Maintenance of variation in virulence and reproduction in populations of an agricultural plant pathogen

Anik Dutta[1], Daniel Croll[2], Bruce A. McDonald[1], and Luke G. Barrett[3,*]

[1]Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland

[2]Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

[3]CSIRO Agriculture and Food, Canberra, Australian Capital Territory, Australia

[*]**Corresponding author:** Luke G. Barrett; E-mail: luke.barrett@csiro.au

Abstract

Genetic diversity within pathogen populations is critically important for predicting pathogen evolution, disease outcomes and prevalence. However, we lack a good understanding of the processes maintaining genetic variation and constraints on pathogen life-history evolution. Here, we analyzed interactions between 12 wheat host genotypes and 145 strains of *Zymoseptoria tritici* from five global populations to investigate the evolution and maintenance of variation in pathogen virulence and reproduction. We found a strong positive correlation between virulence and reproduction, with substantial variation in both traits maintained within each pathogen population. On average, highly virulent isolates exhibited higher fecundity, which might increase transmission potential in agricultural fields planted to homogeneous hosts at a high density. We further showed that pathogen strains with a narrow host range (i.e. specialists) for fecundity were on average less virulent, and those with a broader host range (i.e. generalists) for virulence were on average less fecund on a given specific host. These trade-offs costs associated with host specialization might constrain the directional evolution of virulence and fecundity. We conclude that selection favoring pathogen strains that are virulent across diverse hosts, coupled with selection that maximizes fecundity on specific hosts, may explain the maintenance of these pathogenicity traits within and among pathogen populations.

Keywords: *Zymoseptoria tritici*, wheat, virulence, reproduction, trade-off, host specialization.

Introduction

Plant pathogens typically maintain high intraspecies diversity for key pathogenic traits. These include virulence (defined here as damage caused to the host), host range, and reproduction (Lannou, 2012). Genetic variation underlying phenotypic trait variation (and corresponding resistance traits in their hosts) can be an important determinant of disease epidemiology and can have important consequences for pathogen fitness, host mortality and host reproduction. For example, genetic variation within a pathogen species can facilitate rapid adaptation to control strategies such as fungicide applications and deployment of host resistance (McDonald and Linde, 2002; Zhan *et al*., 2005). However, despite the importance of pathogenicity traits for determining disease incidence, prevalence, and severity, we still lack a clear understanding of how they evolve within populations.

Virulence in plant pathogens can be defined as the degree of damage (e.g., necrosis) and the corresponding fitness reduction in the host following a pathogen infection (Sacristan and Garcia-Arenal, 2008; Barrett *et al*., 2009). Virulence is (at least in part) a direct consequence of host exploitation and is therefore expected to have strong links with pathogen growth and the development of transmission stages needed to infect new hosts (i.e. fecundity). Virulence is thus a key component of pathogen life-history, influencing both the incidence and impact of disease. Populations of plant pathogens typically harbor high levels of genetic variation for both virulence and fecundity (Sacristan and Garcia-Arenal, 2008; Barrett *et al*., 2009). Furthermore, several studies have demonstrated variable expression of these traits according to host and pathogen genetic background, their interaction, and environmental effects in different host-pathogen systems (Pagan *et al*., 2007; Salvaudon *et al*., 2007; Lannou, 2012; Tack *et al*., 2012). However, despite the central importance of these traits to pathogenicity, the

processes underlying their evolution and the maintenance of variation within populations are not well understood.

While fecundity is an important life-history trait, pathogen fitness also critically depends upon the transmission of propagules to a new host (Antonovics, 2017). While increased pathogen fecundity can increase potential for transmission, links between virulence, fecundity and transmission are complicated by the fact that transmission also depends on the host. This complexity is captured by the so called 'trade-off' theory, which assumes that virulence is an unavoidable consequence of pathogen reproduction within the host (Lenski and May, 1994; Frank, 1996), and predicts that intermediate levels of virulence will maximize transmission because higher virulence may significantly reduce the life expectancy of the infected host (Anderson and May, 1982; Frank, 1996; Leggett *et al*., 2013). However, the trade-off theory has largely been developed within the context of unmanaged host populations (e.g. humans, wild animals etc.). Here, we examine the evolution of virulence and fecundity within populations of an agricultural plant pathogen. There are some key properties specific to agroecosystems that might influence expectations for the evolution of virulence. Unlike natural systems, hosts are homogenous and planted at a high density (allowing frequent physical contact among plants), properties that potentially select for increasingly high virulence and reproduction (McDonald and Stukenbrock, 2016). In addition, farmers replace these high density, homogenous resources every year, meaning that there are potentially few negative consequences associated with increasing levels of host damage. The evolution of virulence in agroecosystems can be further influenced by the pleiotropic effect of genes affecting fungicide resistance in pathogen populations. Extensive fungicide application can impose strong directional selection for resistance, which can be positively correlated with virulence (Yang *et al*., 2013). Thus, it might be predicted that in agricultural settings, directional selection may

result in uniformly high levels of virulence and fecundity (Walsh and Blows, 2009; Roff, 2012).

Countering this prediction are frequent reports of high levels of pathogenic diversity within populations of agricultural plant pathogens (Burdon *et al*., 2016). Trade-offs between different components of fitness are often invoked to explain the maintenance of trait variation within species (Stearns, 1989; Thrall *et al*., 2005; Héraudet *et al*., 2008). For host-pathogen interactions, one common prediction is that host specialization will result in the evolution of high levels of fecundity on a restricted subset of hosts (Barrett and Heil, 2012). This outcome implies the existence of trade-offs between the capacity to attack multiple hosts and another component of fitness. A broader host range (i.e. generalism) is expected to increase the number of individual hosts available to infect and lower the risk of extinction should any one host become unavailable, whereas specialization on any individual host (i.e. specialism) comes at the expense of reduced performance on other possible hosts, creating an evolutionary constraint (Kassen, 2002). This specialization trade-off is frequently used to explain why pathogenic traits do not become fixed (i.e. the trade-off maintains trait diversity) in pathogen populations (Brown, 2003). Yet, empirical evidence for costs arising from host specialization are limited (Barrett and Heil, 2012).

Z. tritici is the causal agent of septoria tritici blotch, a major fungal disease of wheat (Dean *et al*., 2012; Fones and Gurr, 2015). The pathogen causes necrotic lesions upon infection and produces asexual fruiting bodies called pycnidia within the lesions. It undergoes several cycles of a/sexual reproduction in a growing season (Karisto *et al*., 2018). Lesion development and pycnidia formation were shown to be two different traits, which can vary according to the particular genotypes involved in each host-pathogen interaction (Karisto *et al*., 2018). In this

study, we used lesion development and pycnidia formation within lesions as proxies for pathogen virulence and reproduction, respectively. We used a set of 12 wheat host cultivars and 145 *Zymoseptoria tritici* strains to address the following questions: To what extent do key pathogenic life-history traits vary within and among populations of an agricultural plant pathogen? How do pathogen virulence and reproductive traits correlate? What is the impact of spatial structure (i.e. hosts and pathogen populations) on variation in virulence and reproduction? Is there any evidence for a trade-off between specialist and generalist strategies, and if so, what other traits are involved?

Materials and Methods

Fungal material

Five genetically different pathogen populations comprising 145 fully sequenced isolates of *Z. tritici* were used in this study. These field populations originated from single wheat fields located in four countries around the world (Zhan *et al*., 2005). The field populations Australia (*n=27*), Switzerland (*n=32*), Israel (*n=30*), and USA (Oregon.R, *n=26*; Oregon.S, *n=30*) were collected in 2001, 1999, 1991 and 1990, respectively. The two populations from Oregon were collected on the same day from two different wheat cultivars, Madsen (Oregon.R) and Stephens (Oregon.S), growing in the same field. All the other populations were sampled from single cultivars. The cultivars Madsen and Stephens were partially resistant and highly susceptible to STB, respectively (Cowger *et al*., 2000). The absence of clones among these isolates and the general absence of pathogen clones within and among populations beyond spatial scales of 1 m was confirmed by previous studies (Linde *et al*., 2002; Zhan *et al*., 2005). After collection, several copies of each isolate were stored and maintained in anhydrous silica gel or 50% glycerol at -80°C.

