

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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3 **between geographically distinct populations**

4

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14 Abstract

15

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

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33

34 Keywords

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

37

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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 nFDS 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,

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

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

111

112 Since no exact data exist about the co-occurrence of wild pathogens and *S. chilense*, we chose
113 to test *S. chilense* disease resistance properties with three widely studies and economically
114 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 ddH₂O
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

173 glasshouse. These lesions were extracted and re-cultivated for several rounds on Potato-
174 Dextrose-Agar (PDA) for clean-up. Microscopic observations and sequence analysis of a cloned
175 Tubulin Beta gene confirmed the genus. Once clean, the *Fusarium* was grown on PDA for a
176 minimum of four days at RT. Spores were harvested by adding ddH₂O and aspirating the liquid.
177 The spores were diluted to 2×10^5 - 5×10^5 spores per ml and we infected the individual leaflets
178 with 5 μ l of this solution.

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

181

182 **Infection assays**

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

198

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

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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

249 each population (Fig 2). The mean and median of the infected fractions range from 0.35 and
250 0.42 for LA3111 to 0.74 and 0.81 for LA4330 or 0.67 and 0.82 for LA2932.

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

256 With *Fusarium* we also see differences between the infected fraction of each population.
257 Interestingly LA3111 is in this case the most infected population (mean: 0.72, median: 0.82)
258 whereas LA4107 is the least susceptible (mean = 0.28, median = 0.11).

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

265

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

273

274 **Pairwise comparisons reveal individual differences between different pathogens**

275 To further determine which populations are different from each other, we performed pairwise
276 comparisons using a variant of Tukey's Honest Significant Difference test. The observed
277 pairwise differences are clearly distinct between the three pathogens. Figure 3 shows a
278 summary heatmap of the differences, with corresponding estimates for each comparison. Cells
279 with significant differences ($p < 0.001$) highlighted in green. All pairwise differences with their
280 95% confidence intervals are plotted in S. figure 3. Of the 63 pairwise comparisons, 32 show a
281 significant difference in infection ratio. Overall, there are more significant differences between
282 populations when it comes to *Fusarium* infection (15) than to *Alternaria* infection (10) or
283 *Phytophthora* (7). Interestingly, some populations show the same result for all pathogens: there
284 are no differences between LA1963 and LA2931 (both central) nor for LA2931 and LA4107
285 (south coast and central) or LA4107 and LA4117 (south coast and south mountain). Also,
286 LA1963 is always more susceptible than LA2932 and LA4117 is always more susceptible than
287 LA4330. In some cases a population in a pair is more resistant to one pathogen and more
288 susceptible to another. LA4330 is more resistant than LA3111 to *Fusarium*, but less resistant to
289 *Alternaria* and *Phytophthora*

290

291

292 **A mix of climatic and geographic variables affect pathogen resistance**

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

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

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

319

320

321 **Discussion**

322

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

333 We hypothesised that pathogen pressures must differ a lot between such diverse geographical
334 locations and as such *S. chilense* should show signs of pathogen adaptations between the
335 different populations. To test our hypothesis we performed infection assays with three global
336 *Solanum* pathogens and with selected *S. chilense* populations. We observe clear differences
337 between the infection success rates of the 3 pathogens on the different *S. chilense* populations,
338 indeed suggesting local pathogen adaptations. We could observe a clear separation between
339 the genotype groups, only for *Alternaria* infection, where the central populations are more
340 susceptible than those from groups in the south. With the other pathogens, within-group
341 differences exist. Pairwise comparisons confirmed that outcomes differ within groups and
342 between pathogens. For example, a pair that shows significant differences for *Phytophthora* and
343 *Alternaria* infection (LA1963-LA4330) does not show this for *Fusarium* or the other way around
344 and very strong pairwise differences can even be seen within the previously identified genotype
345 groups (e.g. LA2932-LA4107 with *Fusarium*). We also showed that there are no generally more
346 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

