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The wild tomato species *Solanum chilense* shows local variation in pathogen resistance between geographically distinct populations

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Wild tomatoes are a valuable source of disease resistance germplasm for tomato (*Solanum lycopersicum*) breeders. Many species are known to possess a certain degree of resistance against certain pathogens, however evolution of resistance traits is yet poorly understood. For some species, like *Solanum chilense,* both differences in habitat and within species genetic diversity is very large. Here we aim to investigate the occurrence of spatially heterogeneous coevolutionary pressures between populations of *S. chilense*. We investigate the phenotypic differences in disease resistance within *S. chilense* against three common tomato pathogens (*Alternaria solani*, *Phytophthora infestans* and a *Fusarium sp*.) and confirm high degrees of variability in resistance properties between selected populations. Using generalised linear mixed models, we show that disease resistance does not follow the known demographic patterns of the species. Models with up to five available climatic and geographic variables are required to best describe resistance differences, confirming the complexity of factors involved in local resistance variation. We confirm that within *S. chilense*, resistance properties against various pathogens show a mosaic pattern and do not follow environmental patterns, indicating the strength of local pathogen pressures. Our study can form the basis for further investigations of the genetic traits involved.

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Abstract

Wild tomatoes are a valuable source of disease resistance germplasm for tomato (*Solanum lycopersicum*) breeders. Many species are known to possess a certain degree of resistance against certain pathogens, however evolution of resistance traits is yet poorly understood. For some species, like *Solanum chilense,* both differences in habitat and within species genetic diversity is very large. Here we aim to investigate the occurrence of spatially heterogeneous coevolutionary pressures between populations of *S. chilense*. We investigate the phenotypic differences in disease resistance within *S. chilense* against three common tomato pathogens (*Alternaria solani*, *Phytophthora infestans* and a *Fusarium sp*.) and confirm high degrees of variability in resistance properties between selected populations. Using generalised linear mixed models, we show that disease resistance does not follow the known demographic patterns of the species. Models with up to five available climatic and geographic variables are required to best describe resistance differences, confirming the complexity of factors involved in local resistance variation. We confirm that within *S. chilense*, resistance properties against various pathogens show a mosaic pattern and do not follow environmental patterns, indicating the strength of local pathogen pressures. Our study can form the basis for further investigations of the genetic traits involved.

Keywords

Host pathogen interaction, resistance, wild tomatoes, alternaria, fusarium, phytophthora, local variation

39 **Background**

40

41 In nature, plants are exposed to a wide range of pathogens and pests. While in most cases the
42 plants appear non-specifically resistant against these threats, drastic or recurrent epidemics do
43 occur (Thrall et al. 2001a, Soubeyrand et al. 2009) and variability in specific resistance to
44 pathogens is observed (Thrall et al. 2001b, Salvaudon et al. 2008). Understanding how
45 reciprocal co-adaptation of hosts and pathogens maintains such diversity has been a key
46 question in theoretical and empirical evolutionary biology. Theoretically, negative direct
47 frequency-dependent selection (ndFDS) is shown to be a necessary condition to maintain long-
48 term stable diversity for resistance in plants and infectivity in pathogens (Tellier and Brown
49 2007). Seed banking, perenniality or polycyclic disease can generate ndFDS, while costs of
50 resistance and infectivity (virulence) are necessary but not sufficient for stable long term
51 polymorphism to occur (Tellier and Brown 2009, Brown and Tellier 2011). Another factor often
52 suggested to maintain diversity is the spatial structure of host and pathogen populations. Spatial
53 structure and migration of hosts and pathogens as well as population sizes and genetic drift
54 generate patterns of local adaptation over space and time (Thrall and Burdon 2002, Gandon
55 and Michalakis 2002). However, a spatial structure with homogeneous environment does not
56 generate ndFDS (Thrall et al. 2002a, Tellier and Brown 2011) Stable long term polymorphism is
57 favoured by spatially heterogeneous environments across which the prevalence and severity of
58 disease or the costs of resistance and infectivity may differ (Gavrilets and Michalakis 2008,
59 Moreno-Gamez et al. 2013).
60 From an ecological perspective, and based on the classic disease triangle from plant pathology
61 (Agrios 2005) the outcome of species interactions are mediated by the abiotic and biotic
62 environment. The influence of the environment generates therefore spatial and temporal
63 variation in evolutionary and coevolutionary dynamics (Thompson 2005), and increasing
64 evidence for geographical variation in coevolutionary dynamics and patterns of local adaptation