Plant material

148 A set of 12 different wheat hosts were used in this study. This host panel included five landraces
149 (Chinese Spring, 1011, 1204, 4391 and 5254), six commercial varieties (Drifter, Gene, Greina,
150 Runal, Titlis, Toronit) and a back-cross line (ArinaLr34). The 1011, 1204, 4391, and 5254
151 landraces were selected from a collection of 199 Swiss wheat landraces from the Swiss national
152 gene bank (www.bdn.ch). This panel was screened for resistance to STB (unpublished results)
153 using four fully sequenced *Z. tritici* isolates, namely 3D1, 3D7, 1E4 and 1A5 (Lendenmann *et*
154 *al*., 2014; Croll *et al*., 2013). The landraces 1011 and 4391 were highly resistant and
155 susceptible, respectively, to all four isolates. The landraces 1204 and 5254 were moderately
156 susceptible to the four isolates. The remaining hosts have diverse genetic backgrounds and
157 were previously used to identify infectivity (i.e. a/virulence) factors in *Z. tritici* (Hartmann *et*
158 *al*., 2017; Zhong *et al*., 2017; Meile *et al*., 2018; Stewart *et al*., 2018). The seeds of Gene and
159 ArinaLr34 were provided by Christopher Mundt (Oregon State University) and Simon
160 Krattinger (KAUST), respectively. The seeds of other hosts were obtained from DSP Ltd.
161 (Delley, Switzerland).
162
163 *Preparation of fungal inoculum*
164 The isolates were regenerated from long term glycerol storage by adding 40µl of concentrated
165 spore suspension into 100 ml Erlenmeyer flasks containing 50ml of yeast sucrose broth (YSB,
166 10 g/L sucrose, 10 g/L yeast extract amended with 50 µg/ml kanamycin sulfate to control other
167 microbial growth). The flasks were kept at 18°C on a continuous shaker at 120 rpm to produce
168 blastospores. Blastospores were harvested after 4-7 days of growth by filtering the liquid media
169 through two layers of sterile cheesecloth. Blastospore pellets were collected by centrifugation
170 (1575 g, 15 minutes, 4°C), washed with sterile water to eliminate any residual growth media
171 and re-suspended in sterile water for subsequent procedures. The spores were counted and
172 adjusted to a final concentration of $5\times10_6$ spores/ml using KOVA counting slides (Hycor

173 Biomedical, Inc., Garden Grove, CA, USA). The spore suspension of each isolate was stored
174 at -20°C until inoculation for between 1-21 days.
175
176 *Phenotyping and data collection*
177 Due to greenhouse space limitations, the whole experiment was divided into two phases
178 consisting of a combination of 6 hosts × 145 isolates in each phase. Three seeds of each host
179 were sown in an individual square pot filled with peat substrate Jiffy GO PP7 (Jiffy Products
180 International, Moerdijk, the Netherlands). Six pots were placed on a tray in a 2×3 array. All the
181 trays were kept in a greenhouse at 22°C (day) and 18°C (night) with 70% relative humidity
182 (RH) and 16-h photoperiod. Plants were inoculated after developing a fully expanded second
183 leaf, at 14 days after sowing. The third leaf and subsequent leaves were trimmed before
184 inoculation and new leaves were trimmed until data collection to facilitate a more uniform
185 distribution of spores and better light penetration onto the inoculated leaf. Before the
186 inoculation day, the spore suspension of each isolate was thawed on ice and kept at 4°C
187 overnight. The final volume of each spore suspension used for inoculation was adjusted to 20
188 ml by adding sterile water supplemented with 0.1% of the Tween 20 surfactant. Each tray
189 containing six hosts was inoculated with an airbrush spray gun (1A Profi Handels GmbH,
190 Wiesbaden, Germany) uniformly until run-off. The spraying was done in a confined area to
191 minimize any chance of cross-contamination. The trays were covered with plastic bags after
192 inoculation to provide 100% RH and transferred into a greenhouse chamber. The entire
193 procedure was repeated three times over three consecutive weeks to generate three independent
194 biological replicates for both phases of the experiment. Spraying of all 145 isolates was
195 performed on a single day. For the landraces 1011, 5254 and 1204, 4391, only one and two
196 plants, respectively, were inoculated in each replicate due to limited seed availability.
197

198 Plastic bags were removed three days post-inoculation (dpi). Environmental conditions
199 otherwise remained identical. To facilitate comparisons among hosts, leaves were harvested
200 between 19-26 dpi because different hosts developed symptoms at different rates. All leaves
201 from each host×isolate combination were collected on the same day. Each second leaf was
202 excised and mounted on A4 paper for scanning as described previously (Karisto *et al*., 2018).
203 Each A4 sheet containing eight inoculated leaves was scanned using a flatbed scanner
204 (CanoScan LiDE 220) for automated image analysis (AIA; Karisto *et al*., 2018). The AIA
205 provided quantitative data on the amount of lesion area caused by the fungus and pycnidia
206 density within the lesions on each leaf. As previously described in Karisto *et al*., (2018), lesion
207 area corresponds to host damage and was used as a proxy for virulence, whereas pycnidia
208 density is a direct measure of pathogen asexual reproduction as pycnidia produce the spores
209 that are eventually transmitted through rain splash or direct contact to neighboring plants.
210
211 *Data analysis*
212 Before analysis, all phenotypic values were log-transformed to fulfill the normality
213 assumptions of ANOVA based on the residual distribution. The log-transformed values were
214 used for all analyses. A combined analysis of variance (ANOVA) was performed to estimate
215 the effect of different factors on the two traits. The following linear model was implemented
216 using the *lm()* function from the package lme4 (Bates *et al*., 2014) in R (R core team, 2019):
217 *Virulence/Reproduction~Host+Population+Isolate:Population+Host:Population+*
218 *Host:Isolate:Population+Replication+Error*
219 All factors were treated as fixed effects and isolates were nested within each population. The
220 least-square mean (LSmean) for each host×isolate combination was extracted by using the
221 function “emmeans” from the package emmeans (Lenth, 2018). Using the LSmean from each
222 host, we computed the global mean for each isolate for virulence and reproduction. Posthoc

multiple comparisons of LSmeans of the host and pathogen population interactions were performed by using Tukey's HSD test at the α=0.05 significance level.

We used the coefficient of variation (CV = standard deviation/mean) of LSmeans for each isolate among 12 hosts as a metric for host specificity (Poissot *et al*., 2012; Caseys *et al*., 2019). Several methods including CV have been proposed for measuring specificity at both individual and species levels (Bolnic *et al*., 2002; Poissot *et al*., 2012). However, each of these has limitations depending on the type of data. While the majority of the specificity metrics assume discrete data, very few are available for continuous data. In our dataset, the use of host resources was more evenly distributed. The majority of isolates readily infected the majority of susceptible hosts, while the degree of virulence and the amount of reproduction varied among the majority of them. Hence we favored the use of CV, which facilitates estimation of the degree of specialism and generalism among the isolates using precise quantitative phenotypic data.

To test for trade-offs, we performed Pearson's correlation analysis between virulence and reproduction, using the overall mean across 12 hosts. Furthermore, we tested the shape of the correlation curve by fitting a polynomial model *(Reproduction~poly (Virulence, 2))*. We were interested in determining how the specificity of each isolate affects its mean virulence and reproduction. Therefore, we performed correlations between mean virulence and the specificity of reproduction as well as between mean reproduction and the specificity of virulence. All analyses were performed in R-studio.