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

381

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

396

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

399 environmental heterogeneity across populations. We conclude indeed on 1) the existence of
400 possible GxGxE interactions for given host-pathogen interactions, 2) heterogeneity in disease
401 incidence and prevalence across habitats, and most interestingly 3) a geographic mosaic of
402 exposure to different pathogens species. The presence-absence of different above- and below-
403 ground pathogens on the same plants may a key component of wild systems generating
404 scenarios such as co-infection (Susi et al. 2015), cross-immunity or facilitation (Tack et al.
405 2015), with consequences for the genomics of pathogens (McMullan et al. 2015). Our research
406 did not yet focus on any genetic differences underlying the variation in infection rates and linking
407 phenotype to genotype should be one of the follow-up projects. Identification of the genes
408 involved in these resistance variations could also help to identify which plant defence
409 mechanisms are affected between populations and if there are indeed evolutionary differences
410 between defence pathways in nonhost resistance compared to resistance variation within or in
411 closely related species (Schulze-Lefert and Panstruga 2011, Stam et al. 2014). We have
412 recently shown that targeted resequencing of genes of interest can be a potent tool to calculate
413 evolutionary parameters of gene families of interest in wild tomato (Stam et al. 2016). Such
414 resequencing studies could thus help to pinpoint how molecular mechanisms are affected by
415 different pathogens as well as climatic variables.

416

417 **Conclusions**

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

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

428

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435

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437

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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.