65 are found in microcosm experiments (Forde et al. 2004, Vogwill et al. 2009, Lopez-Pascua et al.
66 2010)
67 Nevertheless, few field systems exist to study and document the coevolution of plants and their
68 pathogens occurring at short time scales and across several populations. One example is the
69 wild flax – flax rust pathosystem, where local adaptations have been observed and the most
70 resistant varieties of flax generally harboured more virulent strains of rust (Thrall et al. 2002a,
71 Thrall and Burdon 2003). Similarly, the local adaptation of powdery mildew *Podosphaera*
72 *plantaginis* to *Plantago lanceolata* populations spread over different islands off the coast of
73 Sweden showed virulent strains to co-occur with more resistant plants (Laine 2005, Soubeyrand
74 et al. 2009). In the latter plant-pathogen system, several mechanisms theoretically proposed to
75 generate ndFDS have been shown to originate from the environmental heterogeneity across
76 populations: 1) GxGxE interactions (host genotype x pathogen genotype x environment, for
77 example *(Laine 2006)* 2) heterogeneity in disease incidence and prevalence determining thus
78 epidemiological pressures (Soubeyrand et al. 2009)and co-infection (Susi et al. 2015) and 3)
79 different strength of connectivity between populations accelerating or decelerating the speed of
80 coevolution across the landscape (Jousimo et al. 2014). These factors are thus expected to
81 promote and facilitate long term polymorphism at resistance and infectivity loci without
82 unrealistic costs of these alleles. Here we aim to investigate the occurrence of spatially
83 heterogeneous coevolutionary pressures between populations of *Solanum chilense*, a
84 solanaceous wild species, and several pathogens in a relatively small geographical space which
85 exhibits large variation in habitat quality and abiotic environmental factors.
86
87 Wild Solanum species are in general particularly good model species to study between and
88 within species variation, because they occur in diverse geographic and climatic habitats and
89 have a very well studied demography and known evolutionary history (Städler et al. 2005, 2008,

Tellier et al. 2011) Additionally, several studies exist that suggest that at least bacterial resistance-associated genes are under selective pressure (Rose et al. 2005, 2007, 2011). *S. chilense* is native in South America, ranging from southern Peru to central Chile, in a broad range of habitats. *S. chilense* populations have been found from coastal regions, even in slightly alkaline environments, all the way to high altitude (>3000 m) mountain regions. It has been found in extreme dry habitats on the border of the Atacama dessert, as well as near rivers and creeks (Peralta et al. 2008).

S. chilense most likely originated with its sister species *S. peruvianum,* somewhere in south Peru and then migrated south (Städler et al. 2008). A study of the species' demography found four genetically distinct subgroups; one in the north of the range, one in the central region and two in the south (one on the coast and one at high altitudes). Interestingly, the two southern groups are, even though geographically close to each other, more related to the central group than to each other, possibly due to the separating effect of the extremely arid Atacama desert (Böndel et al. 2015) In addition, *S. chilense* shows clear climatic adaptations. Populations from drier regions are responding faster to drought (Fischer et al. 2013) and individual populations found at high altitudes (>3000 m) show higher freezing tolerance (Nosenko et al. 2016) *S chilense* has also been the source of resistance loci against the fungus *Verticilium dahliae (Tabaeizadeh et al. 1999)* and against various viruses (Griffiths and Scott 2001, Ji et al. 2007, Verlaan et al. 2013). Seeing that *S. chilense* occurs in such a wide range of habitats and that the species shows specific signs of local climatic adaptations, we wondered whether we could find variation for pathogen resistance as well.

Since no exact data exist about the co-occurrence of wild pathogens and *S. chilense,* we chose to test *S. chilense* disease resistance properties with three widely studies and economically relevant pathogens, *Alternaria solani*, Phytophthora *infestans* and a *Fusarium* sp.

115 *A. solani* causes early blight and is amongst the most destructive diseases of tomato in tropical
116 and subtropical regions, leading to yield losses of up to 80% in certain regions. *A. solani* has
117 been found in central Peru and is known to cause disease not only on potato - its main host -
118 but also on many other nightshades, including tomato (Song et al. 2011, Kumar et al. 2013). In
119 addition, previous work has shown that *A. solani* resistance can be studied using detached leaf
120 assays (Chaerani and Voorrips 2006, Chaerani et al. 2007).
121 *Fusarium* spp are pathogens that cause very severe disease symptoms on a very wide range of
122 host plants that span almost the entire globe (Agrios 2005) Two *Fusarium* spp are on the top10
123 most important fungi in plant pathology (Dean et al. 2012)The *F. oxysporium* species complex
124 comprises over 100 formae specialis that all infect specific hosts, including tomato (Michielse
125 and Rep 2009). It is widely used to study molecular and genetic mechanisms involved in plant
126 pathogen interactions (Houterman et al. 2008, Ma et al. 2013) and even though it is generally
127 reported to be a vascular pathogen, it has regularly been successfully deployed in detached leaf
128 infection assays (e.g. (Kavroulakis et al. 2007)).
129 *Phytophthora infestans* is an oomycete that causes late blight on potato and tomato. In potato
130 alone the damage amounts up to $1 bn annually (Haverkort et al. 2009)Due to its economic
131 value and the vast amount of molecular and genetic research performed on it, it is considered
132 the most important oomycete plant pathogen (Kamoun et al. 2015). Like the other two
133 pathogens used in this study, P. infestans strains have been sampled in parts of the natural
134 habitat of S. chilense (Perez et al. 2001). The strain EC1 that we used has its origin in Ecuador
135 and is particularly relevant for agriculture as it is a rather aggressive strain that is capable of
136 overcoming certain novel genetic resistances (Foster et al. 2009, Nowicki et al. 2011).
137
138 Here we test the resistance of different *S. chilense* populations from three different regions in
139 Chile and Peru, one central region and two southern regions, one coastal and one mountainous
140 (see Fig1b) against the above mentioned pathogens. These group resemble very distinctive

