation on pathogen fecundity from the main effects for resistance and susceptibility, we included an additional susceptible monoculture treatment, “Ogle” in 2012. Previous work has demonstrated that Ogle is highly susceptible to *P. coronata* (Bruns et al. 2012), but we predicted that pathogen populations

would produce fewer spores on the Ogle treatment than on the other two susceptible monoculture treatment (Colt and Morton) since none of the pathogen strains were collected from fields containing Ogle. While we did find that spore production of all pathogen populations was lower on Ogle than on either Colt or Morton (Figure 3.**8**B), these results were not statistically significant.

The local vs. foreign test of local adaptation predicts that within each host treatment, the pathogen populations collected from the same type of host population in the field (local populations) will have greater fecundity compared to pathogen populations collected from different host populations. Results from the resistant monoculture (Souris) and the mixture treatments did not support this prediction since spore production was strikingly similar among all pathogen populations (Figure 3.**8**). We conclude that pathogens collected in resistant monocultures and mixtures have not evolved greater fecundity on these host populations compared to pathogens collected from other types of host populations. We did find significant variation in spore production among pathogen populations on the susceptible monoculture treatments (Colt and Morton), and some limited evidence of local adaptation. In the 2010 experiment, the pathogen populations originating from Colt (EG-CO) and Morton (AL-MO and TJ-MO) did tend to produce more spores on the Colt and Morton monoculture treatments than pathogen populations originating from resistant Souris monocultures or mixed-genotype variety trials (Figure 3.**8**A). However these trends were not repeated in the 2012 experiments (Figure 3.**8**B). Thus our results do not provide conclusive evidence of local adaptation with respect to fecundity in any pathogen population, and therefore do not support the hypothesis of stronger local adaptation in pathogen populations from monocultures.

Part II. Summary of host diversity experiments

We predicted that genetically diverse host populations would select for pathogens with a greater ability to infect more host genotypes but lower average fecundity across those diverse host genotypes than will host monoculture populations. We find little conclusive evidence to support either hypothesis. While pathogen populations from mixed-genotype variety trials were able to infect more oat cultivars on average and had

greater average virulence levels than pathogen populations from monocultures, just two pathogen populations drove these results. In addition, pathogen populations from host monocultures did not consistently have greater fecundity on their host population of origin than pathogen populations from other sources. However, we did find evidence that both host breadth and average fecundity varied significantly among pathogen populations, and we pursue these results further in the next section.

III. VIRULENCE POPULATION STRUCTURE

Our final goal was to determine the extent to which pathogen populations are structured by differential selection on virulence and fecundity within populations. We focused our analyses on virulence rather than fecundity, because we collected data on the virulence of individual strains whereas we collected fecundity data on population averages. Since we evaluated virulence at multiple loci, we were able to compare the virulence structure of pathogen populations to the genetic structure estimated with microsatellite loci.

Virulence diversity and population structure

Virulence genotype diversity was extremely high. Out of 159 strains tested, we found 142 unique multilocus virulence genotypes. Only 11 of these genotypes were found more than once and no genotype was found in more than four strains. None of the strains that had identical virulence genotypes had the same microsatellite genotype, suggesting that none of the strains we isolated were clones. Virulence genotype diversity was greatest within the CK-VT and RM-VT populations collected from mixed genotype variety trials (Table 3.**7**). Interestingly, while strains from CK-VT demonstrated greater virulence genotype diversity than other populations, this increased virulence diversity did not correspond to an increase in average virulence level (Figure 3. **4**).

We found significant population structure for virulence (ϕ_{ST} = 0.079, p=0.001). The AMOVA results revealed that 8% of the variation at virulence loci occurs among populations (Table 3.**8**). We also found a significant isolation by distance effect (Mantel correlation test; r = 0.3294, p = 0.0450). These results are notably different than the

results of the microsatellite analysis, where we found no evidence of significant population structure (R_{ST} =-0.002), and no evidence of isolation by distance (r = -0.09700, p = 0.7690).

We found significant pairwise differences in ϕ_{ST} values among most populations (Table 3.**9**). Indeed, only seven pairwise population comparisons were not significantly different for ϕ_{ST}. We found no significant difference between the three pairs of populations with the nearest geographic proximity: TJ-MO and TJ-SO, RM-SO and RM-VT, and EG-CO and TW-VT. Interestingly, the CK-VT population, from a mixed-genotype variety trial in the north differed significantly from the other three populations in the north (TJ-MO, TJ-SO, and AL-MO), but did not significantly differ from three of the populations in the center or south regions. The PCA analysis showed that there are roughly three clusters of virulence genotypes (Figure 3.**9**). Individual strains from monoculture populations in the north were largely clustered together (Figure 3.**9**, blue markers) as are the two populations from the south (green markers). Interestingly, individual strains from the variety trials at RM-VT (orange circles) and CK-VT (purple crosses) are distributed widely, suggesting more extensive virulence genotype diversity.

Virulence structure analysis

The STRUCTURE analysis revealed that largest increase in the log probability of the data occurred between K=2 and K=3 clusters and we conclude three ancestral clusters best captures the virulence structure of the data. This result is strikingly different from the structure estimated with the microsatellite loci where we found a single, panmictic population best predicts the observed data. When K=3 the admixture parameter, alpha, ranged from 0.05 to 0.06, indicating low admixture. Allowing alpha to vary among populations did not significantly improve the likelihood. Interestingly, the frequency of strains from the three clusters appeared to vary with geographic region. Three of the populations collected from the north, TJ-MO, TJ-SO, and AL-MO, that were geographically close (Table S3.**1**) appeared to differ significantly from the other populations (Figure 3.**10**A). The next nearest population, CK-VT was 96km away, and the genetic makeup appears to differ. The two populations from the south, TW-VT and

EG-CO, also appear similar. Thus the results from the structure analysis appear to be consistent with the pairwise ϕ_{ST} values described above (Table 3.**9**).

In contrast, if we assume K=3 for the microsatellite results, alpha ranged from 4.7 to 7.9, indicating complete admixture, and there were no discernable differences between populations (Table 3.**8**, B). Thus, while we find little evidence of population structure among microsatellite loci, there appears to be significant structure among pathogen populations with respect to virulence alleles that corresponds with geographic regions.

Characterization of virulence clusters

When K=3, the STRUCTURE analysis assigned 23% of all strains to "Cluster 1" (red lines in Figure 3.**10**A). Individuals with predicted ancestry greater than 70% from Cluster 1 were found in all populations, but the frequency of such individuals assigned to cluster 1 was greatest in the RM-SO and RM-VT populations (Figure 3.**11**) that were from the center sampling region. Cluster 1 was characterized by a higher frequency of virulence at the *v14* and *v* 40 virulence loci, and lower virulence at *v* 36, *v* 56, *v* 55, *v* 39, and *v* 71 (Table 3.**10**). Cluster 2 (green lines in Figure 3.**10**A) was also present in all populations, but was most common in the three monoculture populations from the north region (Figure 3.**11**). Cluster 2 was characterized by a lower frequency of virulence at *v*35 and *v*61 (Table 3.**10**). Cluster 3 (blue lines in Figure 3.**10**A) was most common in the two populations in the southern region (TW-VT and EG-CO) and in the mixture populations in the central and north regions (RM-VT and CK-VT, respectively). Cluster 3 was characterized by a very high frequency of virulence at *v*48 and *v*52 and a lower frequency of virulence at *v*45 (Table 3.**10**).

Variation among populations in fecundity

We could not carry out similar analyses with the pathogen fecundity data since we evaluated fecundity at the population level and do not have information about the genetic variation underlying fecundity. Nevertheless we did observe significant variation in average fecundity among pathogen populations that may indicate underlying genetic differences among populations. Unlike the virulence results, we did not find a significant effect of geographic region on spore production in either the 2010 experiment (Log

likelihood =-100.700, L. ratio =2.286, p=0.3188) or the 2012 experiment (Log likelihood =138.069, L.ratio =0.3126, p= 0.8553). Instead there was considerable variation in pathogen fecundity among populations within a region.

Part III. Summary

Our goal was to determine whether there is evidence for differential selection of virulence among pathogen populations. We expected that if selection at virulence loci did not differ among populations, or was weaker than gene flow, there would be no difference in the structure of populations for microsatellite and virulence loci. However, if there was differential selection among populations and selection was stronger than effects of gene flow, there would be greater population structure in virulence loci than in microsatellite loci. We found evidence supporting the latter prediction: pathogen populations were structured with respect to virulence loci, but not to microsatellite loci. Thus, we conclude that the differences in virulence structure among populations are the result of differential selection rather than demographic processes such as drift.

DISCUSSION

Gene flow and adaptive potential

Genetic variation provides the raw material on which selection acts and is thus a necessary condition for local adaptation. Restricted gene flow and low effective population sizes can lead to a loss of genetic variation within populations due to increased effects of genetic drift, thus limiting the adaptive potential of pathogen populations (Gandon et al. 1996, Gandon and Michalakis 2002). We found no evidence to support restricted gene flow in *P. coronata*. Nearly all of the genetic variation at 12 microsatellite loci was maintained within populations rather than among populations, suggesting high gene flow. Moreover, the results of our genetic cluster analysis showed that all of our sampled pathogen strains likely come from a single, panmictic population.

The low genetic structure that we observed among *P. coronata* populations is similar to the population structure for other wind dispersed agricultural pathogens. For example, Zhan et al. (2003) found that over 90% of the variation in RFLP loci in the wheat pathogen *Mycosphaerella graminicola* was maintained within a field, while only 8% of the variation occurred among regions or continents. Similarly low levels of among population variation were found in *Phaeosphaeria nodorum* (θ = 0.05; Pimentel et al. 2000), and *Tapesia yallundae* (θ = -0.008; Douhan et al. 2002), where θ is an F_{ST} analogue (Weir and Cockerham 1984). In contrast, Kolmer and Ordoñez (2007) found significant genetic structure among wheat leaf rust populations in central Asia (R_{ST} = 0.14) and Barrett et al. (2008a) found considerable levels of genetic structure among natural populations of the *Melampsora lini* (θ = .336). The large population size of *P. coronata* and other agricultural populations likely contributes to the amount of genetic variation within populations, since drift has less effect in large populations (Burdon 1987, Barrett et al. 2008a)

Selection from host genetic diversity

Does host genetic diversity affect the evolution of pathogen host breadth and fecundity? The results of the host breadth and fecundity experiments do not provide

strong support for our hypothesis that host genetic diversity is an important selective force structuring pathogen populations. While we did observe differences in host breadth and fecundity among pathogen populations, the variation between different pathogen populations collected from the same host diversity treatment was substantial.

We hypothesized genetically diverse host populations would select for increased host breadth compared to less diverse monocultures. Results of the host breadth experiments demonstrated that highest average host breadth and virulence level occurred in strains collected from one host mixture, while the lowest average host breadth and virulence level occurred among strains collected from one monoculture. However, since there were no detectable differences in host breadth or virulence level among the other pathogen populations from mixtures and monocultures, we cannot conclude that the difference in host breadth between these two populations is the result of selection from genetically diverse host populations.