Results

Determinants of quantitative variation in pathogen virulence and reproduction

We obtained quantitative data from 11'019 inoculated leaves generated from the interactions between 12 hosts and 145 pathogen strains using AIA to investigate how variation in pathogen life-history traits are maintained. Lesion area and pycnidia density within the lesion area were used as proxies of virulence and reproduction, respectively. Isolates displayed a wide range of phenotypic responses, exhibiting a quantitative distribution in virulence and reproduction on each host (Supplemental figs. 1A and 1B). Using the non-transformed data, virulence ranged from 0 to 100% with an overall mean of 61.4 ± 0.4%. The host 1011 showed the highest resistance on average, followed by Gene and Toronit (Supplemental Table 1). Mean reproduction across 12 hosts ranged from 0 to 671 pycnidia per cm^2 of lesion with an overall mean of 17.8 ± 0.4. The isolates were on average highly virulent and fecund on the most susceptible host 4391.

We applied a linear model to test which factors contributed to the observed quantitative variation in virulence and reproduction. There was significant variation ($P < 2e\text{-}16$) among the hosts, populations, and isolates for both the traits (Table 1). Host identity strongly influenced the observed variation in reproduction ($F = 835.53$, $P < 2e\text{-}16$) and virulence ($F = 232.47$, $P < 2e\text{-}16$). We obtained a highly significant interaction effect between hosts and isolates nested within each population for virulence ($F = 2.11$, $P < 2e\text{-}16$) and reproduction ($F = 2.68$, $P < 2e\text{-}16$). This significant interaction effect indicates the occurrence of host specificity among the isolates for both traits. Multiple comparisons among the populations on each host revealed high variability and changes in ranking among the populations for both traits (Figs. 1A and 1B). The two populations from Oregon had the highest overall mean virulence. The Israeli population exhibited the highest reproduction, followed by Oregon.R and Switzerland.

Table 1. Analysis of Variance (ANOVA) showing the effects of hosts, populations, isolates and the respective interactions on virulence (amount of necrotic lesion area) and reproduction (pycnidia density within lesions) among 145 *Zymoseptoria tritici* isolates from five populations across 12 hosts.

Sources of variation		Virulence			Reproduction		
	DF	SS	F-value	P-value	SS	F-value	P-value
Population	4	77.36	105.42	<2e-16***	98.19	168.16	<2e-16***
Population:Isolate	140	329.50	12.83	<2e-16***	275.80	13.49	<2e-16***
Host	11	469.08	232.47	<2e-16***	1341.63	835.53	<2e-16***
Host:Population	44	171.96	21.30	<2e-16***	387.13	60.27	<2e-16***
Host:Population:Isolate	1540	596.08	2.11	<2e-16***	603.40	2.68	<2e-16***
Replication	2	8.07	22.00	6.7e-16***	4.16	14.23	6.7e-16***
Error	9277	1701.74			1354.21		

*** indicates significance level at 0.01%; DF= degrees of freedom; SS=Sum of square

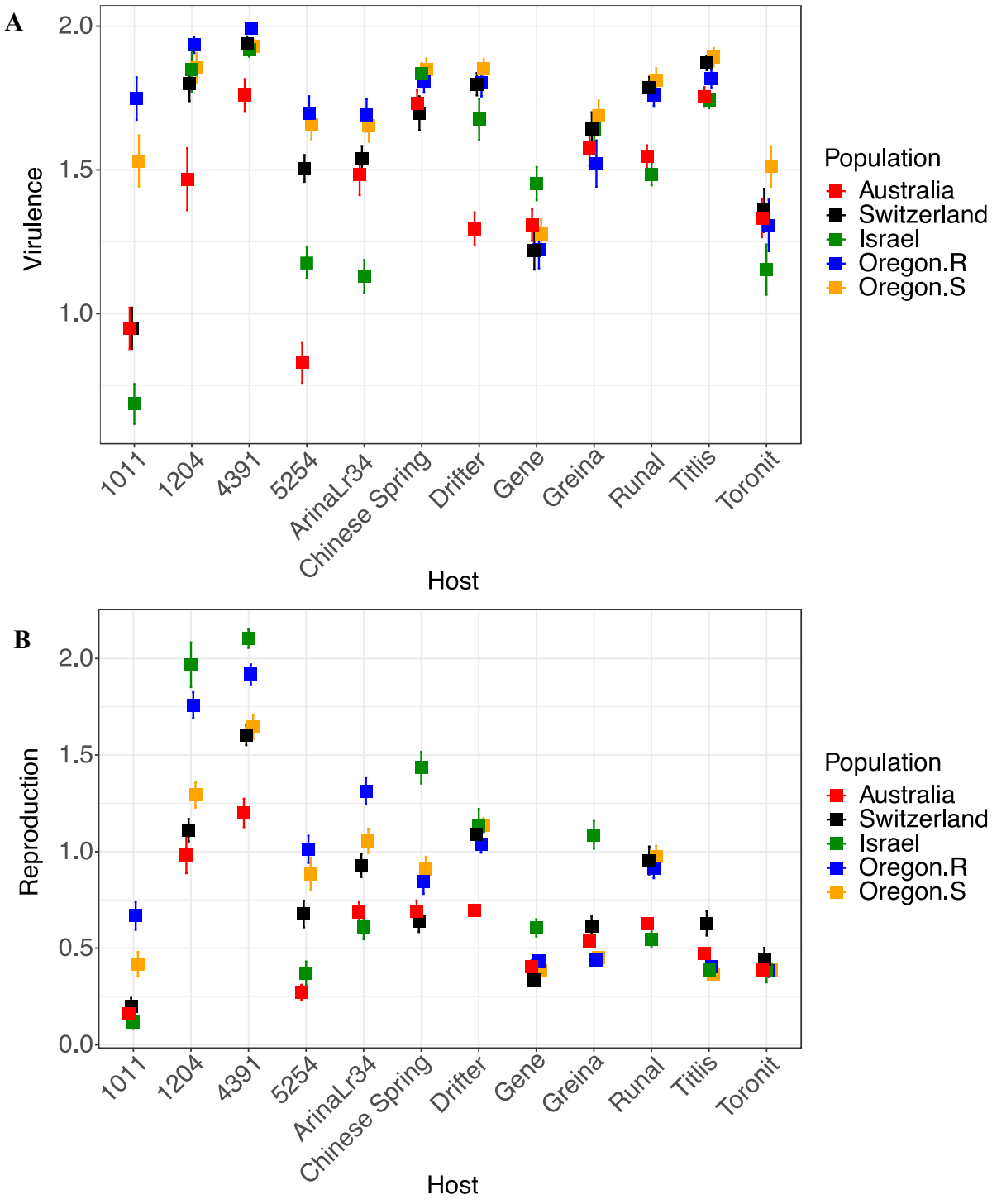


Figure 1. Multiple comparisons for (A) virulence (amount of necrotic lesion area) and (B) reproduction (pycnidia density within lesions) among the five *Zymoseptoria tritici* populations on 12 wheat hosts. Data were log-transformed.