141 habitats and can thus be used to investigate whether we see differences in infection rate
142 throughout the range of the species. We also test whether these differences show a linear
143 pattern when tested against geographical and climatic variables (e.g. north more resistant, high
144 precipitation more resistant) or whether a multitude of factors leads to specific local adaptations
145 to each of the three pathogens.
146

147 **Methods**

148 **Plant growth**

149 Seed batches were obtained from the tomato genomics resource centre (TGRC, Davis, USA).
150 We grew seven different Solanum chilense populations (accession numbers LA1963, LA2931,
151 LA2932, LA3111, LA4107, LA4117 and LA4330) consisting of 10 different plants each and one
152 Solanum pennellii (LA0716) population in our glasshouse from randomly chosen seeds. The
153 plants were grown with 16h light and a minimum temperature of 18°C. Mature plants were cut
154 back at a biweekly interval to assure young leaves of similar age were available at all times for
155 all populations

156

157 **Pathogen propagation and spore production**

158 *Alternaria solani*

159 *A. solani* strains B055 *and* St108 were obtained from the chair of Phytopathology at the TUM
160 (Munich, Germany) and cultivated on SNA plates (at 22°C, 12h UV-A light, 12h darkness
161 (induction of sporulation) and 85% humidity for 3 weeks. We harvested the spores with ddH_20
162 by scratching the mycelium with off the agar. The solution was filtered through 4 layers of mesh
163 and diluted to a concentration of 5000 spores per ml. Each leaflet was infected with a 10µl
164 droplet.

165 *Phytophthora infestans*

166 We obtained late blight pathogen *P. infestans* strain EC1 from the James Hutton Institute
167 (Dundee, UK). It was cultivated on RyeB agar, incubated 6 days at RT in darkness, 3 days at
168 RT and daylight. We scratched the mycelium with ice cold water with a pipette tip from the plate
169 and store at 4°C until further use (up to 3 hours). The solution was diluted to 2000-3000
170 sporangia per ml and the leaflets were infected with 5µl of this solution.

171 *Fusarium* sp.

172 *Fusarium* infected lesions were identified on a few detached *S. chilense* leaves from our

glasshouse. These lesions were extracted and re-cultivated for several rounds on Potato-Dextrose-Agar (PDA) for clean-up. Microscopic observations and sequence analysis of a cloned Tubulin Beta gene confirmed the genus. Once clean, the *Fusarium* was grown on PDA for a minimum of four days at RT. Spores were harvested by adding ddH_2O and aspirating the liquid. The spores were diluted to $2x10^5$-$5x10^5$ spores per ml and we infected the individual leaflets with 5µl of this solution.

All protocols for pathogen cultivation, including ingredients for the growth media can be found in more detail on https://www.protocols.io/view/Plant-Pathogen-Cultivation-fmkbk4w

Infection assays

To minimise the effect of variation between plants within one population, we collected leaves of same age randomly from 8 to 10 plants per population and shuffled them. We then drew the leaves randomly from that mix to distribute them over up to 9 boxes for each infection experiment. Each box contained 16 leaves (4 rows), from four different populations and each box contained different combinations of populations. Box number and leaf position were marked to later rule out possible effects. To eliminate the possible confounding effect of difference in surface coating composition between the different populations and remove any pathogens that accumulated on the plants during the growth time in the glass house, we washed them for 10 seconds with 70% Ethanol to sterilize the surface and remove natural wax layers before washing with ddH2O. We assured the leaf surface was dry before drop inoculation. For each pathogen 16-24 leaves - about 100 leaflets - were infected for each population and the experiments were repeated four times, accumulating to about 450 – 500 infection events per pathogen. The *Alternaria* infections were done on the axial side of the leaf, *Phytophthora* and *Fusarium* infections were done on the abaxial side of the leaf. The leaves were incubated at RT and scored after 6 to 8 days, dependent on temperature and growth conditions in the lab.