We hypothesized that genetically diverse host populations would constrain the evolution of increased fecundity on any one host, due to trade-offs in pathogen performance on different host genotypes (Rausher 1984, Futuyma and Moreno 1988, Joshi and Thompson 1995). We expected that pathogen populations from single-genotype monocultures would show a stronger pattern of local adaptation to their host population of origin than pathogen populations from mixtures. However we found no consistent evidence of local adaptation in any of the pathogen populations with respect to fecundity due to the large amount of variation within pathogen by host population combinations and among fecundity experiments. For example, two of the pathogen populations collected from susceptible Morton monocultures produced more spores on Morton monoculture treatments in greenhouse experiments than other pathogen populations in 2010, but since these results were not consistent across the two fecundity experiments they do not provide conclusive support of local adaptation. We found no evidence in either experiment to suggest that pathogens from resistant Souris monocultures have evolved increased fecundity on Souris compared to pathogens from other sources. Thus we cannot draw conclusions about the effect of host genetic diversity on pathogen local adaptation.

It may also be that size of the host populations in variety trials were simply too small to exert any significant selection pressure. Indeed, we assumed that local adaptation in this system occurs at the scale of a single host field and over the time frame of a single oat-growing season, but it may be that in a widely dispersed pathogen like *P. coronata,* the spatial scale at which local selection drives evolution is much larger than a single population (Imhoof and Schmid-hempel 1998, Laine 2005, Cogni and Futuyma 2009).

Relative effects of selection and gene flow

Does selection structure pathogen populations? Observed differences in virulence population structure within different regions are most often attributed to selection (Kolmer 1989, Bousset et al. 2002, Leonard 2003), but virulence structure could also result from drift (Barrett et al. 2008). Here we provide evidence that natural selection structures the variation at virulence loci in populations of *P. coronata.* If the variation among populations at virulence loci were simply the result of demographic processes such as drift and migration, the virulence structure should be similar to that described for variation at microsatellite loci. Instead, we found significant structure among populations with respect to virulence loci, but no evidence of population structure with respect to neutral microsatellite loci. Thus, we conclude that the regional differences in virulence we observe are the result of differential selection.

Strong gene flow can potentially restrict local adaptation if selection is not strong enough to overcome the constant influx of non-adapted genotypes (Holt and Gomulkiewicz 1997). However, our results demonstrate that pathogen populations respond to selection in the face of strong gene flow. The extremely low R_{ST} estimate from microsatellite data indicates that gene flow in this system is extremely high, yet pathogen strains from the north and south regions appear to be differentiated with respect to virulence. We conclude that the strength of selection on these loci is strong enough to overcome the homogenizing effect of gene flow and that adaptation of *P. coronata* is not limited by high gene flow.

The spatial scale of local adaptation can vary widely among systems (Imhoof and Schmid-hempel 1998, Thrall et al. 2002, Laine 2005, Cogni and Futuyma 2009) and the

appropriate scale is not always obvious ahead of time (Levin 1992). For example, Cogni and Futuyma (2009) found no evidence that the specialized seed predator *Uteheisa ornatrix* was locally adapted to its legume host at a regional level, but found evidence of local adaptation at a continental scale. Thrall et al. (2002) found some evidence that strains of the flax rust pathogen, *Linum marginale* were locally adapted at the scale of individual host populations within a region, but found that patterns of local adaptation were much stronger at a regional scale. In the host diversity experiments, we made the assumption that local adaptation occurred at the scale of a single host field, but our analysis of pathogen virulence structure revealed that geographic location was more important to virulence structure than the genotype or genetic diversity of the host population where the pathogens were collected. Thus, we argue that the scale at which pathogen local adaptation occurs in this system is likely larger than an individual field. There are several factors that could vary at a regional level and affect selection on virulence loci, such as regional differences in the most commonly planted cultivars, or the abundance of the wild hosts such as the common agricultural weed, *Avena fatua* (Oates et al. 1983).

CONCLUSIONS

Local adaptation is expected when the strength of selection within local environments is stronger than gene flow, assuming that the adaptive potential of pathogen populations is not limited by a lack of genetic variation. However, the spatial scale at which local adaptation occurs is not always obvious. We asked whether there was evidence that *P. coronata* strains collected from individual oat fields were locally adapted to their host populations of origin and whether local adaptation was stronger in pathogens from single-genotype monocultures. We found little evidence of local adaptation within pathogen populations, and no evidence to support the hypothesis that host genetic diversity affects the evolution of host breadth and pathogen fecundity. However, we observed significant regional differences in virulence loci that were not explained by historical population structure, suggesting selection. We conclude that local adaptation in this agricultural system structures pathogen populations at a regional level.

FUNDING AND ASSISTANCE

We gratefully acknowledge the many people who helped with the greenhouse and lab work. We are especially grateful to Jeremy Lund who helped with the greenhouse isolations, infection trials, and fecundity experiments. Elizabeth Guhl helped with the microsatellite project and the second fecundity experiment. As always, we are grateful to Jerry Ochocki at the USDA CDL for his help growing and maintaining rust strains. Mike Paetz helped with collections and fecundity experiments. Valerie Wong, Aaron David, Paul Nelson, Gina Quiram, and Tom Giarla helped with fecundity experiments. We thank Roger Caspers for allowing us access to the variety trials and providing seeds, and all of the oat farmers who graciously allowed us to sample their fields. The work was funded by a Hatch Grant (MIN-71-024) from the Minnesota Agricultural Experiment Station and Department of Plant Biology at the University of Minnesota, and by the Center for Community Genetics, and the Dayton Natural History Fund.

TABLES & FIGURES

Table 3. 1. Collection site information and number of single pustule strains.

Field	Region[1]	Diversity[2]	Cultivar[3]	Acres	Incidence[4]	Stems counted	SP strains [5]	Microsat. analysis [6]	Host breadth [7]	Virulence [8]
TJ-SO	North	Mono	Souris	25	16%	50	33	25	26	23
TJ-MO	North	Mono	Morton	30	100%	50	31	23	29	21
AL-MO	North	Mono	Morton	60	100%	50	33	22	31	21
CK-VT	North	Mix	Mix	<1	67%	70	31	26	27	27
RM-SO	Central	Mono	Souris	6	12%	50	19	17	18	11
RM-VT	Central	Mix	Mix	<1	59%	155	28	20	27	22
EG-CO	South	Mono	Colt	13	67%	115	30	23	29	19
TW-VT	South	Mix	Mix	<1	37%	150	28	26	27	15

[1] Geographic region of field

[2] Genetic diversity of the host population

[3] Cultivar planted in the monoculture fields

[4] Proportion of plants that were infected with crown rust

[5] Number of single pustule strains isolated

[6] Number of strains genotyped at all 12 microsatellite loci

[7] Number of strains tested for host breadth on all 10 host cultivars

[8] Number of strains tested for virulence on all 28 oat differential lines

Table 3.2. Number of alleles, size, observed and heterozygosity, and average population differentiation of 13 microsatellite loci

Locus	Num.[1]	N_a[2]	Size range	H_O[3]	H_E[4]	HW test[5]	R_{ST}[6]	
A18	180	3	4	0.022	0.033	**0.017**	0.016	
A26	181	5	10	0.028	0.369	**<0.001**	0.124	**
A36	179	3	6	0.503	0.476	0.241	0.004	
A75	180	8	16	0.472	0.479	0.421	0.018	
B78	177	3	4	0.09	0.088	0.699	0.040	
C33	182	3	10	0.368	0.464	**0.004**	-0.001	
B2	182	3	4	0.071	0.085	**0.028**	-0.009	
B15	181	3	4	0.343	0.358	0.289	0.019	
B25	177	6	21	0.316	0.304	0.283	-0.004	
A35	181	6	10	0.663	0.642	0.219	-0.016	
A59	178	6	8	0.472	0.491	0.515	-0.005	
B13	172	8	12	0.221	0.229	0.408	0.000	
B54	181	4	36	0.37	0.354	0.239	-0.004	

[1] Number of genotypes successfully amplified out of 182

[2] Number of alleles

[3] Observed heterozygosity

[4] Expected heterozygosity

[5] P-value of Hardy-Weinberg test comparing observed and expected heterozygosity.

[6] RST value generated from locus x locus AMOVA. Asterisks indicate significant R_{ST} at $p<0.05$

Table 3.3. Microsatellite diversity indices by pathogen population

Population	Num[1]	Num genotypes[3]	Polym. loci [2]	Shannon's Index (I)[4]	Ave. N_a[5]	Allelic size range	θ_H [6]	H_e[7]
TJ-SO	25	24	10	0.624	3.385	9.692	1.736	0.337
TJ-MO	23	23	11	0.670	3.308	10.833	1.653	0.367
AL-MO	22	21	11	0.576	2.692	8.833	1.73	0.339
CK-VT	26	25	9	0.549	2.462	10.6	1.886	0.299
RM-SO	17	16	11	0.586	2.846	9.231	1.715	0.344
RM-VT	20	19	11	0.550	2.846	8.833	1.861	0.305
EG-CO	23	23	12	0.543	2.846	8.833	1.82	0.314
TW-VT	26	25	11	0.586	3.154	8.833	1.746	0.334

[1] Number of strains

[2] Number of polymorphic loci

[3] Number of unique multilocus genotypes

[4] Shannon's diversity index where $I = -\Sigma\, p_i ln(p_i)$, where p_i is the frequency of the *ith* virulence genotype**.**

[5] Average number of alleles

[6] Population parameter θ, estimated using the stepwise mutation model

[7] Expected heterozygosity

Table 3.4. AMOVA summary for 12 microsatellite loci

	DF	Sum of squares	Variance component	Percentage of variation
Among populations	7	816.333	-0.2498	-0.2
Within populations	356	45552.669	127.957	100.2
Total	363	46,369,003	127.707	

Locus A26 was excluded from the analysis.

Table 3.5. Summary of ANOVA results for 2010 fecundity experiment

Source	DF	Type II SS	MS	F	p	
A. *Infection incidence*						
Block	2	1.1389	0.569	12.376	0.0000	***
Host treatment	3	7.4699	2.490	54.113	0.0000	***
Pathogen population	7	0.211	0.030	0.655	0.7094	
Host * Pathogen	21	1.017	0.048	1.052	0.4100	
Residuals	108	4.9235	0.046			
B. *Spore production*						
Block	2	15.7239	7.8620	34.797	<0.0001	***
Average leaf area	1	1.0377	1.0377	4.593	0.0344	*
Host treatment	3	24.9532	8.3177	36.814	<0.0001	***
Pathogen population	7	4.5822	0.6546	2.897	0.0082	**
Host * Pathogen	21	6.5922	0.3139	1.389	0.1399	
Residuals	107	24.1753	0.2259			
C. *Spore production| incidence*						
Block	2	9.1768	4.588	20.204	0.0000	***
Incidence	1	1.5735	1.574	6.929	0.0097	**
Host treatment	3	8.0367	2.679	11.796	0.0000	***
Pathogen population	7	4.0028	0.572	2.518	0.0195	*
Host * Pathogen	21	5.9287	0.282	1.243	0.2318	
Residuals	107	24.303	0.227			

Results of three separate ANOVA analysis to determine the effect o host treatment and pathogen population of origin on A) infection incidence (proportion of infected leaves) B) total spore production, and C) total spore production (grams of dry spores) after accounting for incidence. Incidence was arcsine-square-root transformed and total spore production was log transformed. Average leaf area did not have a significant effect on incidence or spore production given incidence and was dropped from these models.