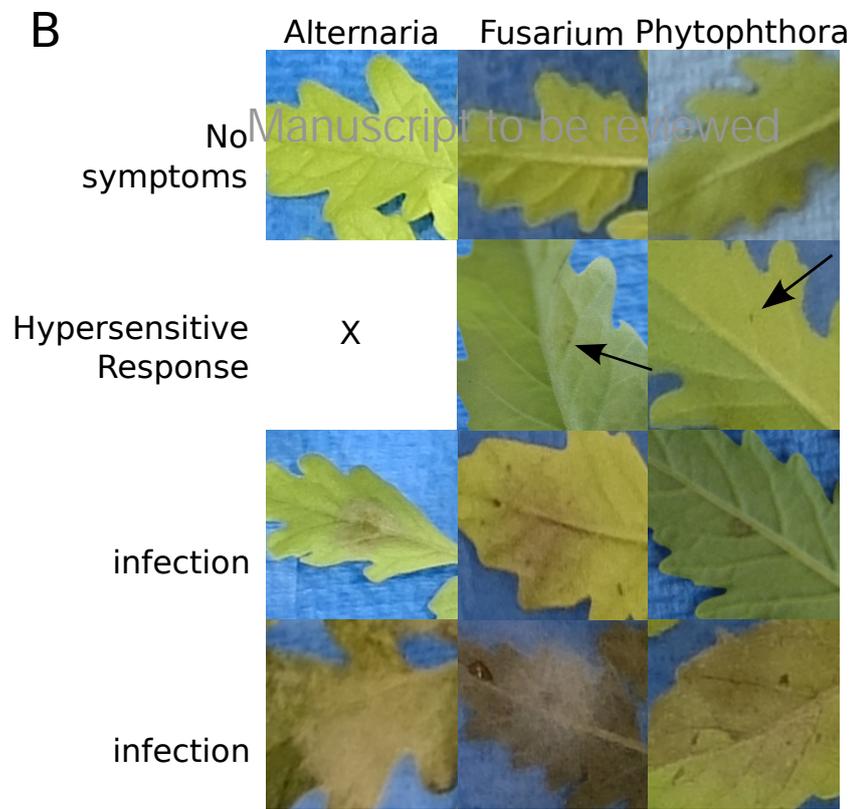


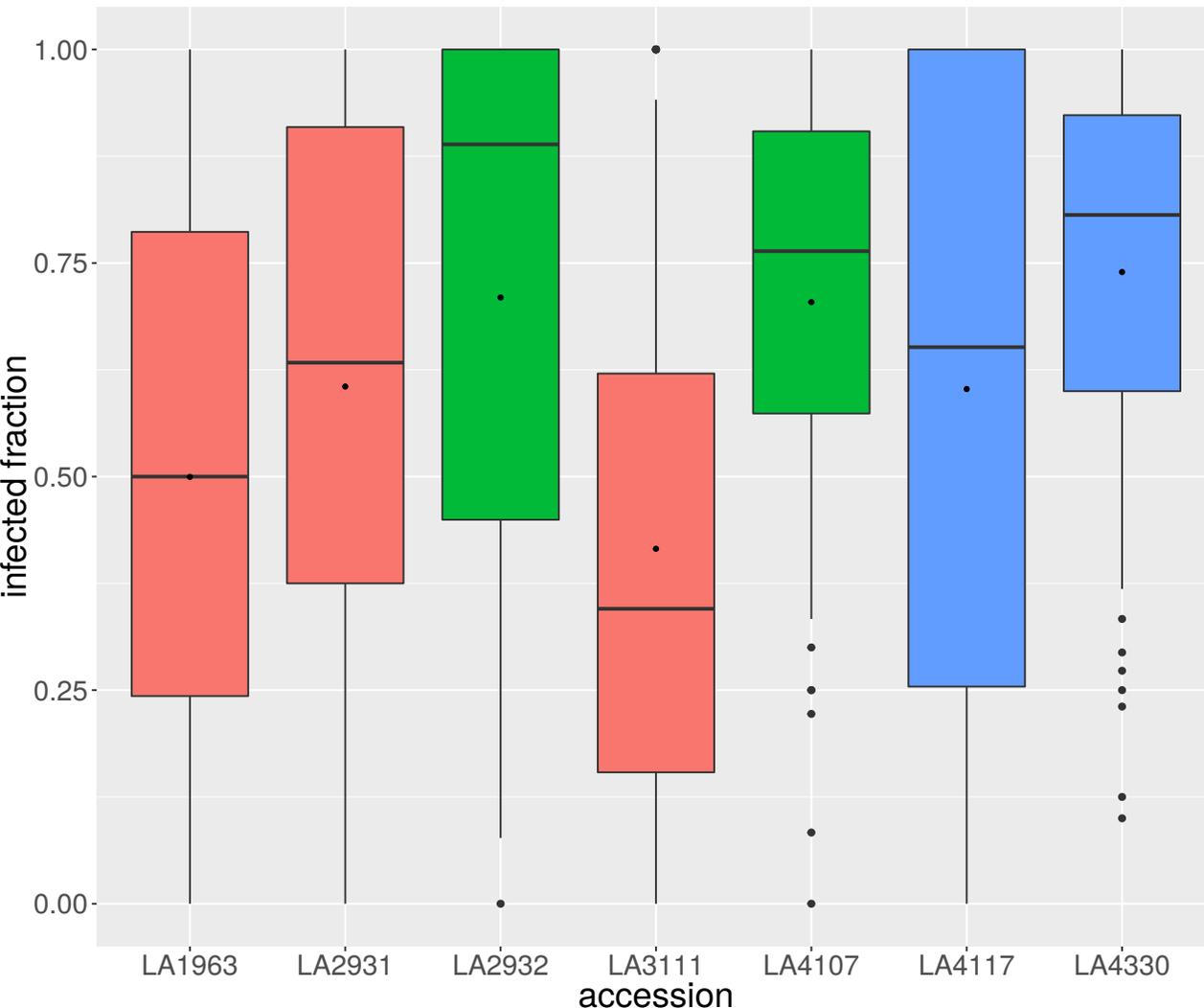
Figure 2 (on next page)

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.