199 **Scoring and Data analysis**

200 All data analysis was done using R (R foundation for statistical computing). Generalised Linear
201 Mixed Models were made using the glmer option from the package lme4. To construct GLMM
202 we used a binomial variable (y) consisting of the number of successful and unsuccessful
203 infection events per leaf. The GLMM were constructed taking the leaf position in the box (leaf)
204 and a combination of the box number and experimental date (exp:box) into account as random
205 effects. For our first model populations names were used as fixed effects. (model1 = y ~
206 accession +(1|leaf)+(1|exp:box)). For the second model, we hierarchically tested different
207 climatic and geographical parameters (e.g. model2 = y ~ geographic1 + climatic1 + climatic 2 +
208 (1|leaf)+(1|exp:box)). Pairwise comparisons were examined using an implementation of Tukey
209 Honest Significant Difference test as provided by function glht from the the R package
210 multcomp. The boxplots were drawn using the package ggplot2 and the heatmap using gplots.
211 All packages are available through CRAN.
212
213 **Distribution map and geographical characteristics**
214 Geographical data for all populations were obtained from the Tomato Genome Resource
215 Centre. Climatic data were extracted from the http://worldclim.org/ database. The species
216 distribution map was drawn using the maps package in R. All geographic and climate data used
217 can be found in S. data 1.
218
219
220
221
222

223 **Results**

224

225 **S. chilense populations show different resistant properties against different pathogens**

226 We selected seven populations that represent three previously described genotype groups
227 (Böndel et al. 2015). Two populations originate from the central range (LA1958, LA3111), two
228 from the coastal regions (LA2932, LA4107) and two from the southern mountainous region
229 (LA4117, LA4330). A seventh population is geographically in the middle between the southern
230 mountain and the central group (LA2931). Böndel et al. group it with the central populations, but
231 assign properties of both groups to it. Figure 1A shows the species distribution and highlights
232 the selected populations.

233

234 In some wild tomato species (e.g. *S. pennellii)*, thick and sticky surface coating have a dramatic
235 effect on pathogen ingress. In *S. chilense,* surface coatings are notably less thick, and resemble
236 those of cultivated tomato, however to minimise the effect of difference in coating, as well as to
237 sterilise the leaves, we washed all leaves briefly in 70% ethanol before infection. The effects of
238 *S. chilense surface* sterilisation is noticeable during infection, but not as dramatic as with *S*
239 *pennellii* (S Figure 1).
240 We infected individual leaflets for up to 16 leaves of each population per experiment with
241 *Alternaria solani* (str 108) and counted the occurrence of infected leaflets per leaf, as this
242 represents the success rate of the pathogen to establish itself and overcome genetic resistance.
243 Infection events, were scored as either negative (no infection or clear small necrotic lesions,
244 indicating a hypersensitive response) or positive (ranging from growth just outside the droplet
245 area up to full infection of the leaflet) (Fig 1B). We observed variation within each population. In
246 almost all instances at least one leaf was fully infected whereas another was completely
247 resistant. These outliers have large effect on the calculated mean fraction. To allow good
248 judgement we report the 1st and 3rd quantile, the median value as well as the mean value for

each population (Fig 2). The mean and median of the infected fractions range from 0.35 and 0.42 for LA3111 to 0.74 and 0.81 for LA4330 or 0.67 and 0.82 for LA2932.

To test the robustness of our method, we did an additional infection with a second strain of *Alternaria* (B055). The overall infection rates are lower in this set of experiments (median of 0.54 compared to 0.62), however Figure S2 shows that just like for strain st108, LA3111 is the least infected population with a mean of 0.40 and LA4330 and LA2932 have a high median, with an infected fraction of 0.70 or 0.73 respectively.

With *Fusarium* we also see differences between the infected fraction of each population. Interestingly LA3111 is in this case the most infected population (mean: 0.72, median: 082) whereas LA4107 is the least susceptible (mean = 0.28, median = 0.11).

Finally, for *P infestans,* the infected fractions again show a different pattern. The data show a larger spread as can be seen by the larger distance between the 1st and 3rd quartile and the lowest and highest mean and median fraction were closer together ranging from 0.30 and 0.21 for LA3111 to 0.60 and 0.70 for LA4330 (Fig 2C). LA3111, one population that seems particularly resistant against *Alternaria* and *Phytophthora* seems to be the most susceptible to *Fusarium*.

To test the significance of the differences and the effect of the different populations on infection, we constructed a general linearised mixed model (glmm). We assigned experimental parameters (data, box and leaf number) as random effects and tested whether there were significant differences between the populations for each infecting species by looking at the infection counts (y) per leaf. These models show that indeed there are highly significant differences ($p<0.00001$) in infection rates between some populations for all three pathogens tested (S Data 2).