Table 3.6. Summary of ANOVA results for 2012 fecundity experiment

Source	DF	SS	MS	F	p	
A. *Infection incidence*						
Greenhouse bench	2	0.263	0.1314	5.850	0.0045	***
Host treatment	4	13.551	3.3878	150.886	<0.0001	***
Pathogen population	6	0.683	0.1139	5.073	0.0002	***
Host *Pathogen	24	0.770	0.0321	1.429	0.1274	
Residuals	68	1.527	0.0225			
B. *Spore production*						
Greenhouse bench	2	0.5854	0.2927	76.468	<0.0001	***
Average leaf area	1	0.0272	0.0450	7.1117	0.0096	**
Host treatment	4	0.6163	0.1541	40.249	<0.0001	***
Pathogen population	6	0.2384	0.0397	10.377	<0.0001	***
Host *Pathogen	24	0.0989	0.0041	1.076	0.3933	
Residuals	67	0.2565	0.0039			
C. *Spore production | incidence*						
Greenhouse bench	2	0.5854	0.293	77.026	<0.0001	***
Incidence	1	0.6950	0.695	182.895	<0.0001	***
Average leaf area	1	0.0633	0.063	16.658	0.0001	***
Host treatment	4	0.0242	0.006	1.592	0.1868	
Pathogen population	6	0.1250	0.021	5.482	0.0001	***
Host *Pathogen	24	0.0791	0.003	0.867	0.6414	
Residuals	66	0.2506	0.004			

Results of three separate ANOVA analysis to determine the effect o host treatment and pathogen population of origin on A) infection incidence (proportion of infected leaves) B) total spore production, and C) total spore production (grams of dry spores) after accounting for incidence. Incidence was arcsine-square-root transformed and total spore production was square-root transformed. Average leaf area did not have a significant effect on disease incidence and was removed from the model.

Table 3.7. Virulence genotype diversity within populations

Pop.	Region	Diversity	Cultivar	Num. strains	Num. genotypes[1]	Num. polym. loci[2]	Shannon's Index (I)[3]
TJ-SO	North	Mono	Souris	23	22	20	0.3
TJ-MO	North	Mono	Morton	21	21	21	0.301
AL-MO	North	Mono	Morton	21	17	17	0.263
CK-VT	North	Mixture	Mix	27	25	24	0.348
RM-SO	Center	Mono	Souris	11	10	15	0.275
RM-VT	Center	Mixture	Mix	22	20	22	0.384
EG-CO	South	Mono	Colt	19	18	17	0.307
TW-VT	South	Mixture	Mix	15	14	13	0.25

[1] Number of unique multilocus virulence genotypes

[2] Number of polymorphic loci (out of 28)

[3] Shannon's diversity index where $I = -\Sigma\, p_i ln(p_i)$, where p_i is the frequency of the *ith* virulence genotype**.**

Table 3.8. AMOVA summary for 28 virulence loci

Source	Df	SS	MS	Variance component	Percent variation
Among Populations	7	53.643	7.663	0.262	8%
Within Populations	134	409.816	3.058	3.058	92%
Total	141	463.459		3.320	100%

Table 3.9. Pairwise ϕ_{ST} estimates for virulence loci

	TJ-SO	TJ-MO	AL-MO	CK-VT	RM-SO	RM-VT	EG-CO	TW-VT
TJ-SO	0							
TJ-MO	0.0332	0						
AL-MO	0.0819*	0.0523*	0					
CK-VT	0.0928*	0.0858*	0.0463*	0				
RM-SO	0.2282*	0.1241*	0.0844*	0.0539	0			
RM-VT	0.1478*	0.0873*	0.0588*	0.0297	0.0028	0		
EG-CO	0.0514*	0.0384*	0.0741*	0.0070	0.1060*	0.0532*	0	
TW-VT	0.1950*	0.1034*	0.1849*	0.0838*	0.0842	0.1169*	0.0444	0

Asterisks indicate significant ϕ_{ST} at $p<0.05$.

Table 3.10. Virulence frequencies within each genetic cluster

Diff. line	Cluster 1	Cluster 2	Cluster 3
v94	0.018	0.001	0.04
v62	0.042	0.002	0.017
v48	0.053	0.003	0.948
v52	0.103	0.008	0.95
v68	0.151	0.012	0.091
v14	0.56	0.016	0.281
v40	0.551	0.017	0.204
v 96	0.085	0.018	0.048
v54	0.106	0.019	0.016
v64	0.104	0.034	0.067
v50	0.122	0.039	0.191
v53	0.218	0.08	0.159
v60	0.427	0.129	0.416
v35	0.457	0.193	0.267
v61	0.44	0.196	0.377
v46	0.502	0.436	0.648
v67	0.632	0.525	0.63
v58	0.751	0.637	0.824
v59	0.751	0.637	0.824
v51	0.848	0.837	0.864
v36	0.853	0.948	0.99
v56	0.851	0.95	0.992
v45	0.908	0.993	0.807
v63	0.91	0.993	0.928
v55	0.8	0.996	0.956
v39	0.812	0.997	0.992
v71	0.815	0.997	0.992
v38	0.982	0.999	0.959

Heat map showing the average frequency of the virulence genotype at 28 virulence loci within each of the three genetic clusters identified by STRUCTURE. Virulence was determined by assessing infection against differential oat lines that carried different crown rust resistance genes (Pc genes). Thus *v94* above refers to the inferred pathogen virulence locus that corresponds to the *Pc94* host resistance gene.

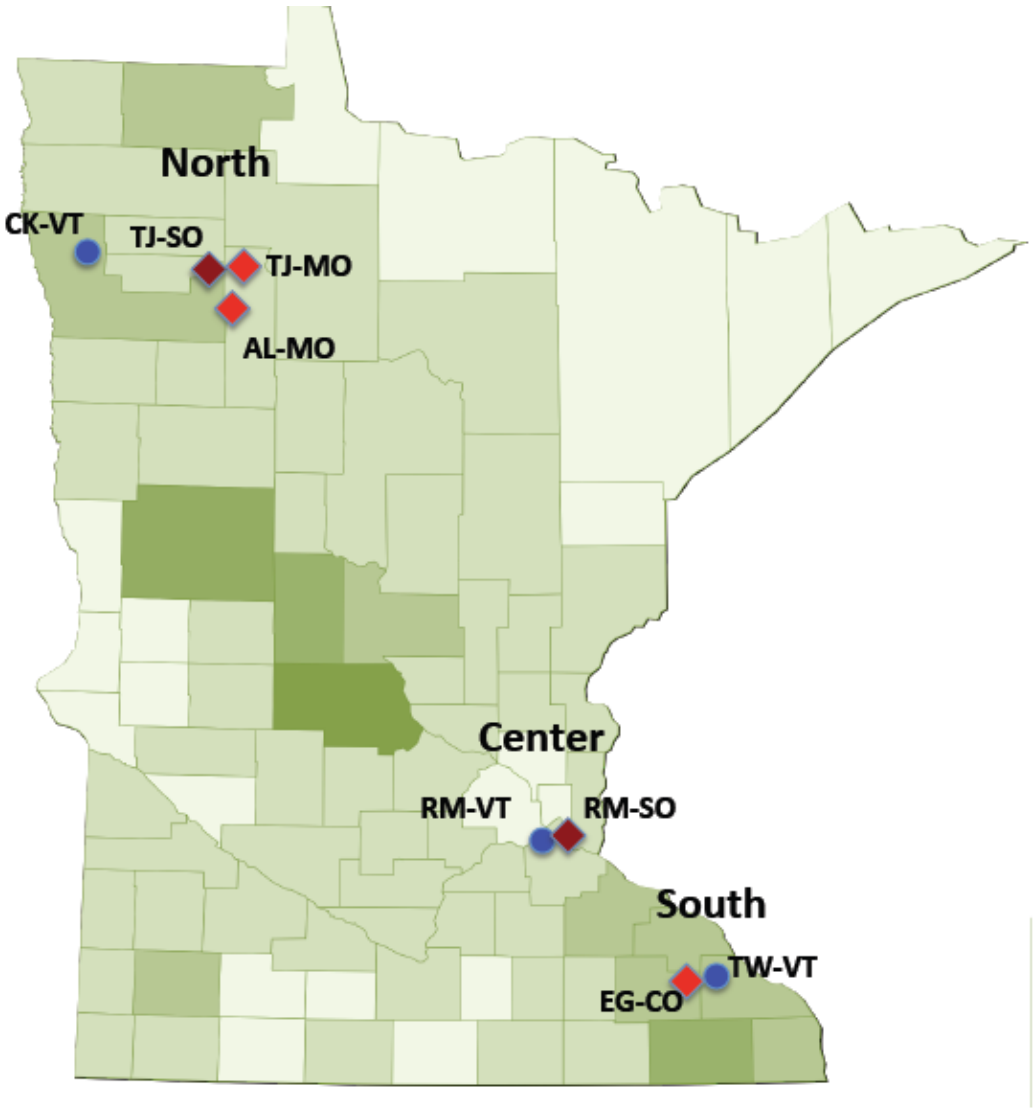

Figure 3.1. Location of oat fields in Minnesota where crown rust was collected. Blue circles indicate mixed-cultivar variety trials and red circles indicate single-cultivar monocultures. The green shading represents oat production per county as reported to the USDA. Distances between populations are not strictly to scale. A matrix of pairwise distances based on GPS coordinates can be found in (Table S3.**1**).

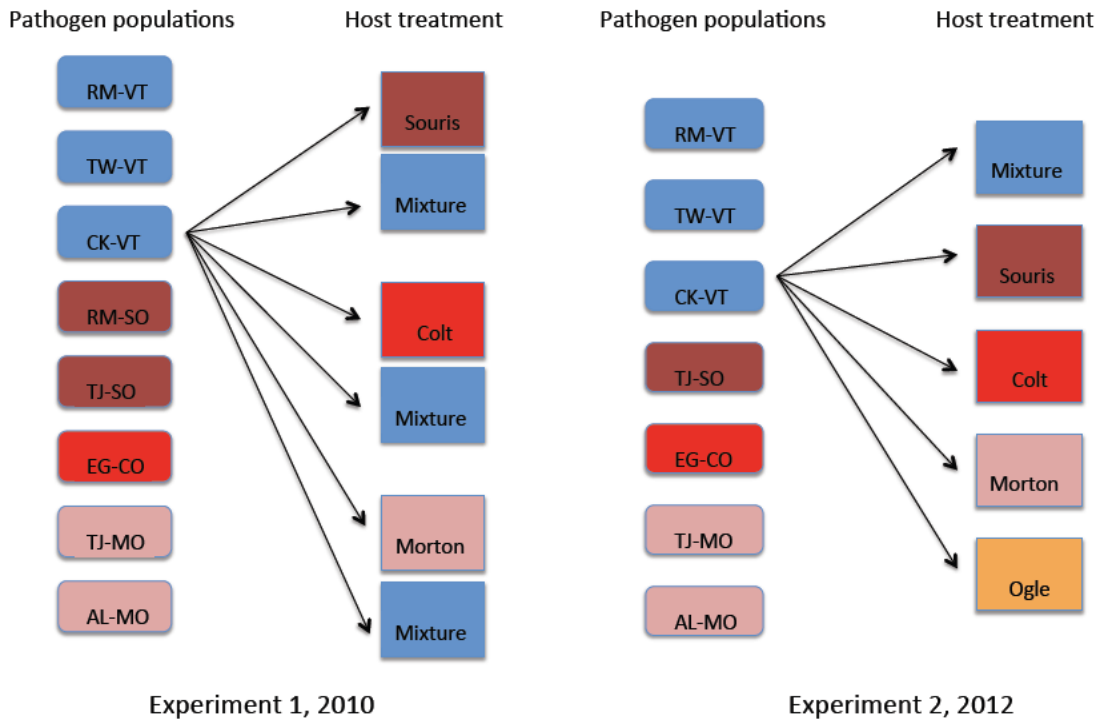

Figure 3.2. Experimental design of cross-inoculation experiments to measure fecundity.