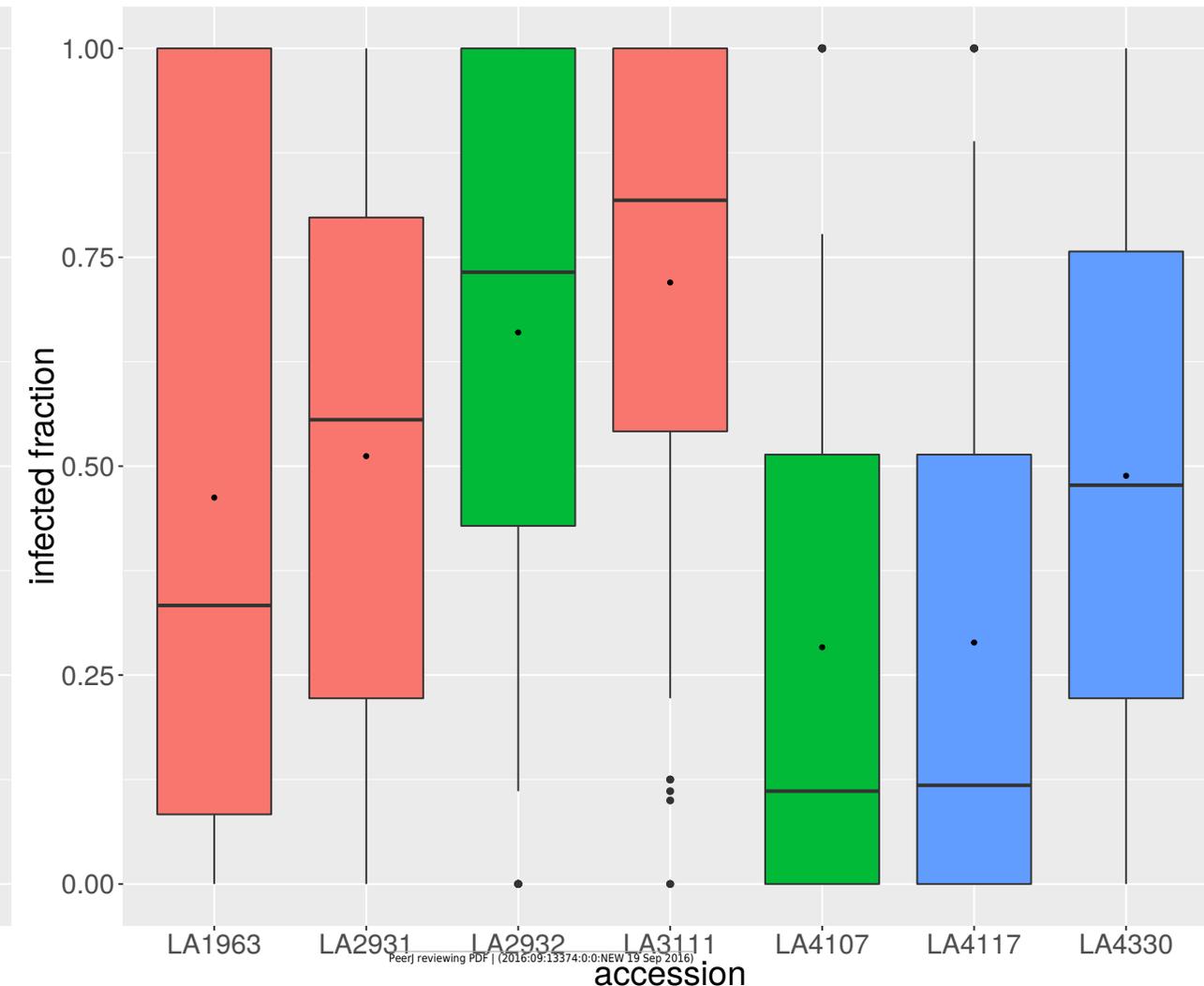
A

Alternaria infected leaves



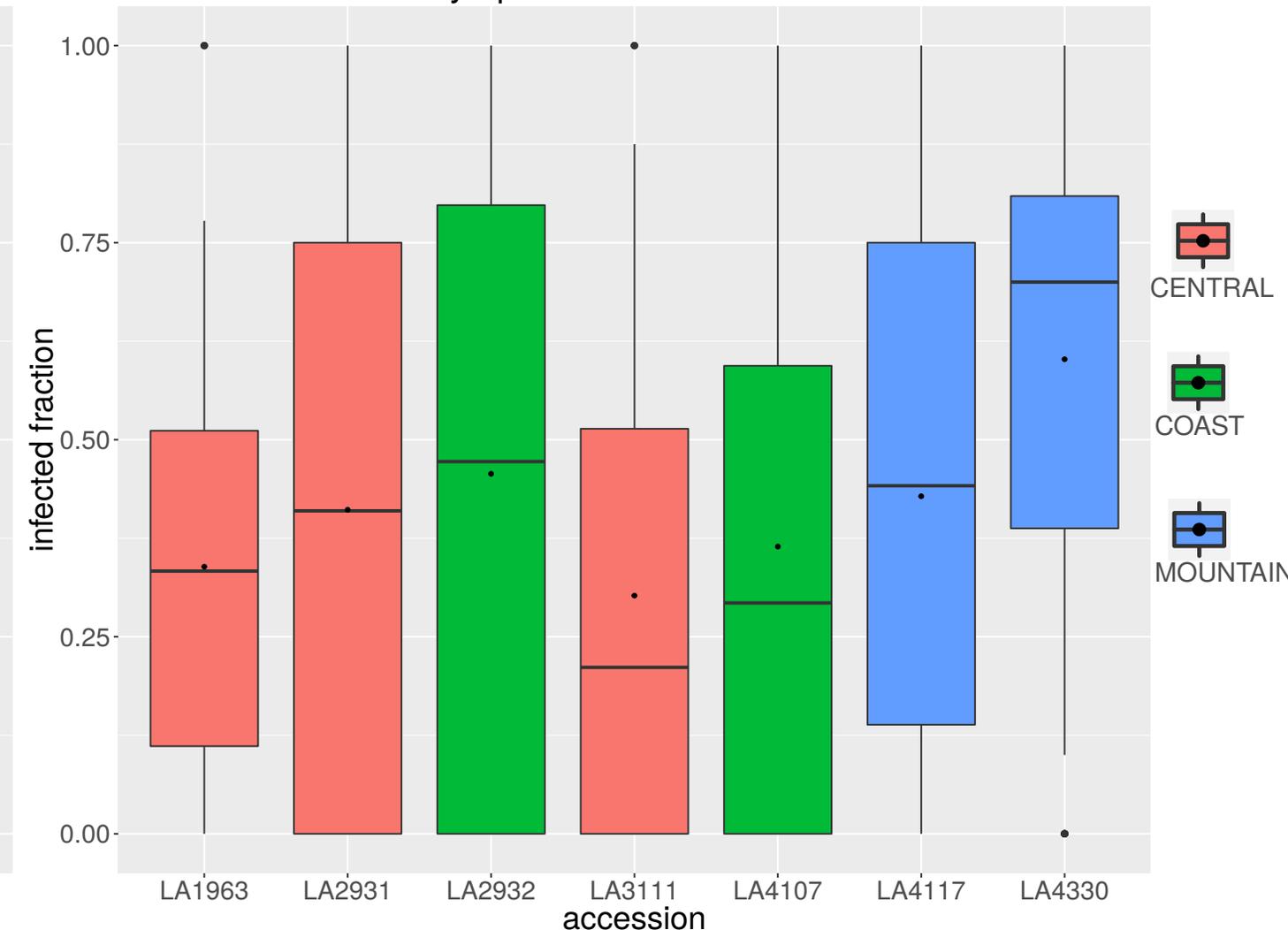
B

Fusarium infected leaves



C

Phytophthora infected leaves



CENTRAL

COAST

MOUNTAIN

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 *Phytophthora 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
-0.71550	0.71985	-0.26015	LA1963 - LA4107
-0.16418	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

Manuscript to be reviewed

Table 1 (on next page)

Table 1

Summary of GLMM results

1	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