Pairwise comparisons reveal individual differences between different pathogens

To further determine which populations are different from each other, we performed pairwise comparisons using a variant of Tukey's Honest Significant Difference test. The observed pairwise differences are clearly distinct between the three pathogens. Figure 3 shows a summary heatmap of the differences, with corresponding estimates for each comparison. Cells with significant differences ($p < 0.001$) highlighted in green. All pairwise differences with their 95% confidence intervals are plotted in S. figure 3. Of the 63 pairwise comparisons, 32 show a significant difference in infection ratio. Overall, there are more significant differences between populations when it comes to Fusarium infection (15) than to Alternaria infection (10) or Phytophthora (7). Interestingly, some populations show the same result for all pathogens: there are no differences between LA1963 and LA2931 (both central) nor for LA2931 and LA4107 (south coast and central) or LA4107 and LA4117 (south coast and south mountain). Also, LA1963 is always more susceptible than LA2932 and LA4117 is always more susceptible than LA4330. In some cases a population in a pair is more resistant to one pathogen and more susceptible to another. LA4330 is more resistant than LA3111 to *Fusarium*, but less resistant to *Alternaria* and *Phytophthora*

A mix of climatic and geographic variables affect pathogen resistance

To see whether a change in certain geographic and climatic conditions can be linked to an increase or decrease of resistance rates between populations, we built new glmm using such data. First we made a simple model for *Alternaria*, testing the infection counts (y) against either latitude or longitude, a combination of both or an interaction of both. This showed that both latitude and longitude have a significant effect ($p < 0.001$). A model with both parameters shows a better fit, whereas a model with an interaction does not. We extended the model to include both parameters (longitude + latitude) and to fit various environmental parameters (Table 1, S. Data 2). We obtained the best AIC (2641.8) for a model containing altitude, annual precipitation,

the temperature in the wettest and the temperature in the coldest quarter. Additions of other climatic data did not yield an improvement of the model. Table 1 shows that of all effects, longitude is the strongest effect, followed by the mean minimum temperature in winter, the annual precipitation and altitude. It should be noted that models that only take temperature effects into account do not account for significance. A glmm with the infection counts set against the previously identified genetic groups (y ~ group), yields a high AIC (2705). The model with the populations yields an as good AIC as the one with all available variables. This suggests that no single variable has a strong, exclusive correlation to infection rate and that each population represents its own micro environment with specific geographic and climate parameters that are all of influence.

Similar to *Alternaria*, we tested all variables for *Phytophthora* and *Fusarium*. The pattern seen for *Phytophthora* is almost identical to that of *Alternaria.* The AIC values are generally lower, but the trends are the same. Interestingly, *Fusarium* shows a slightly different picture. Whereas longitude is still the strongest effect, its significance is lower and the temperature in the coldest quarter of the year has a relatively large effect. The effect of altitude is not significant and differences in annual precipitation have a nearly negligible effect as well. As with *Alternaria*, the model testing for the group effect shows a lesser fit than the model per population (results for selected models can be found in S data 2).

Discussion

The wild tomato *Solanum chilense* grows in a variety of habitats in Chile and Peru, ranging from lower coastal areas to very high altitudes (>3000m). These populations experience considerable variation in geographic parameters like precipitation and temperatures. It is known that *S. chilense* has a clear demographic pattern and signs of adaptations to climatic differences between different populations (Fischer et al. 2011, 2013, Nosenko et al. 2016). A demographic pattern of North-South colonisation is observable with larger and more diverse populations in the north of the range and smaller and less diverse populations in the south. In addition, there is little to no genetic exchange between some of the southern most populations that are separated by the extremely dry Atacama desert. This lead to the conclusion that *S. chilense* can be divided in a northern, a central and two southern genotype groups (Böndel et al. 2015).

We hypothesised that pathogen pressures must differ ~~a lot~~ between such diverse geographical locations and as such *S. chilense* should show signs of pathogen adaptations between the different populations. To test our hypothesis we performed infection assays with three global *Solanum* pathogens and with selected *S. chilense* populations. We observe clear differences between the infection success rates of the 3 pathogens on the different *S. chilense* populations, indeed suggesting local pathogen adaptations. We could observe a clear separation between the genotype groups, only for Alternaria infection, where the central populations are more susceptible than those from groups in the south. With the other pathogens, within-group differences exist. Pairwise comparisons confirmed that outcomes differ within groups and between pathogens. For example, a pair that shows significant differences for *Phytophthora* and *Alternaria* infection (LA1963-LA4330) does not show this for *Fusarium* or the other way around and very strong pairwise differences can even be seen within the previously identified genotype groups (e.g. LA2932-LA4107 with *Fusarium*). We also showed that there are no generally more resistant or more susceptible population. For example LA3111 is particularly resistant against

347 *Fusarium*, but the most susceptible to *Phytophthora* and *Alternaria*.