In both experiments each pathogen population was inoculated onto each host treatment, but for simplicity, only the inoculations for the CK-VT population are illustrated. Colors indicate the host treatment and the host population of origin. Blue= host genotype mixtures, Red= monoculture of either Colt, Souris, or Morton. In 2010, three times as many mixtures were inoculated as monoculture treatments. In 2012, we did not use the RM-SO pathogen population, the Ogle monoculture treatment was added, and the mixture treatment was replicated at the same level as the other host treatments.

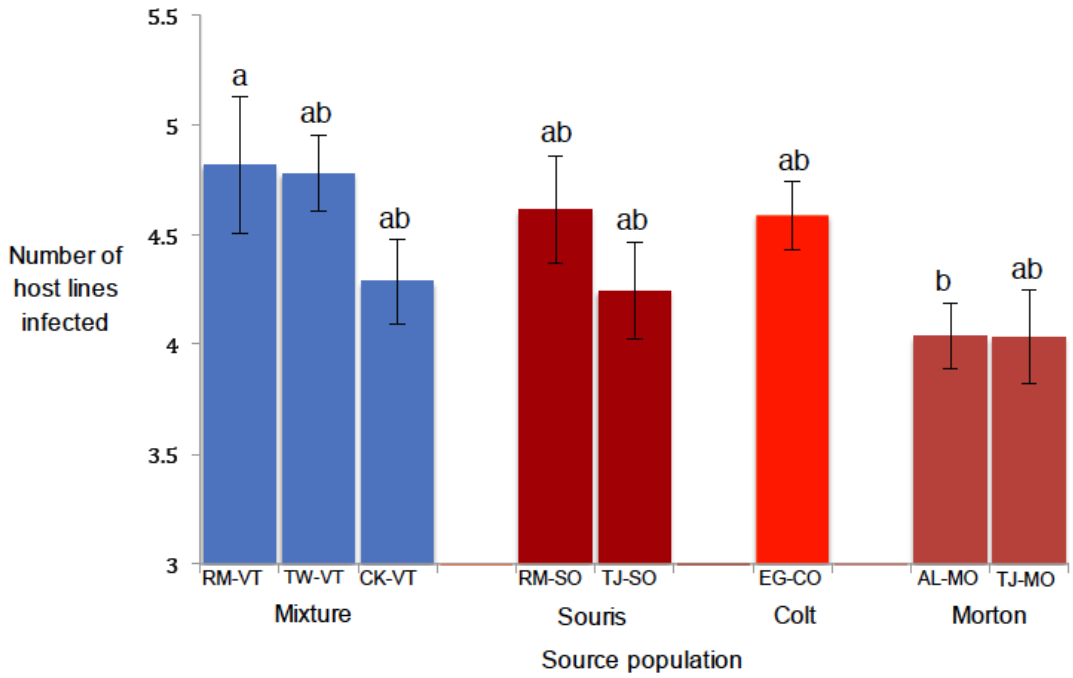

Figure 3.3. Average host genotype breadth of eight pathogen populations.

Host genotype breadth was measured as the number of host lines infected out of a set of seven different oat cultivars. Pathogen populations collected from host mixtures are shown in blue, while populations from monocultures are shown in red. Populations from monoculture are grouped by host genotype. Populations that significantly differ in host genotype breadth do not share letters (Tukey HSD test, $p<0.05$). The cultivar identity of each monoculture population is indicated. Error bars represent ± 1 SE.

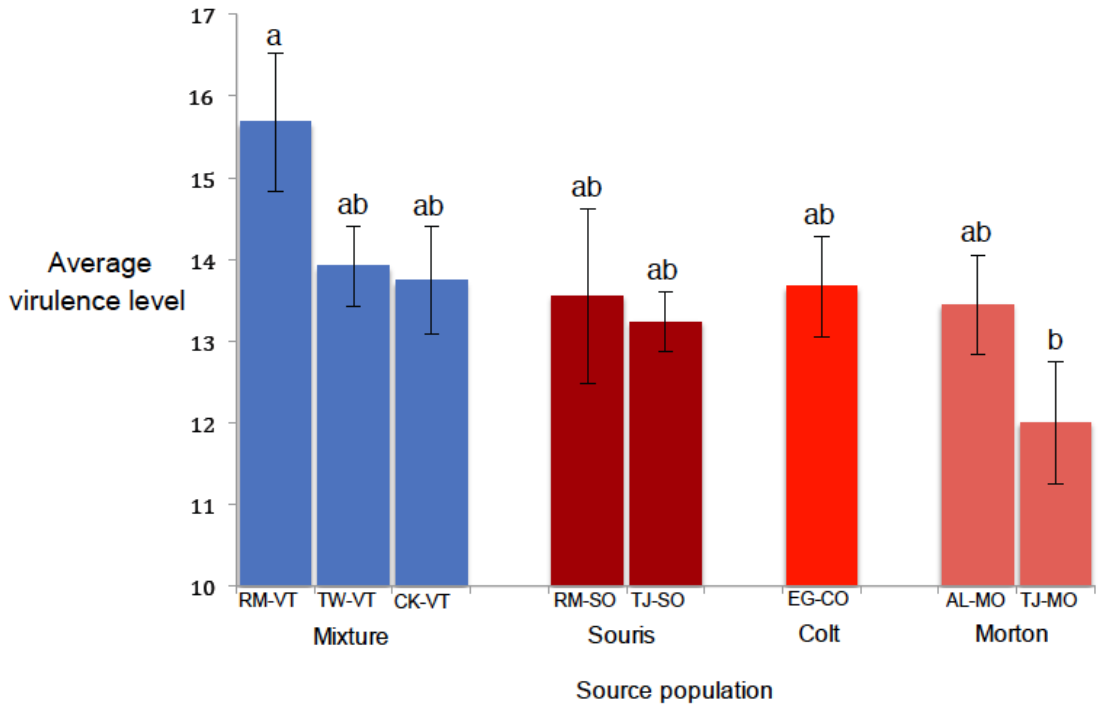

Figure 3. 4. Average virulence level of eight pathogen populations

Average virulence level was measured as the number of oat differential lines infected out of a set of 28 lines. Pathogen populations collected from host mixtures are shown in blue, while populations from monocultures are shown in red. Populations from monoculture are grouped by host genotype. Populations that significantly differ in host genotype breadth do not share letters (Tukey HSD test, $p<0.05$). The cultivar identity of each monoculture population is indicated. Error bars represent ± 1 SE.

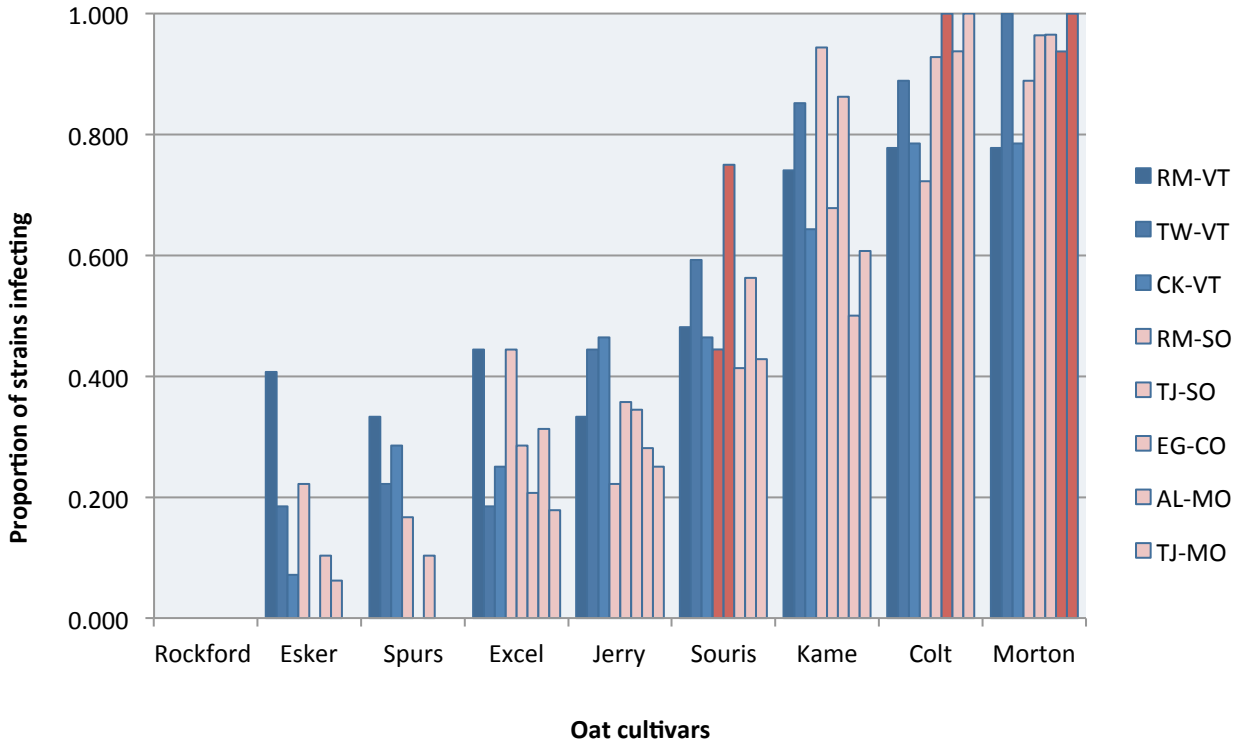

Figure 3.5. Proportion of strains from each population able to infect nine different oat cultivars.

Pathogen populations from mixed-genotype host populations are shown in blue. Populations from single-genotype monocultures are shown in red. Within a cultivar, 'local' pathogen populations that originated on a monoculture of the same cultivar in the field are highlighted in dark red.