297 *Absence of a trade-off between pathogen virulence and reproduction*

298 We performed Pearson's correlation between virulence and reproduction to determine whether
299 or not there is a trade-off between these traits. The overall mean values of each isolate across
300 12 hosts were used in this analysis. Overall, we detected a significant positive correlation (r =
301 0.62, $P < 2.2e\text{-}16$; Fig. 2A) between the two traits. This indicates that highly virulent isolates
302 also had high levels of fecundity. However, the polynomial regression did not show evidence
303 for any saturating point (results not shown) on the curve, indicating an increasing trend for both
304 traits. The positive correlation was consistent within each population, although the strength of
305 the correlation within each population varied considerably, with the ISR population showing
306 the highest correlation coefficient (r = 0.49 to 0.85, P = 0.0045 to 3.5e-09; Fig. 2B). The
307 variation in the correlation indicated that individual isolates might differ in their strategy to
308 exploit certain hosts.
309

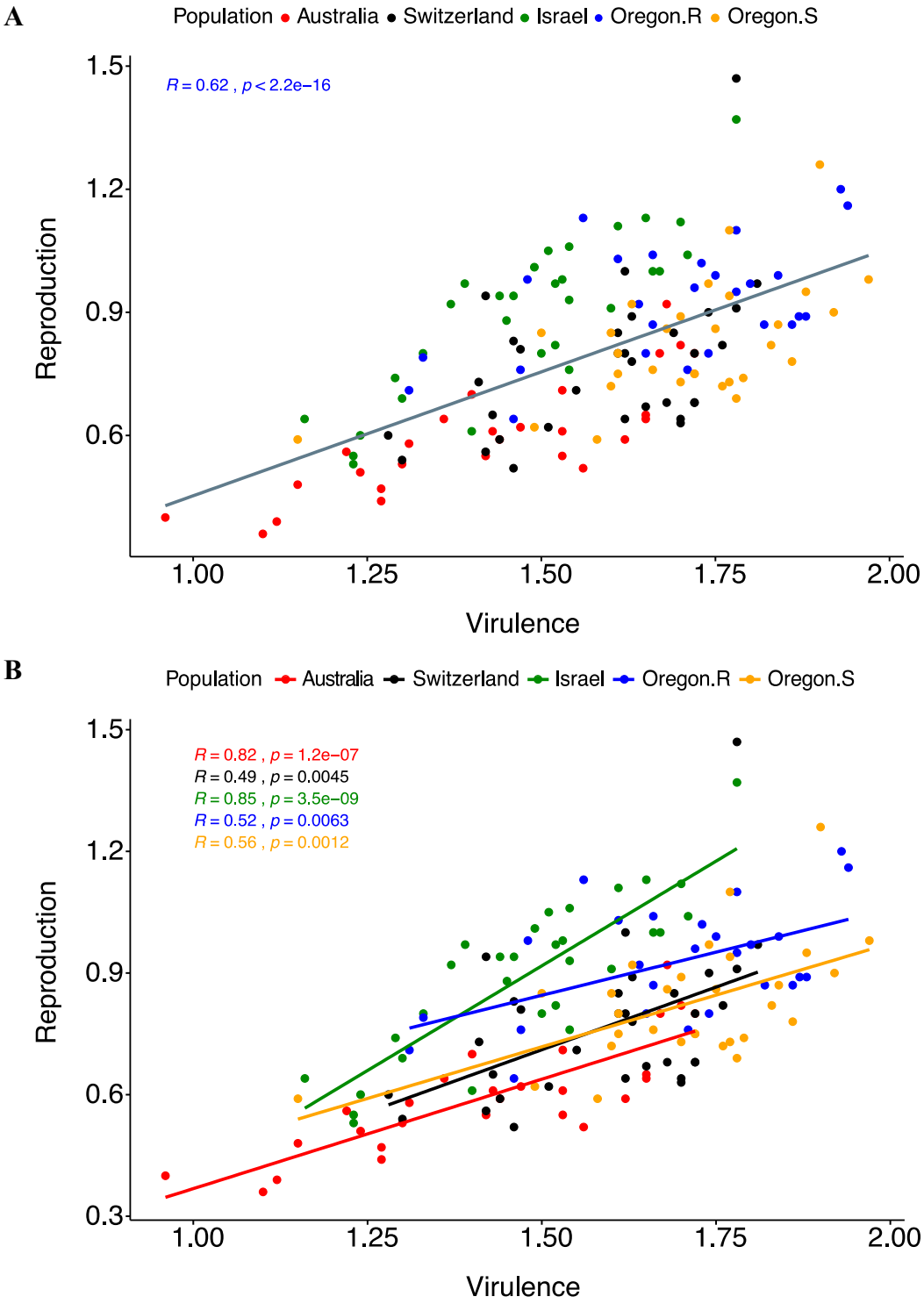


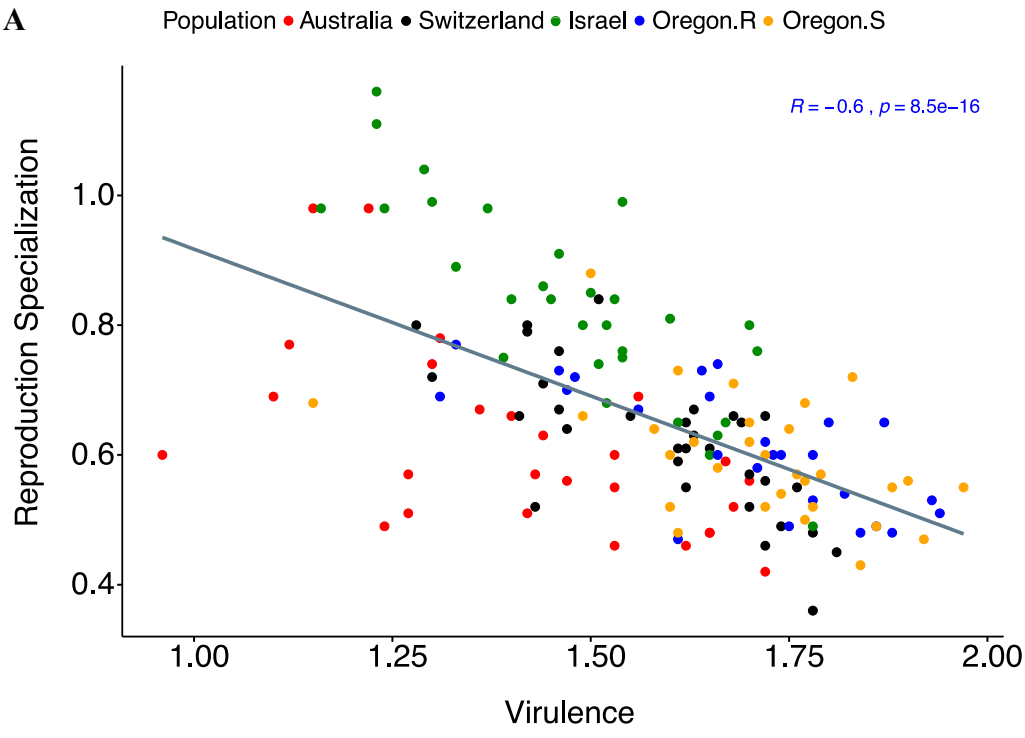
Figure 2. Correlation between virulence (amount of necrotic lesion area) and reproduction (pycnidia density within lesions; A) overall and (B) within each population, among 145 *Zymoseptoria tritici* isolates from five populations. Each point represents the overall.

318 *Host specialization reduces the mean trait performance*

319 We investigated the influence of host specialization on the overall virulence and reproduction
320 and the maintenance of genetic diversity in the field. Using CV as a specificity metric, we
321 detected a significant negative correlation (r = -0.60, P = 8.5e-16, Fig. 3A) between
322 specialization for reproduction and mean virulence. This pattern indicates that isolates that are
323 generalists for reproduction have higher mean virulence across the 12 hosts. We also found a
324 significant negative correlation (r = -0.42, P = 1.3e-07, Fig. 3B) between specialization for
325 virulence and mean reproduction, indicating that isolates that exhibit host specialization for
326 virulence have an overall lower rate of reproduction across the 12 hosts. It is evident that most
327 of the isolates from the populations in Oregon and Israel are generalists or specialists,
328 respectively, for both traits (Figs. 3A & 3B). Importantly, the positive correlation (r = 0.46, P
329 = 6.8e-09, Fig. 4) between specialization for reproduction and maximum reproduction indicate
330 that there is a benefit associated with being a specialist.