348

349 We used a glmm to test which factors might contribute to these differences. Interestingly,
350 whereas the species as a whole, shows a strong north-south demography, our analyses show
351 that not latitude, but longitude is a very strong effect. This could at the one hand be explained
352 due to the absence of the northern most group in our analysis, but a more likely explanation is
353 the bigger geographic and associated climatic difference in the east-west gradient of the
354 species, with low altitude coastal areas in the west, and high mountains in the east.
355 Temperature differences can have large effects on the prevalence of pathogen populations as
356 shown for wild plant-pathosystems (Laine 2008) and also on crops, pathogens show adaptation
357 to different temperature regimes (Mboup et al. 2012, Stefansson et al. 2013) The mountainous
358 areas in our study have particularly cold winters and fairly low mean temperatures in summer,
359 which could be detrimental for pathogen survival or slow its growth and thus reduce pathogen
360 pressure.
361 Our results show indeed that temperature in winter as well as temperature in the wettest quarter
362 have a significant effect on infection rate. The importance of overwintering inoculum has
363 previously been shown to be a main predictor for *Podosphaera plantaginis* epidemics on
364 *Plantago lanceolata* in the next growing season (Soubeyrand et al. 2009). However, it must be
365 noted that models that only incorporate winter temperature or indeed any other single climatic
366 variables effects did not show any significance. This is in line with a between species
367 comparison for wild potato (Spooner et al. 2009) and might be related to the fact that some
368 higher altitude locations also have the highest annual precipitation rates. For example for *P.*
369 *infestans* a relative high humidity has large effects on successful sporulation (Harrison and
370 Lowe 1989)

371

372 Our climate data were extracted from worldclim.org and might not provide the whole picture. For

example, precipitation data might be accurate, but do not take into account a common sea-fog phenomenon, that can be observed along the coast of Chile and Peru (Cereceda and Schemenauer 1991, Schemenauer and Cereceda 1992) This fog increases the local humidity for several hours up to several days in certain “fog basins”. Similarly, no data is available on any nearby streams, rivers or irrigation canals for any of the populations. For some populations, a note is available for the state of the site at the time of collection (e.g. “dry quebrada”), but it remains unknown whether these features are a constant or changed in the time before collection.

The best fitted models incorporate five climatic and geographic variables. Adding more variables did not improve the model, mainly due to the correlations between the available climate data. The strongest effects were observed for combinations of longitude and latitude together with climatic variables, indicating that one or two variables alone do not determine pathogen resistance. The latitude effect, which can be observed in the evolution of the species as a whole, seems to be less strong in our analyses, where longitude plays a larger role. Overall, our results indicate that indeed S. *chilense* shows local variations, which are possibly the result of adaptations to local pathogen pressures. The mosaic like structure of our results indicate that these resistances are likely caused by a multitude of factors. These findings are in line with several inter species studies in wild potato, where no correlation could be found between geographical location of the species and resistance against *P. infestans (Khiutti et al. 2015)* or *A. solani* (Jansky et al. 2008) To further unravel the combination of factors contributing to local variations, new sampling excursions would be required, that not just collect plant and pathogen, but also measure local geographic and climatic parameters.

In this study, several mechanisms theoretically proposed to generate stable long term polymorphism at host resistance and pathogen infectivity loci are shown to originate from the

environmental heterogeneity across populations. We conclude ~~indeed on~~ 1) the existence of possible GxGxE interactions for given host-pathogen interactions, 2) heterogeneity in disease incidence and prevalence across habitats, and most interestingly 3) a geographic mosaic of exposure to different pathogens species. The presence-absence of different above- and below-ground pathogens on the same plants may a key component of wild systems generating scenarios such as co-infection (Susi et al. 2015), cross-immunity or facilitation (Tack et al. 2015), with consequences for the genomics of pathogens (McMullan et al. 2015). Our research did not yet focus on any genetic differences underlying the variation in infection rates and linking phenotype to genotype should be one of the follow-up projects. Identification of the genes involved in these resistance variations could also help to identify which plant defence mechanisms are affected between populations and if there are indeed evolutionary differences between defence pathways in nonhost resistance compared to resistance variation within or in closely related species (Schulze-Lefert and Panstruga 2011, Stam et al. 2014). We have recently shown that targeted resequencing of genes of interest can be a potent tool to calculate evolutionary parameters of gene families of interest in wild tomato (Stam et al. 2016). Such resequencing studies could thus help to pinpoint how molecular mechanisms are affected by different pathogens as well as climatic variables.

Conclusions

Differences in pathogen disease resistance have been well described between many wild crop relatives. Here we presented a phenotypic study that shows specific pathogen adaptations between populations of one wild tomato species *S. chilense*. We showed that there are clear differences between individual populations. Using generalised linear mixed models, we show that this variation does not follow a simple geographical cline, that multiple climatic factors are needed to explain parts of the variation and that even within previously identified genotype groups resistance properties can differ dramatically. Our study confirms a mosaic pattern in

resistance properties within one species and can form the starting point for studies unravelling environmental effects on said properties as well as the genetic and molecular mechanisms involved in plant-pathogen coevolution.