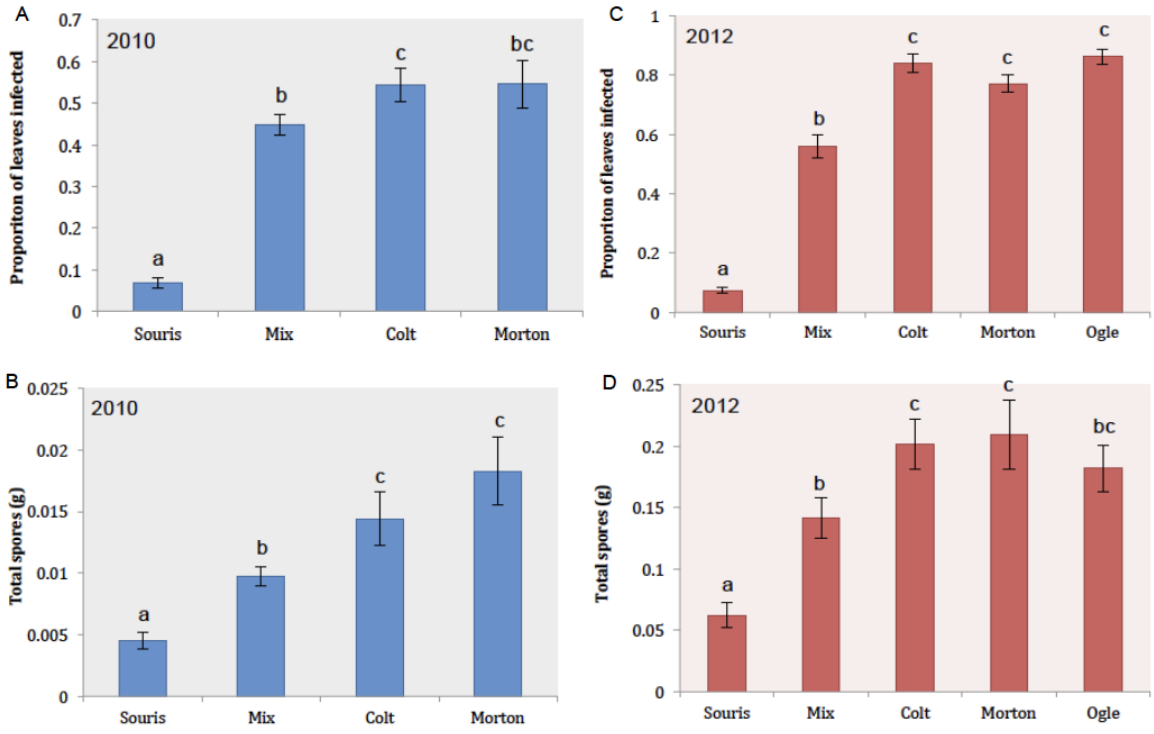

Figure 3.6. Main effects of host treatment on pathogen incidence and spore production.

Infection incidence (A,C) and average spore production (B, D) on different host treatments in the 2010 (A,B) and 2012 (B,D) experiments. Spore production is significantly different on host treatments that do not share letters (Tukey HSD test, $p<0.05$). Error bars represent ± 1 SE.

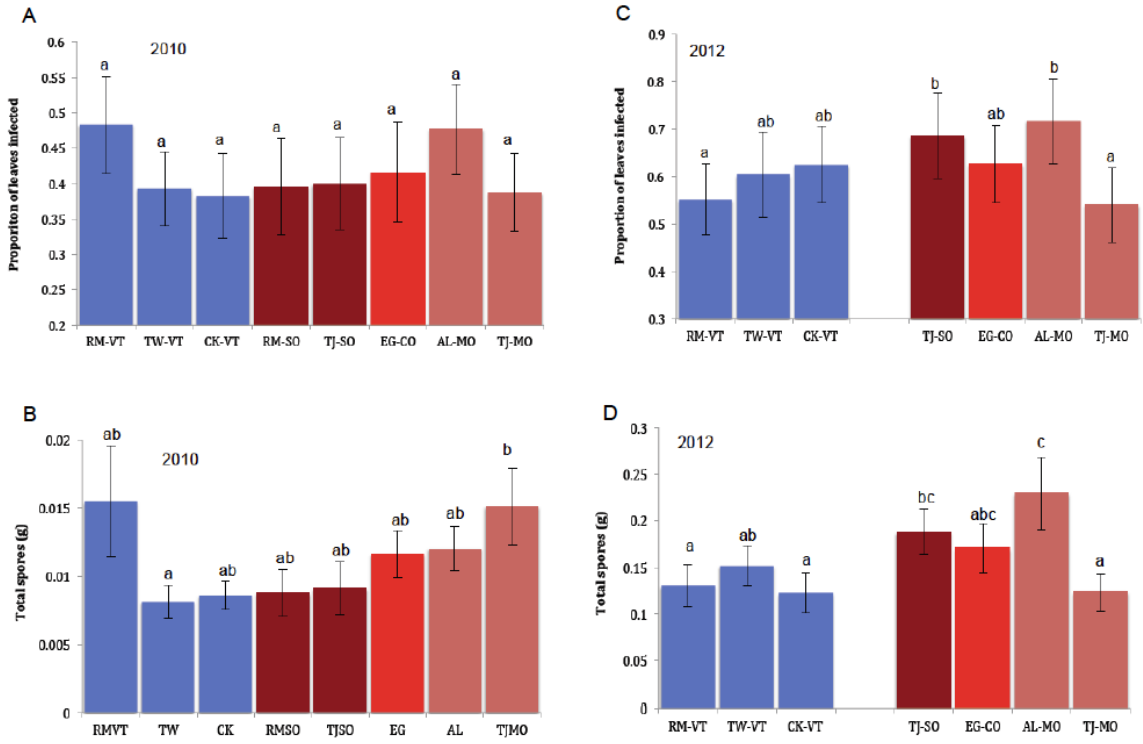

Figure 3.7. Main effects of pathogen population on incidence and spore production

Average infection incidence (A, C) and spore production (B, D) of pathogen populations originating from host cultivar mixtures (blue) or single-cultivar monocultures (red) in the 2010 (A, B) and 2012 (C, D) experiments. Pathogen populations with the same shade of red were collected from fields planted with the same host cultivar. Within each graph populations that do not share letters differ significantly (Tukey HSD test, $p<0.05$). Error bars represent ± 1 SE.

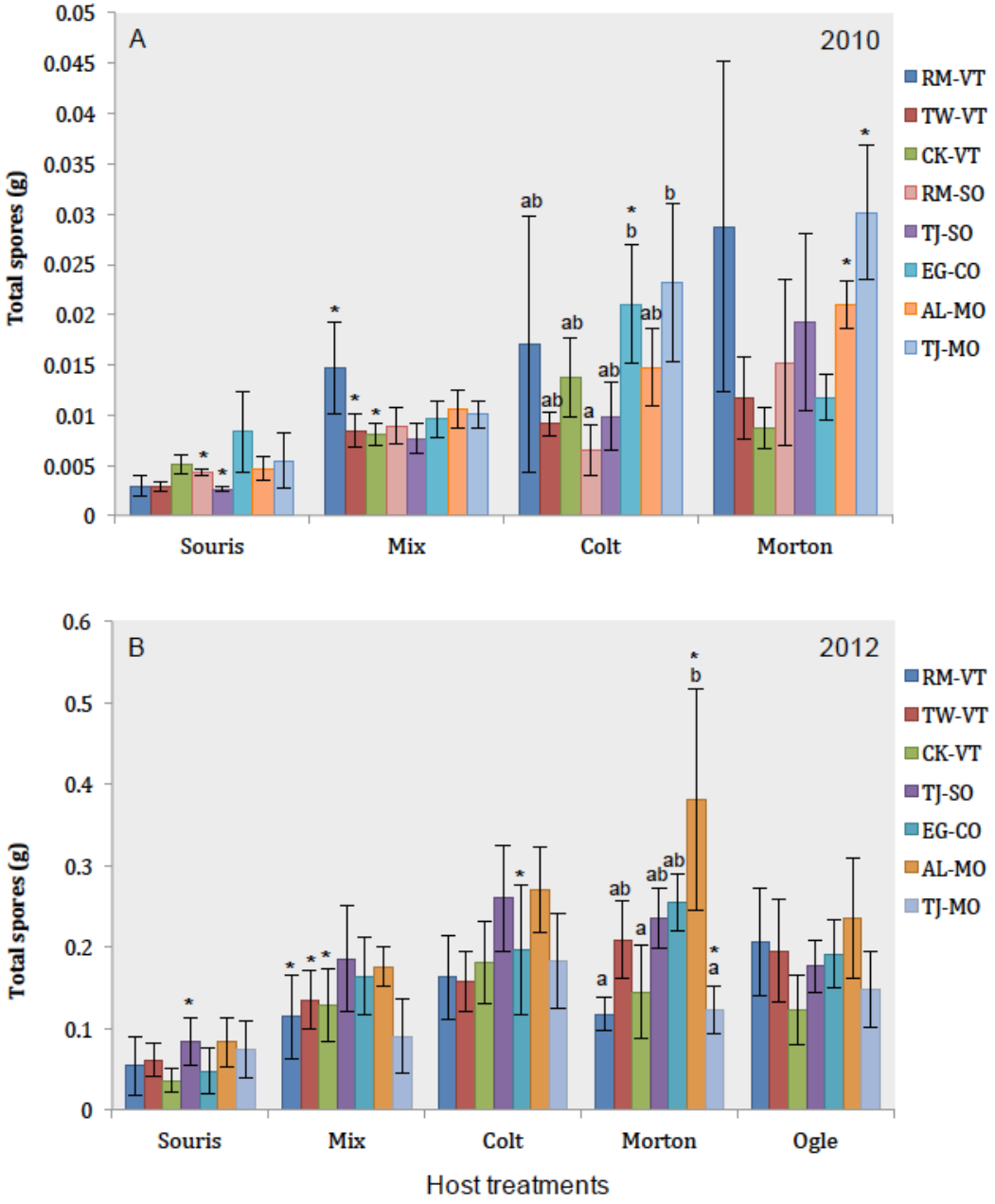

Figure 3.8. Spore production of pathogen populations within each host treatment. In 2010 (A) significant differences in spore production among pathogen populations were only observed on the Colt monoculture treatment, while in 2012 (B) significant differences were only observed on the Morton monoculture. Within a host treatment spore production is significantly different among pathogen populations that do not share letters (Tukey HSD test, $p<0.05$). On each host treatment, 'local' pathogen populations are marked with an asterisk. Error bars represent ± 1 SE.

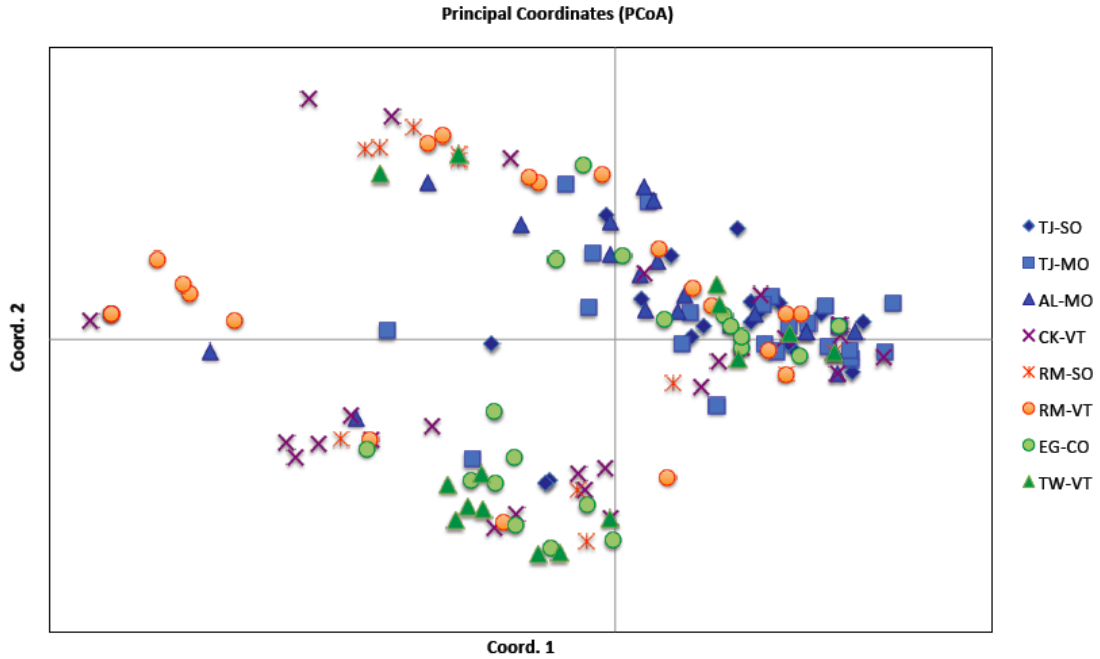

Figure 3.9. PCA based on genetic distance between multilocus virulence genotypes. Individuals from the northern region are shown in blue (CK-VT is in purple), the central region is shown in orange, and the southern region is shown in green.