331

332



333

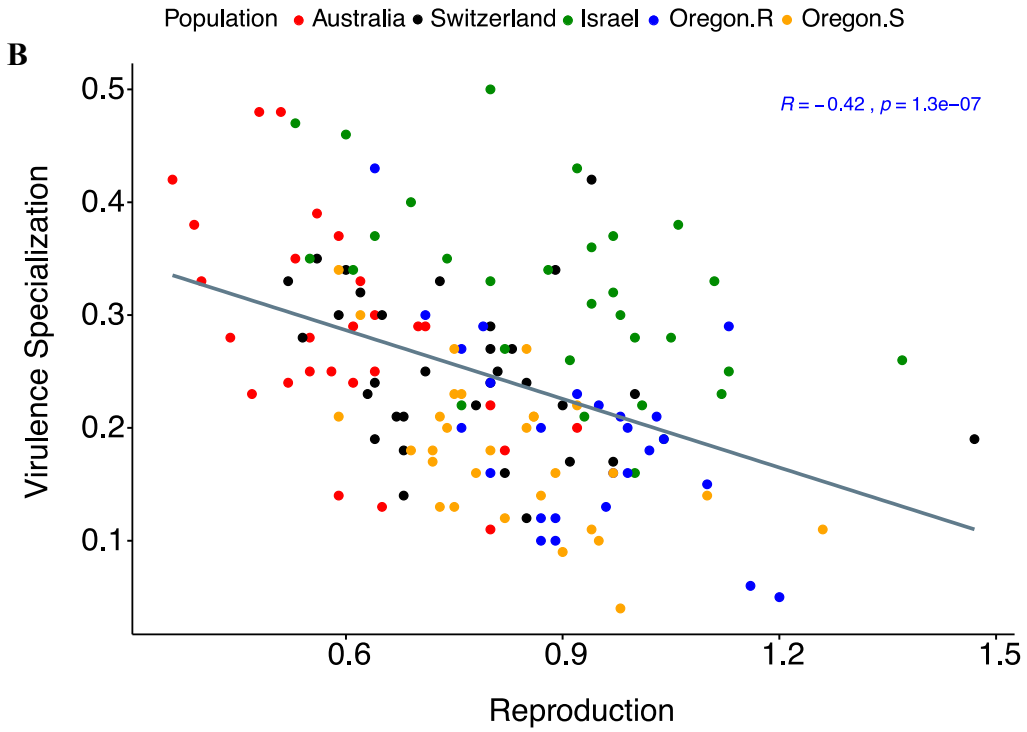


Figure 3. Correlation between (A) overall mean virulence (amount of necrotic lesion area) and reproduction specialization, (B) overall reproduction (pycnidia density within lesions) and virulence specialization among 145 *Zymoseptoria tritici* isolates from five populations. Specialization represents the estimates of coefficient of variation of means across 12 hosts for each trait. Higher specialization indicates preference for specific hosts to maximize trait performance. Data were log-transformed.

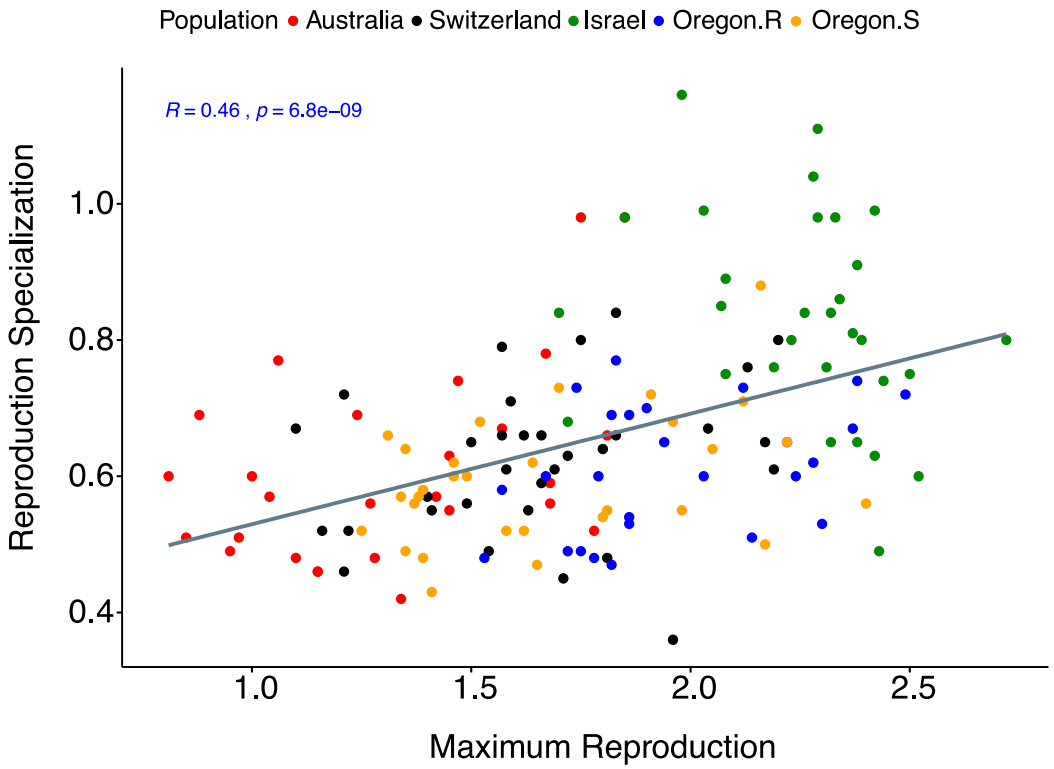


Figure 4. Correlation between maximum reproduction (maximum pycnidia density within the lesion area produced by each isolate across 12 hosts) and reproduction specialization (measured as the coefficient of variation of means for pycnidia density within lesions across 12 hosts) among 145 *Zymoseptoria tritici* isolates from five populations. Data were log-transformed.

Discussion

Understanding the processes that maintain the variation in pathogenicity is of central importance to predict pathogen evolution and understand disease dynamics (Burdon and Thrall, 2008). Using a panel of 12 diverse hosts and 145 pathogen strains, we demonstrated that individual estimates of virulence and reproduction varied quantitatively, and that host genotype and interactions with pathogen genotype are major contributors to the observed variation in virulence and reproduction. We observed a strong positive correlation between the two traits with substantial variation within each population. Furthermore, the data show a continuum of host specialization among *Z. tritici* isolates, where isolates ranged between high and low fecundity depending on the specific host infected. This may constrain directional evolution of both virulence and fecundity and contribute to the maintenance of trait variation.

Host diversity as a key determinant of pathogenicity trait variation

Infection outcomes in many pathosystems are determined by genetic interactions among hosts and pathogens (e.g. Pagan *et al*., 2007; Pariaud *et al*., 2009; Lannou, 2012; Tack *et al*., 2012). Earlier models of pathogen evolution often assumed that variation in infectivity is solely controlled by pathogen strains (Restif and Koella, 2003). Here, in addition to the significant host-isolate interactions, the effect of host cultivar on reproduction was almost 3-fold higher than that observed for virulence, indicating that host genetic background strongly influences resource allocation for reproduction following pathogen colonization (Vale *et al*., 2011; Attisano *et al*., 2012). This result is consistent with previous studies, which show that even after successful infection, host immune response, nutrient availability, and quantitative resistance may hamper pathogen reproduction (Karisto *et al*., 2018). The high level of dependency on host genotype for reproductive fitness is consistent with results demonstrating that different isolates maximize reproductive fitness on specific hosts and that host specific

patterns of fecundity are potentially important for the maintenance of pathogen diversity (see discussion below).