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Figure 1(on next page)

S. chilense populations and phenotypic observations

A) A map showing the populations used in this study, belonging to the central (red), southern mountainous (blue) or southern coastal (green) region. The geographic range of whole species is depicted in the background (grey dots). B) The phenotypic observations after infection range from no visible symptoms (first row) and small black necrotic lesions resembling the Hypersensitive Response (HR, second row), both scored as 'not infected', to intermediate and strong infection (third and fourth row), both scored as 'infected'. In the columns from left to right: infection with Alternaria, Fusarium and Phytophthora. We could not observe HR in the Alternaria infections.

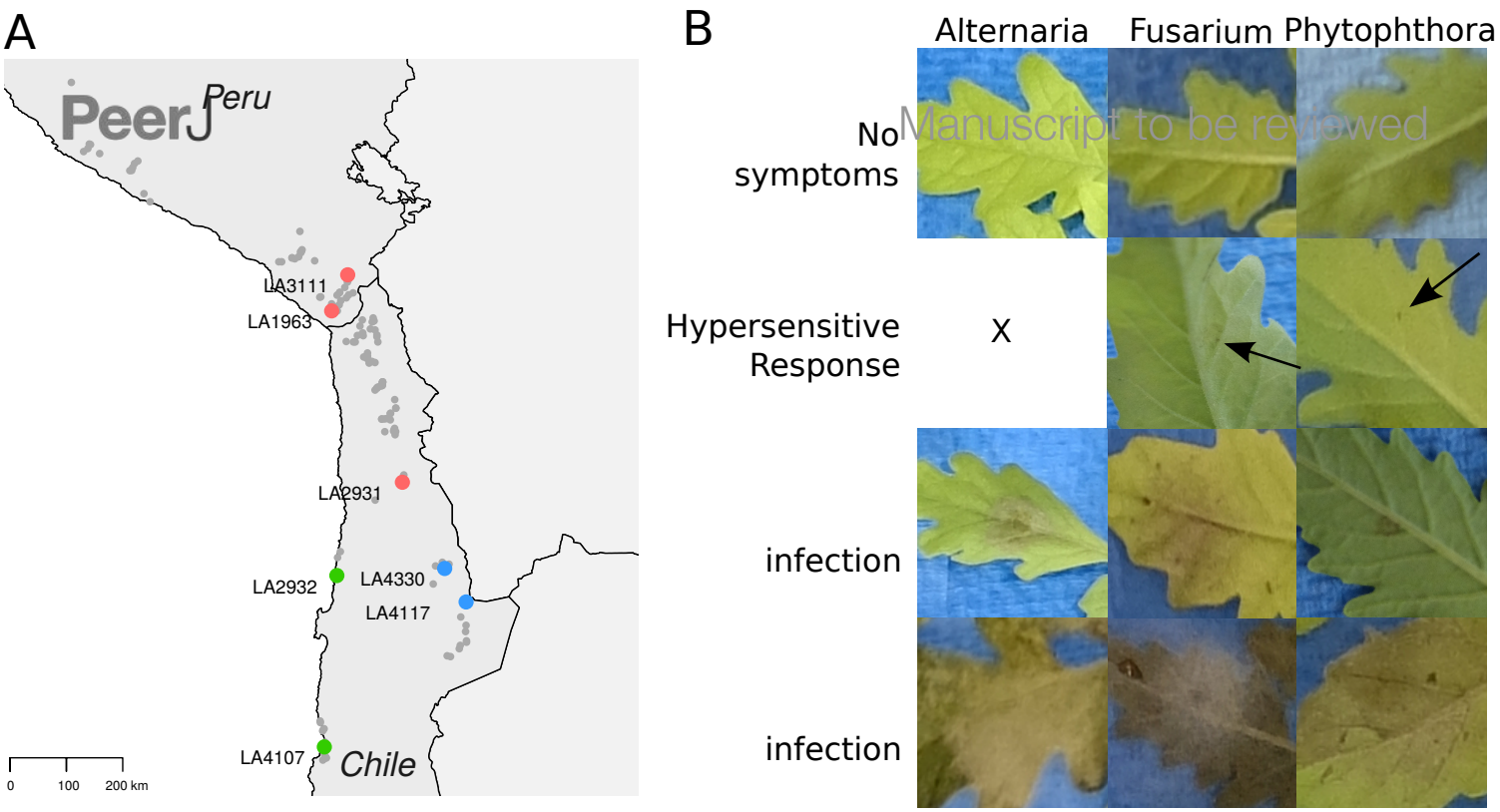

Figure 2(on next page)

Infected leaf fraction for different S. chilense populations

The boxplots show the median and 1st and 3rd quartile of the infected fractions per leaf for A) Alternaria, B) Fusarium and C) Phytophthora. The black dots represent the mean value for the infections. The Y axis ranges from 0 (no infected leaflets on a leaf) to 1 (all leaflets show infection). On the X axis, each population is represented. The colours correspond to the geographic regions as depicted in figure 1.