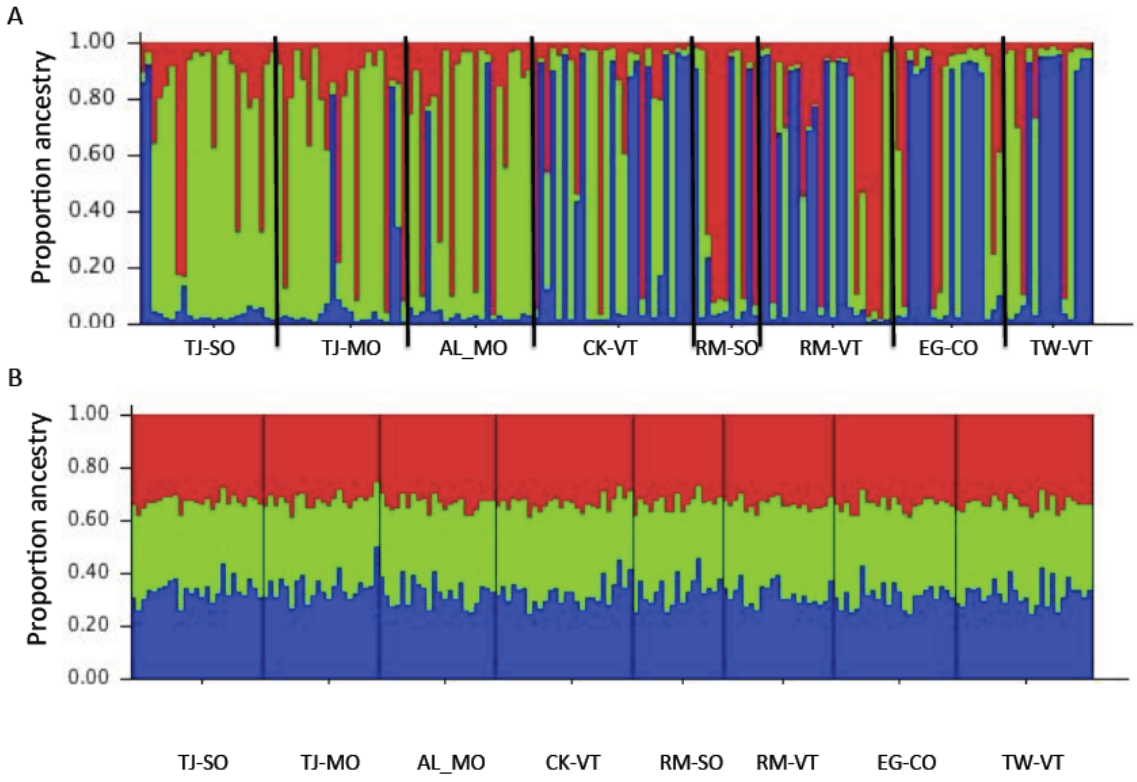

Figure 3.10. STRUCTURE analysis of variation at virulence and microsatellite loci. Estimated proportion of ancestry of each pathogen strain to three different clusters based on A) 28 virulence loci and B) 12 microsatellite loci. Each strain is represented by a vertical bar and the three colors represent the three genetic clusters.

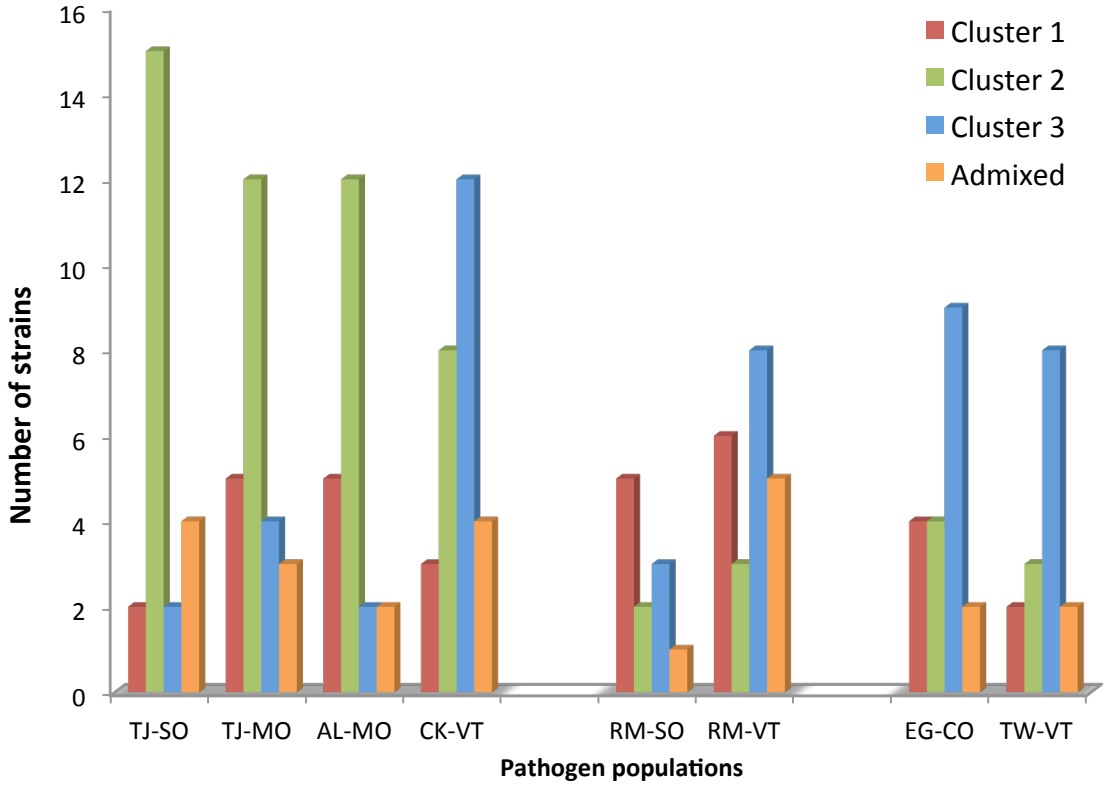

Figure 3.11. Proportion of strains from each population assigned to each of three genetic clusters.

Genetic clusters were determined by STRUCTURE, and the colors of the three clusters correspond to those depicted in Figure 3.**10**A. Strains where less than 70% of the ancestry was assigned to a single cluster are categorized as admixed (orange bars). Pathogen populations grouped by region; north, center and south.

SUPPLEMENTAL TABLES & FIGURES

Table S3.1. Pairwise distance (km) between pathogen collection sites

	CK-VT	TJ-SO	TJ-MO	AL-MO	RM-SO	RM-VT	EG-CO	TW-VT
CK-VT	0							
TJ-SO	83	0						
TJ-MO	84	1.72	0					
AL-MO	96.5	15.2	15.2	0				
RM-SO	436	371	370	353	0			
RM-VT	436	371	370	353	0.06	0		
EG-CO	559	487	486	471	122	122	0	
TW-VT	559	488	487	471	126	126	6.87	0

Table S3.2. Release date, State, and pedigree of eight oat cultivars present in the variety trail collection sites

Cultivar	Release date	State	Parental genotypes
Colt	2009	SD	SD97575 x Morton
Morton*	2001	ND	ND88092 x IAB805-X
Kame	2005	WI	10712 x IAB805-X
Spurs	2003	IL	Jay x Rodeo
Esker	2003	WI	Jim x Gem
Excel	2007	ID	P9741A41-4-6 self
Jerry*	1997	ND	Valley x ND810458
Souris*	2006	ND	ND90141 x ND900118
Ogle**	1983	IL	Brave x 336

*Not present in TW-VT site

** Not present in either site

Table S3.3. Coefficient of coancestry (*f*) based on cultivar pedigrees from POOL (Pedigrees Of Oat Lines).

	Colt	**Morton**	**Kame**	**Spurs**	**Esker**	**Excel**	**Jerry**	**Souris**
Colt	---							
Morton	0.25[po]	---						
Kame	0.0313	0.125[hs]	---					
Spurs	0	0	0.0078	---				
Esker	0	0	0.0156	0.0313	---			
Excel	0	0	0	0.125		---		
Jerry	0	0	0	0	0	0	---	
Souris	0	0	0	0	0	0	0	---
Ogle	0	0	0.0313	0.125	0.0938	0	0	0

[po] parent-offspring
[hs] half-sibs

Table S3. 4. Microsatellite marker accessions and size range

Locus	GenBank Accession	PCR size range (bp)	N_A	Allelic size range
A18	EU056534	233-237	3	4
A26	EU056535	155-162	5	7
A36	EU056537	150-156	3	6
A75	EU056544	178-182	4	4
B78	EU056559	143-151	3	8
C33	EU056564	182-192	2	10
B2	EU056546	233-235	3	2
B15	EU056550	190-194	3	4
B25	EU056553	160-164	5	4
A35	EU056536	196-214	4	18
A59	EU056540	214-249	5	35
B13	EU056548	241-243	2	2
B54	EU056557	142-152	4	10

From Dambroski and Carson (2008)

Table S3. 5. Incidence on specific cultivars within mixture populations

Field	Colt	Esker	Excel	Kame	Morton	Rockford	Souris	Spurs
CK-VT	dead	100%	100%	100%	100%	0%	50%	70%
RM-VT	100%	100%	50%	100%	100%	3%	20%	100%
TW-VT	70%	7%	43%	43%	NA	NA	NA	17%

Disease incidence on seven sampled oat cultivars from three host-mixture populations. The TW population did not contain the Morton, Rockford, or Souris cultivars.

Table S3. 6. Population pairwise R_{ST} determined by the sum of squared size of difference.

	TJ-SO	TJ-MO	AL-MO	CK-VT	RM-SO	RM-VT	EG-CO	TW-VT
TJ-SO	0							
TJ-MO	-0.0183	0						
AL-MO	-0.0136	-0.0184	0					
CK-VT	-0.0104	-0.0104	-0.0152	0				
RM-SO	-0.0095	-0.0095	-0.0046	-0.0103	0			
RM-VT	-0.0096	-0.0096	-0.0106	-0.0163	0.0056	0		
EG-CO	-0.0050	-0.0050	0.0155	0.0147	0.0021	0.0150	0	
TW-VT	0.0018	0.0018	-0.0012	0.0038	0.0055	-0.0018	0.0429*	0

* indicates $p<0.05$. Locus A26 was not included.