Evolution of pathogen virulence and fecundity in agroecosystems

Here we show that highly virulent isolates of *Z. tritici* on average exhibit greater reproductive potential. This likely reflects general links between pathogen induced necrosis and nutrient release in this system (Zhan *et al*., 2005; Kelm *et al*., 2012). We did not detect any evidence for a saturation point on the correlation curve generated from our dataset, suggesting that both traits follow a continuous range. On a susceptible host, high levels of fecundity and virulence are likely to be advantageous because highly virulent isolates can outcompete co-infecting strains (Zhan *et al*., 2015; McDonald and Stukenbrock, 2016). However, despite this seeming advantage, highly virulent and fecund isolates did not dominate any population. Rather, we observed that considerable variation for both virulence and fecundity is maintained within field populations.

Trade-offs between transmission and virulence have been predicted to contribute to the maintenance of variation for virulence. This reflects the assumption that increased virulence decreases the longevity of the infected host so that the transmission period decreases, creating a trade-off (Ebert and Bull, 2003; de Roode *et al*., 2008). In this agricultural ecosystem (characterized by high density monocropping with relatively homogeneous hosts), higher reproduction (and higher virulence) may be predicted to increase transmission potential as the reproductive units (pycnidia) contain spores that are dispersed by rain-splash throughout the field, and hosts are replaced by farmers each growing season. However, it should be noted that although fecundity can influence transmission, they are not synonymous. Under natural field conditions, transmission depends on other factors such as the quantity, viability, and infection

efficiency of spores, as well as climatic conditions and, most importantly, the availability of a susceptible host. How these traits and outcomes interact to determine virulence evolution is not well understood.

We found considerable variation among populations in overall levels of virulence and reproduction on a diverse set of hosts. We also found a strong correlation between these traits within populations. These results suggest that selection for these traits can differ among populations. One possible explanation for these findings is that the differential patterns of virulence and reproduction reflect specific patterns of adaptation to the most common host genotypes planted in the different wheat fields from which these pathogens were sampled. However, the Swiss population displayed relatively low levels of virulence and fecundity, which was surprising considering that the bulk of the tested hosts originated from Swiss breeding programs. It is possible that the intensive breeding for STB resistance and frequent introductions of new varieties from neighboring countries (Fossati and Brabant, 2003; Brabant *et al*., 2006) in Switzerland may have hampered selection for higher trait performance and adaptation to a specific host. Thus, imposing diversifying selection on local pathogen populations by recurrent changing of wheat cultivars and specific resistance traits might be a useful strategy to disrupt evolution towards increased virulence and fecundity (Burdon *et al*., 2014). Furthermore, in addition to quantitative resistance to *Z. tritici* (Yates *et al*., 2019), wheat varieties across the world often share common major resistance genes (Brown *et al*., 2015), which can be overcome by convergent evolution generating similar virulent mutations independently in different geographical populations, even in the absence of gene flow among populations (Croll and McDonald, 2017). These processes could explain the observed higher performance of the Israeli and Oregon populations on Swiss hosts.

Role of host specialization in the evolution of pathogenicity trait variation

Does host specialization limit the emergence of "super-pathogen" strains combining higher virulence, higher fecundity, and a broader host range? Here, all the hosts exhibited a wide spectrum of resistance and susceptibility towards pathogen virulence and reproduction. For example, the wheat genotypes 1011, Gene and Toronit showed greater resistance to virulence and suppressed reproduction of some isolates, while other wheat genotypes were moderately to highly susceptible to virulence and enabled higher reproduction of some isolates. Therefore, we assessed patterns of host specificity by examining patterns of quantitative variation for virulence and reproduction among the many host-isolate interactions in our experiment. We found significant negative correlations between overall virulence and reproduction specificity, indicating that isolates with high host specificity for reproduction (i.e. higher pycnidia production on some hosts and a smaller number of pycnidia on other hosts) were on average less virulent across all hosts (i.e. produced a lower average lesion area). This observation is consistent with the hypothesis that increased specificity for fecundity results in decreased virulence (Kirchner and Roy, 2002). This pattern further indicates a cost associated with host specialization, which is suggested to result from antagonistic pleiotropy or non-overlapping loci controlling trait performance on different hosts (Kawecki, 1994; Legros and Koella, 2010; Hartmann *et al*., 2017).

The observed positive relationship between specialization for reproduction and maximum reproduction suggests that isolates following a generalist strategy fail to maximize their reproductive fitness on a specific host, thus invoking the principle of "jack of all trades-master of none" (Remold, 2012). This trade-off could explain why specialist isolates are maintained within populations and why 'super-pathogen' strains do not dominate (Kassen, 2002). This is consistent with the results reported by Thrall and Burdon (2003), where generalist isolates with

458 a broader host range had lower overall fecundity. Such specificity for reproduction may be
459 advantageous under scenarios involving host heterogeneity, competition during multiple
460 infection, and disruptive selection (Jaenike, 1990; Barrett *et al*., 2009).
461
462 The precise scenario(s) generating heterogeneity, disruptive selection and promoting the
463 maintenance of variation for host specialization within and among populations remain
464 unknown. Specialists could outperform generalists on a given host (Garamszegi, 2006; Romero
465 and Elena, 2008) because generalists suffer from unequal selection pressure imposed by
466 different hosts (González *et al*., 2019). For example, in a wheat field planted to a susceptible
467 cultivar (typically grown in a monoculture), being highly specialized in reproduction could be
468 beneficial because competition among isolates can favour selection for higher reproduction
469 instead of broader host range. In contrast, strains with high average levels of fecundity across
470 different host genotypes may be expected to have an advantage in environments with higher
471 levels of host genetic heterogeneity (May and Anderson, 1983; Frank, 1996). For example, in
472 areas where multiple cultivars are planted in relatively close proximity, generalist pathotypes
473 may have an advantage. Indeed, the maintenance of variation may reflect conflicts between
474 selection for fecundity and transmission within fields planted to a single susceptible cultivar
475 and selection for ability to infect multiple cultivars planted across an agricultural landscape. In
476 our case, whether the diversity of wheat host genotypes present within agricultural landscapes
477 is alone sufficient to explain the maintenance of generalist pathotypes given the presumed
478 advantage to specialists remains an interesting but open question.
479
480 **Conclusion**
481 Here, we report how different processes regulate life-history trait variation, which ultimately
482 improves our understanding of pathogen evolution and disease dynamics. Host diversity and

differential quantitative interactions with pathogen strains are likely key determinants of variation in virulence and reproduction. Trade-offs for reproduction encountered by specialist vs generalist isolates reinforce the general importance of costs in maintaining pathogenicity trait variation. These mechanisms prevent the fixation of super-pathogen strains in pathogen populations while indicating that diversifying the host in agricultural fields might be a useful strategy to decelerate virulence evolution. Many approaches can be used to introduce dynamic diversity into agricultural ecosystems and reduce selection for increased virulence (McDonald, 2014). A low tech approach that was shown in independent, replicated, field experiments to impose diversifying selection on populations of three cereal pathogens is growing cultivar mixtures (Zhan and McDonald, 2013). Implementing such dynamic diversity at the field scale may impose such trade-offs on generalist isolates that lowers the ability to maximize reproduction on any particular host (McDonald, 2014). We conclude that context-dependent selective forces operating on pathogen populations located in different geographic locations will likely play an ongoing role in contributing to the maintenance of variation in virulence and reproduction in this and other pathosystems. While increased reproduction is likely to provide more opportunities for transmission and virulence evolution in the environments that typify much of modern agriculture, we still lack a deeper understanding of the impact of other life-history traits that have an impact on epidemiology and disease dynamics, such as latent period, spore quantity and infection efficiency.

Data availability

Raw data used for this study are available at: https://doi.org/10.5061/dryad.j3tx95x9m.

Author contributions

LGB conceived the idea of the research. AD conducted the experiment, collected, and analyzed the phenotypic data, wrote the manuscript with LGB. BAM and DC provided funding and corrected the manuscript. All authors approved the final version of the manuscript.