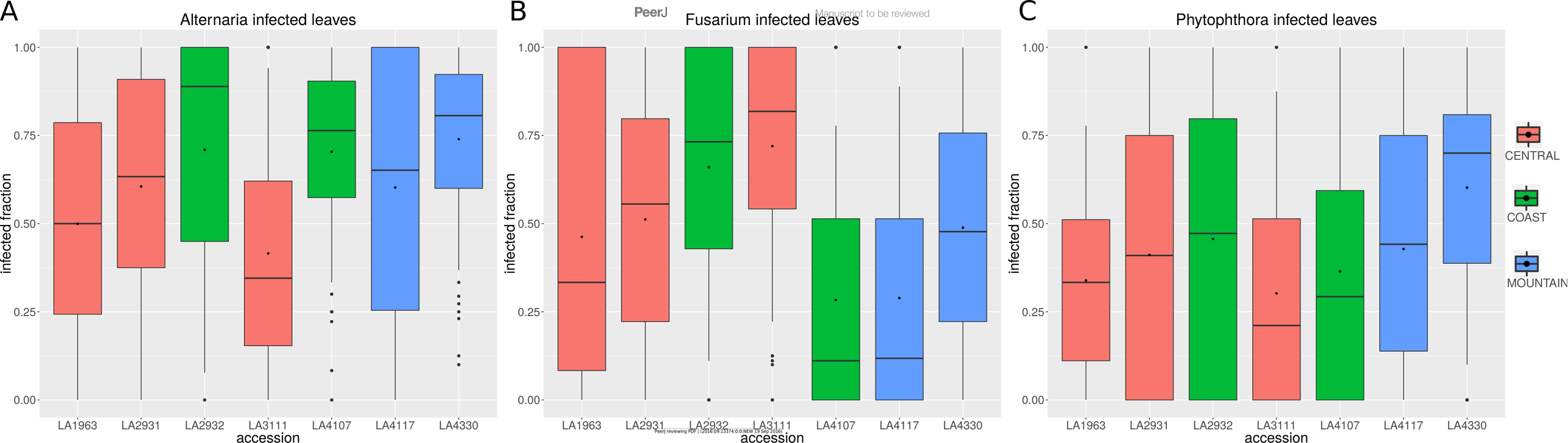

Figure 3(on next page)

Populations with significant different infected fractions

Heatmap depicting whether a pairwise difference shows a significant result for Alternaria solani (left column), Fusarium sp. (middle column) and Phytophthera infestans (right column). Each row represents a pairwise comparison. Green cells represent a significant difference ($p < 0.001$ after multiple testing correction) and the numbers represent the estimated effect, with negative numbers indicating that the population mentioned on the left is less resistant than the one on the right.

Alternaria	Fusarium	Phytophthora	
-0.55183	0.10738	-0.26040	LA1963 – LA2931
-0.87557	-0.78253	-0.73009	LA1963 – LA2932
0.29545	-1.11537	0.26290	LA1963 – LA3111
-0.[illegible]50	0.71985	-0.26015	LA1963 – LA4107
-0.[illegible]648	0.88139	-0.42327	LA1963 – LA4117
-1.37044	0.02266	-1.23225	LA1963 – LA4330
-0.32373	-0.88991	-0.46969	LA2931 – LA2932
0.84728	-1.22275	0.52330	LA2931 – LA3111
-0.29967	0.61247	0.00025	LA2931 – LA4107
0.08695	0.77401	0.16287	LA2931 – LA4117
-0.81860	-0.08472	-0.97185	LA2931 – LA4330
1.17102	-0.33285	0.99300	LA2932 – LA3111
0.02407	1.50237	0.46994	LA2932 – LA4107
0.41069	1.66391	0.30681	LA2932 – LA4117
0.49487	0.80518	-0.50215	LA2932 – LA4330
-1.14695	1.83522	-0.52305	LA3111 – LA4107
-0.76033	1.99676	-0.68618	LA3111 – LA4117
-1.66588	1.13803	-1.49516	LA3111 – LA4330
0.38662	0.16154	-0.16312	LA4107 – LA4117
-0.51894	-0.69719	-0.97210	LA4107 – LA4330
-0.90556	-0.85873	-0.80897	LA4117 – LA4330

Table 1(on next page)

Table 1

Summary of GLMM results

1
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	Model	Alternaria	Fusarium	Phytophthora
1	y~accession	**2641.8**	**2307.6**	**1893.3**
2	y~Lat	2708.6	2431.3	1958.3
3	y~Long	2815.1	2490.8	1965.8
4	y~Long+Lat	2703.9	2420.6	1945.4
5	y~Long*Lat	2705.8	2419.1	1947.4
6	y~Long+Lat+Alt+AnnPrecip+TempA+TempB	**2641.8**	**2307.6**	**1893.3**
7	y~Altitude	2843.3	2503.5	1985.1
8	y~Temp	2843.5	2506.5	1984.1
9	y~AnnPrecip	2757.2	2457.1	1968.4
10	y~group	2705.0	2489.7	1930.2

2