Table S3. 7. Composition of pathogen populations in 2010 and 2012 fecundity experiments

	Num. hosts [1]		**Souris** [2]		**Colt** [2]		**Morton** [2]	
	2010	*2012*	*2010*	*2012*	*2010*	*2012*	*2010*	*2012*
Mixtures								
RM-VT	3.95	3.40	0.50	0.40	0.75	0.65	0.75	0.65
TW-VT	3.76	3.40	0.57	0.50	0.76	0.70	0.90	0.85
CK-VT	2.68	2.35	0.36	0.30	0.59	0.50	0.59	0.50
Monocultures								
RM-SO	3.50	---	0.05	---	0.07	---	0.08	---
TJ-SO	3.50	3.28	0.68	0.61	0.86	0.83	0.82	0.78
EG-CO	2.90	2.35	0.45	0.35	0.75	0.65	0.60	0.50
AL-MO	2.39	2.00	0.48	0.40	0.65	0.50	0.61	0.50
TJ-MO	3.05	3.00	0.45	0.44	0.82	0.83	0.91	0.89

[1] Average number of host cultivars infected by experimental pathogen populations in 2010 and 2012.

[2] Proportion of each pathogen population able to infect Souris, Colt, and Morton cultivars.

Table S3.8 (1-8): Pathogen strains included in 2010 and 2012 fecundity experiments

Table S3.8.1 Strains from RM-VT used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
559.2	0	0	0	0	1	0	0	0	1	
534.1	0	0	1	0	1		0	0	2	
550	0	0	1	0	1	0	0	0	2	
565	0	0	1	1	0	0	0	0	2	
534.3	0	0	0	0	0	1	1	1	3	
595	0	0	0	0	0	1	1	1	3	
541	0	0	1	0	0	1	1	1	4	
544	0	1	0	0	0	1	1	1	4	
558	0	0	1	0	0	1	1	1	4	
559.1	0	1	0	0	0	1	1	1	4	
503	0	1	1	0	0	1	1	1	5	
535	1	0	0	0	1	1	1	1	5	
574	1	0	1	0	0	1	1	1	5	
575	0	0	1	0	1	1	1	1	5	
560	1	0	0	1	1	1	1	1	6	
596	1	0	0	1	1	1	1	1	6	
585.2	1	1	0	1	1	1	1	1	7	
594	0	0	0	0	0	1	0	0	1	*
534.2	0	1	0	0	1	1	1	1	5	*
593	0	0	1	1	1		1	1	5	*
536	0	0	0	0	0	0	0	0	0	**
536.2	0	0	0	0	0	0	0	0	0	**
542	0	0	0	0	0	0	0	0	0	**

Infection ability of each strain on eight different oat cultivars. A "0" indicates no infection and a "1" indicates infection. [a] Total number of cultivars infected by each strain. * Strain included in 2010 experiment only. ** Strain included in 2012 experiment. All other strains were included in both experiments.

Table S3.8.2 Strains from TW-VT used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
56	0	0	0	0	0	0	0	0	0	
1	0	1	0	0	0	0	0	1	2	
7	0	0	0	0	0	1	1	1	3	
8	0	0	0	0	1	0	1	1	3	
15	0	0	1	0	0	1		1	3	
41	0	0	0	0	0	1	1	1	3	
14	0	0	0	0	1	1	1	1	4	
21	0	0	0	0	1	1	1	1	4	
24	0	0	0	1	1	1	0	1	4	
25	0	1	0	0	0	1	1	1	4	
30	0	0	0	0	1	1	1	1	4	
46	1	0	0	0	0	1	1	1	4	
47	0	0	0	0	1	1	1	1	4	
53	0	0	1	0	0	1	1	1	4	
1	0	0	0	1	1	1	1	1	5	
18	0	0	1	0	1	1	1	1	5	
12	0	0	1	1	1	1	1	1	6	
27	1	0	0	1	1	1	1	1	6	
22	0	0	0	0	0	1	1	1	3	*
42	1	1	0	0	1	0	0	0	3	*
9	0	0	0	1	1	1	1	1	5	*
10	0	0	0	0	0	0	0	0	0	**
49	0	0	0	0	0	0	0	0	0	**

See table legend on page 145

Table S3.8.3 .Strains from CK-VT used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
604	0	0		0	0	1	0	0	1	
603	0	0	1	1	0	0	0	0	2	
624	0	1	0	1	0	0	0	0	2	
628	0	0	0	0	0	0	1	1	2	
640	0	0	0	0	0	0	1	1	2	
661	0	0	1	0	0	0	1	0	2	
663	0	0	0	1	1	0	0	0	2	
637	0	0	0	0	1	0	1	1	3	
665	0	0	0	0	1	1	0	1	3	
681	0	0	0	1	1	1	0	0	3	
682	0	0		0	0	1	1	1	3	
608	0	0	0	1	0	1	1	1	4	
627	0	0	0	1	0	1	1	1	4	
654	0	0	0	0	1	1	1	1	4	
680	0	0	1	0	0	1	1	1	4	
645	0	1	0	1	1	1	1	1	6	
679	0	0	0	0	0	1	0	0	1	*
606	0	0	0	1	1	0	0	0	2	*
647	0	0	0	0	0	0	1	1	2	*
683	0	0	0	0	0	0	1	1	2	*
695	0	0	0	1	1	0	0	0	2	*
694	0	0		0	0	1	1	1	3	*
1201	0	0	0	0	0	0	0	0	0	**
1203	0	0	0	0	0	0	0	0	0	**
1205.1	0	0	0	0	0	0	0	0	0	**
1205.3	0	0	0	0	0	0	0	0	0	**

See table legend on page 145

Table S3.8.4 .Strains from RM-SO used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
162	0	0	0	0	0	1	0	0	1	*
168.1	0	0	0	0	1	1	0	0	2	*
175.2	0	0	0	0	0	1	0	1	2	*
168.2	0	0	0	0	0	1	1	1	3	*
175.3	0	0	0	0	0	1	1	1	3	*
164.2	0	0	0	0	1	1	1	1	4	*
179.2	0	0	0	1	0	1	1	1	4	*
162.2	0	1	0	0	1	1	1	1	5	*
175.1	0	0	1	0	1	1	1	1	5	*
177	0	0	1	0	1	1	1	1	5	*

See table legend on page 145

Table S3.8.5 .Strains from TJ-SO used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
1052	0	0	0	0	1	0	0	0	1	
1019.1	0	0	0	0	1	0	0	1	2	
1026	0	0	0	0	1	0	1	0	2	
1046	0	0	0	0	0	0	1	1	2	
1048	0	0	0	1	1	0	0	0	2	
1005	0	0	1	0	0	0	1	1	3	
1011.2	0	0	0	1	0	0	1	1	3	
1019.2	0	0	0	1	0	1	1	0	3	
1037	0	0	0	0	0	1	1	1	3	
1053	0	0	0	1	0	0	1	1	3	
1002		0	0	0	1	1	1	1	4	
1002.2	0	0	0	1	0	1	1	1	4	
1011.1	0	0	0	0	1	1	1	1	4	
1013.1	0		0	0	1	1	1	1	4	
1030	0	0	0	0	1	1	1	1	4	
1038	0	0	0	0	1	1	1	1	4	
1036	0	0	1	0	1	1	1	1	5	
1022	0	0	1	1	1	1	1	1	6	
1021	0	0	0	0	1	0	1	1	3	*
1043	0	0	0	0	1	1	1	1	4	*
1015.1	0	0	0	1	1	1	1	1	5	*
1023	0	0	1	1	1	1	1	1	6	*
1051.1	0	0	0	0	1	1	1	1	4	**
1051.2	0	0	0	0	1	1	0	1	3	**

See table legend on page 145

Table S3.4.8.6 Strains from EG-CO used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
135	0	0	0	0	0	0	0	0	0	
139	0	0	0	0	1	0	0	0	1	
117	0	0	0	0	0	1	1	0	2	
118	0	0	0	0	0	1	1	0	2	
134	0	0	0	0	0	0	1	1	2	
151	0	0	0	0	0	0	1	1	2	
152	0	0	0	0	0	1	1	0	2	
110	0	0	0	0	0	1	1	1	3	
120	0	0	0	0	1	1	1	0	3	
150.1	0	0	0	0	1	1	0	1	3	
105	0	0	0	1	0	1	1	1	4	
124.1	0	0	0	0	1	1	1	1	4	
137	0	0	0	0	1	1	1	1	4	
108	0	0	1	1	0	1	1	1	5	
127	0	0	0	1	1	1	1	1	5	
155	0	0	0	1	1	1	1	1	5	
123	0	0	0	0	0	1	0	0	1	*
157	0	0	0	0	0	1	0		1	*
121	0	0	0	0	1	1	1	1	4	*
160.2	0	0	0	1	1	1	1	1	5	*
116	0	0	0	0	0	0	0	0	0	**
124.2	0	0	0	0	0	0	0	0	0	**
140	0	0	0	0	0	0	0	0	0	**
160	0	0	0	0	0	0	0	0	0	**

See table legend on page 145

Table S3.8.7 Strains from AL-MO used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
1140.1	0	0	0	0	0	0	0	0	0	
1152.2	0	0	0	0	0	0	0	0	0	
1104	0	0	0	0	1	0	0	0	1	
1119	0	0	0	0	1	0	0	0	1	
1143.1	0	0	0	0	0	0	0	1	1	
1152.1	0	0	0	0	0	0	1	0	1	
1117	0	0	1	1	0	0	0	0	2	
1120.2	0	0	0	0	1	1	0	0	2	
1125.1	0	0	0	0	0	0	1	1	2	
1136	0	0	0	0	0	0	1	1	2	
1121.1	0	0	0	0	1	1	1	0	3	
1124.1	0	0	0	0	1	0	1	1	3	
1124.2	0	0	0	0	0	1	1	1	3	
1139.1	0	0	0	0	1	0	1	1	3	
1142	0	0	0	0	0	1	1	1	3	
1121.2	0	0	0	1	1	1	0	1	4	
1137	0	0	0	0	1	1	1	1	4	
1135	0	0	1	1	0	1	1	1	5	
1115	0	0	0	0	0	0	1	1	2	*
1146	0	0	0	0	1	0	1	0	2	*
1110	0	0	0	0	0	1	1	1	3	*
1113	0	0	0	0	1	1	1	1	4	*
1143.2	0	0	1	0	1	0	1	1	4	*
1116	0	0	0	0	0	0	0	0	0	**
1140.2	0	0	0	0	0	0	0	0	0	**

See table legend on page 145

Table S3.8.8 .Strains from TJ-MO used in spore production experiment

Strain	Esker	Spurs	Excel	Jerry	Souris	Kame	Colt	Morton	Total	
940.1	0	0	0	0	0	0	0	1	1	
901.2	0	0	0	0	0	0	1	1	2	
907.2	0	0	0	0	0	1	1	0	2	
910.2	0	0	0	0	0	0	1	1	2	
920	0	0	0	0	0	1	1	0	2	
933	0	0	0	0	0	0	1	1	2	
903	0	0	0	0	1	1	0	1	3	
907.1	0	0	0	0	0	1	1	1	3	
921	0	0	0	0	1	1		1	3	
923	0	0	0	0	0	1	1	1	3	
940.2	0	0	0	0	1	0	1	1	3	
947	0	0	0	0	0	1	1	1	3	
905.2	0	0	1	0	1	0	1	1	4	
910.1	0	0	0	1	0	1	1	1	4	
911	0	0	0	0	1	1	1	1	4	
936	0	0	0	0	1	1	1	1	4	
948	0	0	0	0	1	1	1	1	4	
930	0	0	1	1	1	0	1	1	5	
950.1	0	0	0	0	1	0	0	1	2	*
939	0	0	0	0	1	0	1	1	3	*
901.1	0	0	1	0		1	1	1	4	*
916	0	0	0	1	0	1	1	1	4	*
947.1	0	0	0	1	0	1	1	1	4	**
947.2	0	0	0	1	1	1	1	1	5	**