Acknowledgments

This work was supported by the Swiss Federal Office for Agriculture (BLW) in the framework of NAP-PGREL (national plan of action for the conservation and sustainable utilization of plant genetic resources) Project Nr. 627000640. Peter Thrall read an earlier version and made useful comments and suggestions that improved the manuscript.

538 **References**

539 Anderson, R. M., & May, R. M. (1982). Coevolution of hosts and parasites. *Parasitology*,
540 *85*(2), 411-426.

541 Antonovics, J. (2017). Transmission dynamics: critical questions and challenges. Philosophical
542 Transactions of the Royal Society B: Biological Sciences, 372(1719), 20160087.

543 Attisano, A., Moore, A. J., & Moore, P. J. (2012). Reproduction-longevity trade-offs reflect
544 diet, not adaptation. *Journal of Evolutionary Biology*, *25*(5), 873-880.

545 Barrett, L. G., & Heil, M. (2012). Unifying concepts and mechanisms in the specificity of
546 plant–enemy interactions. *Trends in plant science*, *17*(5), 282-292.

547 Barrett, L. G., Kniskern, J. M., Bodenhausen, N., Zhang, W., & Bergelson, J. (2009). Continua
548 of specificity and virulence in plant host–pathogen interactions: causes and
549 consequences. *New Phytologist*, *183*(3), 513-529.

550 Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). lme4: Linear mixed-effects models
551 using Eigen and S4. R package version 1.1–7. 2014.

552 Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M., & Svanbäck, R. (2002). Measuring
553 individual-level resource specialization. *Ecology*, *83*(10), 2936-2941.

554 Brabant, C., Fossati, D., & Kleijer, G. (2006). Selection of spring wheat in Switzerland (La
555 sélection du blé de printemps en Suisse). *Revue suisse Agric*, *38*(2), 73-80.

556 Brown, J. K. M. (2003). Little else but parasites. *Science*, *299*(5613), 1680-1681.

557 Brown, J. K., Chartrain, L., Lasserre-Zuber, P., & Saintenac, C. (2015). Genetics of resistance
558 to *Zymoseptoria tritici* and applications to wheat breeding. *Fungal Genetics and*
559 *Biology*, *79*, 33-41.

560 Burdon, J. J., & Thrall, P. H. (2008). Pathogen evolution across the agro-ecological interface:
561 implications for disease management. *Evolutionary Applications*, *1*(1), 57-65.

562 Burdon, J. J., Barrett, L. G., Rebetzke, G., & Thrall, P. H. (2014). Guiding deployment of
563 resistance in cereals using evolutionary principles. *Evolutionary Applications*, 7(6),
564 609-624.

565 Burdon, J. J., Zhan, J., Barrett, L. G., Papaïx, J., & Thrall, P. H. (2016). Addressing the
566 challenges of pathogen evolution on the world's arable
567 crops. *Phytopathology*, *106*(10), 1117-1127.

568 Caseys, C., Shi, G., Soltis, N., Gwinner, R., Corwin, J., Atwell, S., & Kliebenstein, D. (2020).
569 Quantitative interactions drive Botrytis cinerea disease outcome across the plant
570 kingdom. *bioRxiv*, 507491.

571 Cowger, C., Hoffer, M. E., & Mundt, C. C. (2000). Specific adaptation by *Mycosphaerella*
572 *graminicola* to a resistant wheat cultivar. *Plant pathology*, *49*(4), 445-451.
573 Croll, D., & McDonald, B. A. (2017). The genetic basis of local adaptation for pathogenic
574 fungi in agricultural ecosystems. *Molecular ecology*, *26*(7), 2027-2040.
575 Croll, D., Zala, M., & McDonald, B. A. (2013). Breakage-fusion-bridge cycles and large
576 insertions contribute to the rapid evolution of accessory chromosomes in a fungal
577 pathogen. *PLoS Genetics*, *9*(6).
578 Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P.
579 D., ... & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant
580 pathology. *Molecular plant pathology*, *13*(4), 414-430.
581 Ebert, D., & Bull, J. J. (2003). Challenging the trade-off model for the evolution of virulence:
582 is virulence management feasible?. *Trends in microbiology*, *11*(1), 15-20.
583 Fones, H., & Gurr, S. (2015). The impact of Septoria tritici Blotch disease on wheat: An EU
584 perspective. *Fungal Genetics and Biology*, *79*, 3-7.
585 Fossati, D., & Brabant, C. (2003). Selection of wheat in Switzerland: the federal stations'
586 programme (La selection du ble en Suisse: le programme des stations federales.). *Revue*
587 *Suisse d'agriculture*, *35*(4), 169-180.
588 Frank, S. A. (1996). Models of parasite virulence. *The Quarterly review of biology*, *71*(1), 37-
589 78.
590 Garamszegi, L. Z. (2006). The evolution of virulence and host specialization in malaria
591 parasites of primates. *Ecology letters*, *9*(8), 933-940.
592 González, R., Butković, A., & Elena, S. F. (2019). Role of host genetic diversity for
593 susceptibility-to-infection in the evolution of virulence of a plant virus. *Virus*
594 *evolution*, *5*(2), vez024.
595 Hartmann, F. E., Sánchez-Vallet, A., McDonald, B. A., & Croll, D. (2017). A fungal wheat
596 pathogen evolved host specialization by extensive chromosomal rearrangements. *The*
597 *ISME journal*, *11*(5), 1189-1204.
598 Héraudet, V., Salvaudon, L., & Shykoff, J. A. (2008). Trade-off between latent period and
599 transmission success of a plant pathogen revealed by phenotypic
600 correlations. *Evolutionary Ecology Research*, *10*(6), 913-924.
601 Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and*
602 *Systematics*, *21*(1), 243-273.

603 Karisto, P., Hund, A., Yu, K., Anderegg, J., Walter, A., Mascher, F., ... & Mikaberidze, A.
604 (2018). Ranking quantitative resistance to Septoria tritici blotch in elite wheat cultivars
605 using automated image analysis. *Phytopathology*, *108*(5), 568-581.
606 Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance
607 of diversity. *Journal of evolutionary biology*, *15*(2), 173-190.
608 Kawecki, T. J. (1994). Accumulation of deleterious mutations and the evolutionary cost of
609 being a generalist. *The American Naturalist*, *144*(5), 833-838.
610 Kelm, C., Ghaffary, S. M. T., Bruelheide, H., Röder, M. S., Miersch, S., Weber, W. E., ... &
611 Saal, B. (2012). The genetic architecture of seedling resistance to Septoria tritici blotch
612 in the winter wheat doubled-haploid population Solitär× Mazurka. *Molecular*
613 *Breeding*, *29*(3), 813-830.
614 Kirchner, J. W., & Roy, B. A. (2002). Evolutionary implications of host–pathogen specificity:
615 fitness consequences of pathogen virulence traits. *Evolutionary ecology research*, *4*(1),
616 27-48.
617 Lannou, C. (2012). Variation and selection of quantitative traits in plant pathogens. *Annual*
618 *review of phytopathology*, *50*.
619 Leggett, H. C., Buckling, A., Long, G. H., & Boots, M. (2013). Generalism and the evolution
620 of parasite virulence. *Trends in ecology & evolution*, *28*(10), 592-596.
621 Legros, M., & Koella, J. C. (2010). Experimental evolution of specialization by a
622 microsporidian parasite. *BMC evolutionary biology*, *10*(1), 159.
623 Lendenmann, M. H., Croll, D., Stewart, E. L., & McDonald, B. A. (2014). Quantitative trait
624 locus mapping of melanization in the plant pathogenic fungus Zymoseptoria tritici. *G3:*
625 *Genes, Genomes, Genetics*, *4*(12), 2519-2533.
626 Lenski, R. E., & May, R. M. (1994). The evolution of virulence in parasites and pathogens:
627 reconciliation between two competing hypotheses. *Journal of theoretical*
628 *biology*, *169*(3), 253-265.
629 Lenth, R., Singmann, H., & Love, J. (2018). Emmeans: Estimated marginal means, aka least-
630 squares means. *R package version*, *1*(1).
631 Linde, C. C., Zhan, J., & McDonald, B. A. (2002). Population structure of *Mycosphaerella*
632 *graminicola*: from lesions to continents. *Phytopathology*, *92*(9), 946-955.
633 May, R. M., & Anderson, R. M. (1983). Epidemiology and genetics in the coevolution of
634 parasites and hosts. *Proceedings of the Royal society of London. Series B. Biological*
635 *sciences*, *219*(1216), 281-313.

McDonald, B. A. (2014). Using dynamic diversity to achieve durable disease resistance in agricultural ecosystems. *Tropical Plant Pathology*, *39*(3), 191-196.

McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual review of phytopathology*, *40*(1), 349-379.

McDonald, B. A., & Stukenbrock, E. H. (2016). Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1709), 20160026.

Meile, L., Croll, D., Brunner, P. C., Plissonneau, C., Hartmann, F. E., McDonald, B. A., & Sánchez-Vallet, A. (2018). A fungal avirulence factor encoded in a highly plastic genomic region triggers partial resistance to septoria tritici blotch. *New Phytologist*, *219*(3), 1048-1061.

Pagán, I., Alonso-Blanco, C., & García-Arenal, F. (2007). The relationship of within-host multiplication and virulence in a plant-virus system. *PLoS One, 2*(8).

Pariaud, B., Ravigné, V., Halkett, F., Goyeau, H., Carlier, J., & Lannou, C. (2009). Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology*, *58*(3), 409-424.

Poisot, T., Canard, E., Mouquet, N., & Hochberg, M. E. (2012). A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, *3*(3), 537-544.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Remold, S. (2012). Understanding specialism when the jack of all trades can be the master of all. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1749), 4861-4869.

Restif, O., & Koella, J. C. (2003). Shared control of epidemiological traits in a coevolutionary model of host-parasite interactions. *The American Naturalist*, *161*(6), 827-836.

Roff, D. A. (2012). *Evolutionary quantitative genetics*. Springer Science & Business Media

Romero, P. A., & Elena, S. F. (2008). The degree of plant resilience to infection correlates with virus virulence and host-range. *Spanish Journal of Agricultural Research*, *6*(S1), 160-169.

de Roode, J. C., Yates, A. J., & Altizer, S. (2008). Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. *Proceedings of the National Academy of Sciences*, *105*(21), 7489-7494.

Sacristan, S., & García-Arenal, F. (2008). The evolution of virulence and pathogenicity in plant pathogen populations. *Molecular plant pathology*, *9*(3), 369-384.

670 Salvaudon, L., Héraudet, V., & Shykoff, J. A. (2007). Genotype-specific interactions and the
671 trade-off between host and parasite fitness. *BMC Evolutionary Biology*, *7*(1), 189.
672 Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional ecology*, *3*(3), 259-268.
673 Stewart, E. L., Croll, D., Lendenmann, M. H., Sanchez-Vallet, A., Hartmann, F. E., Palma-
674 Guerrero, J., ... & McDonald, B. A. (2018). Quantitative trait locus mapping reveals
675 complex genetic architecture of quantitative virulence in the wheat pathogen
676 *Zymoseptoria tritici*. *Molecular plant pathology*, *19*(1), 201-216.
677 Tack, A. J. M., Thrall, P. H., Barrett, L. G., Burdon, J. J., & Laine, A. L. (2012). Variation in
678 infectivity and aggressiveness in space and time in wild host–pathogen systems: causes
679 and consequences. *Journal of evolutionary biology*, *25*(10), 1918-1936.
680 Thrall, P. H., & Burdon, J. J. (2003). Evolution of virulence in a plant host-pathogen
681 metapopulation. *Science*, *299*(5613), 1735-1737.
682 Thrall, P. H., Barrett, L. G., Burdon, J. J., & Alexander, H. M. (2005). Variation in pathogen
683 aggressiveness within a metapopulation of the *Cakile maritima–Alternaria brassicicola*
684 host–pathogen association. *Plant pathology*, *54*(3), 265-274.
685 Vale, P. F., Wilson, A. J., Best, A., Boots, M., & Little, T. J. (2011). Epidemiological,
686 evolutionary, and coevolutionary implications of context-dependent parasitism. *The*
687 *American Naturalist*, *177*(4), 510-521.
688 Walsh, B., & Blows, M. W. (2009). Abundant genetic variation+ strong selection= multivariate
689 genetic constraints: a geometric view of adaptation. *Annual Review of Ecology,*
690 *Evolution, and Systematics*, *40*, 41-59.
691 Yang, L., Gao, F., Shang, L., Zhan, J., & McDonald, B. A. (2013). Association between
692 virulence and triazole tolerance in the phytopathogenic fungus *Mycosphaerella*
693 *graminicola. PLoS One*, *8*(3).
694 Yates, S., Mikaberidze, A., Krattinger, S. G., Abrouk, M., Hund, A., Yu, K., ... & Karisto, P.
695 (2019). Precision phenotyping reveals novel loci for quantitative resistance to septoria
696 tritici blotch. *Plant Phenomics*, *2019*, 3285904.
697 Zhan, J., & McDonald, B. A. (2013). Field-based experimental evolution of three cereal
698 pathogens using a mark–release–recapture strategy. *Plant Pathology*, *62*, 106-114.
699 Zhan, J., Linde, C. C., Jürgens, T., Merz, U., Steinebrunner, F., & McDonald, B. A. (2005).
700 Variation for neutral markers is correlated with variation for quantitative traits in the
701 plant pathogenic fungus *Mycosphaerella graminicola*. *Molecular Ecology*, *14*(9),
702 2683-2693.

703 Zhan, J., Thrall, P. H., Papaïx, J., Xie, L., & Burdon, J. J. (2015). Playing on a pathogen's
704 weakness: using evolution to guide sustainable plant disease control strategies. *Annual*
705 *review of phytopathology*, *53*, 19-43.
706 Zhong, Z., Marcel, T. C., Hartmann, F. E., Ma, X., Plissonneau, C., Zala, M., ... & Amselem,
707 J. (2017). A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence
708 on wheat cultivars carrying the *Stb6* resistance gene. *New Phytologist*, *214*(2), 619-631.
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731 **Figures legends**
732 **Figure 1.** Multiple comparisons for (A) virulence (amount of necrotic lesion area) and (B)
733 reproduction (pycnidia density within lesions) among the five *Zymoseptoria tritici*
734 populations on 12 wheat hosts. Data were log-transformed.
735

736 **Figure 2.** Correlation between virulence (amount of necrotic lesion area) and reproduction
737 (pycnidia density within lesions; A) overall and (B) within each population, among 145
738 *Zymoseptoria tritici* isolates from five populations. Each point represents the overall
739 mean of virulence and reproduction combined over 12 hosts. Data were log-
740 transformed.
741
742 **Figure 3**. Correlation between (A) overall mean virulence (amount of necrotic lesion area)
743 and reproduction specialization, (B) overall reproduction (pycnidia density within
744 lesions) and virulence specialization among 145 *Zymoseptoria tritici* isolates from five
745 populations. Specialization represents the estimates of coefficient of variation of
746 means across 12 hosts for each trait. Higher specialization indicates preference for
747 specific hosts to maximize trait performance. Data were log-transformed.
748
749 **Figure 4.** Correlation between maximum reproduction (maximum pycnidia density within
750 the lesion area produced by each isolate across 12 hosts) and reproduction
751 specialization (measured as the coefficient of variation of means for pycnidia density
752 within lesions across 12 hosts) among 145 *Zymoseptoria tritici* isolates from five
753 populations. Data were log-transformed.