See table legend on page 145

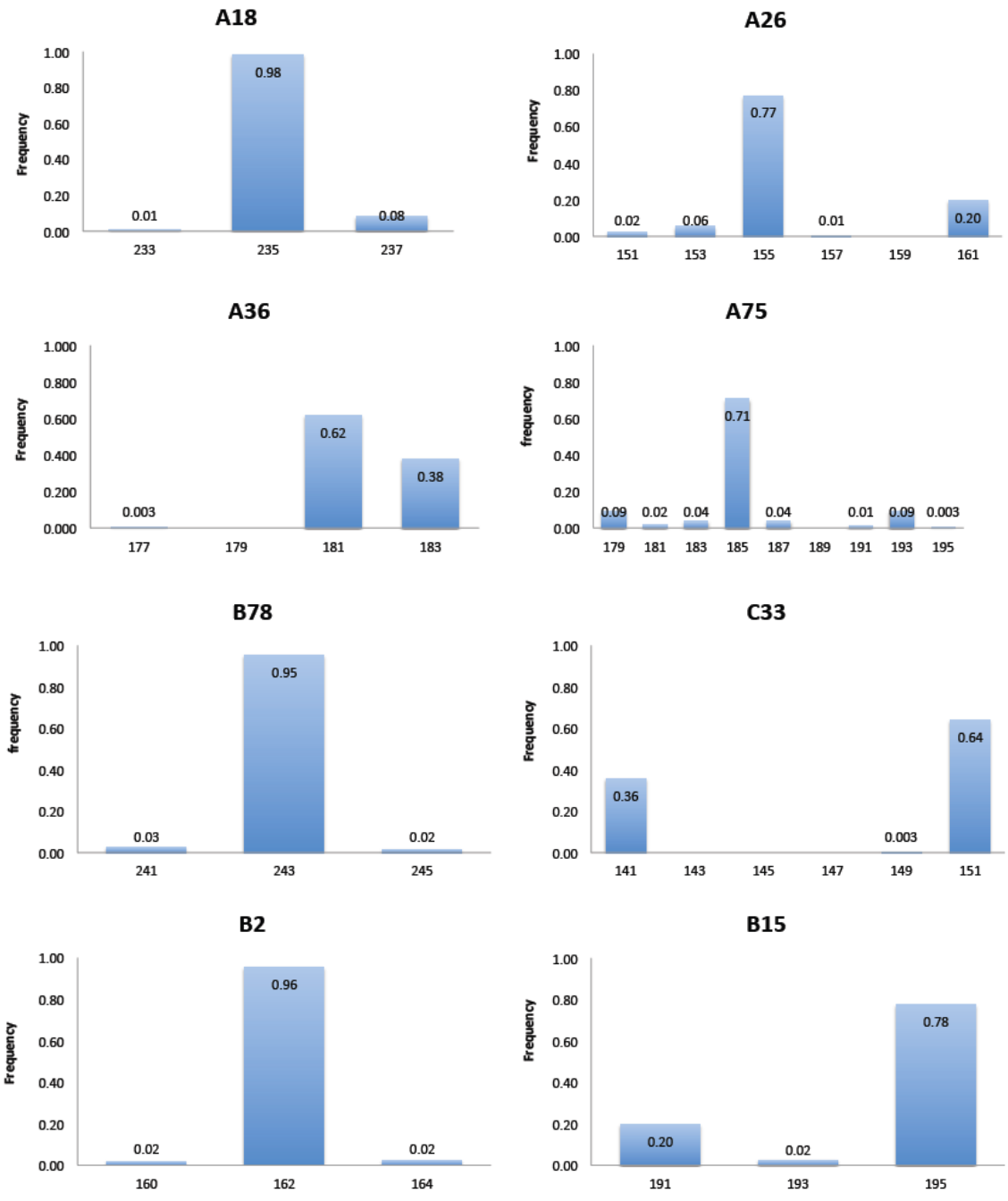

Figure S3. 1. Frequency of alleles at microsatellite loci

Allele sizes are indicated on the x-axis. The figure is continued on the next page.

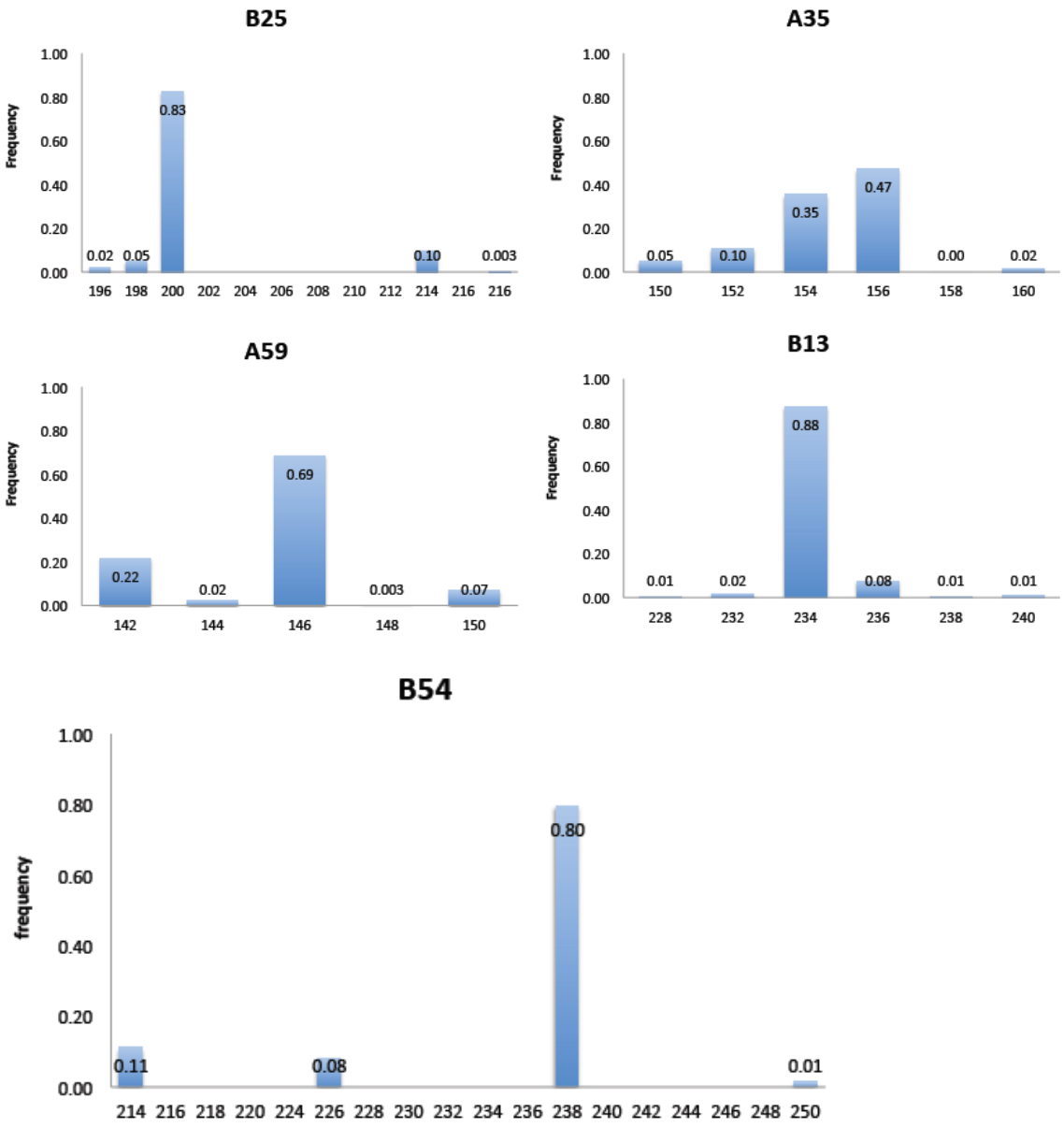

Figure S3. **1** continued.

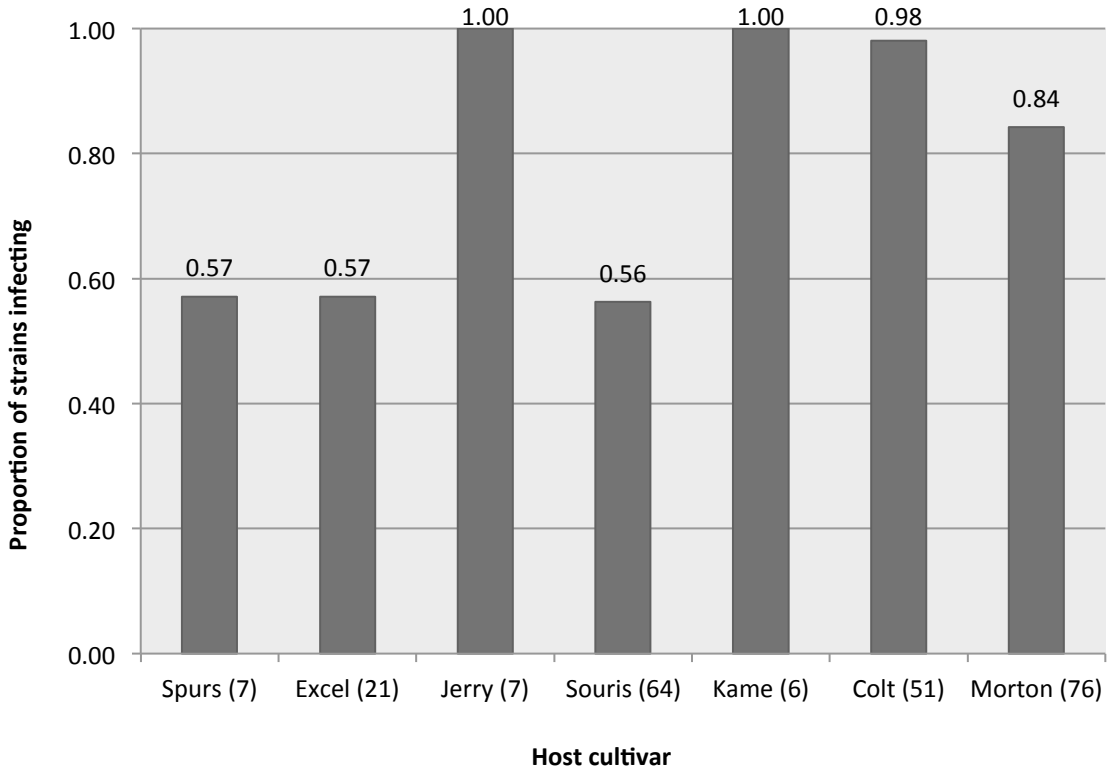

Figure S3. 2. Proportion of strains from each host cultivar that were able to re-infect their home host cultivar.

The total number of strains from each cultivar is given in parentheses. Strains were pooled from across all populations.

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