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1 **The level of specialization of *Phytophthora infestans* to potato and tomato is**
2 **a biotrophy-related, stable trait**

3

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9

10 Short title: Biotrophy drives host adaptation in *Phytophthora infestans*

11

12 **Abstract**

13 Despite their ability to infect both plant species, natural populations of *Phytophthora infestans*, the
14 pathogen causing late blight on potato and tomato, are usually separated into genetically distinct
15 lineages that are mainly restricted to either host. Laboratory cross-inoculation tests revealed a host-
16 related local adaptation between genotypes, with asymmetric fitness performance between
17 generalist lineages, mainly present on tomato, and specialist lineages confined to potato. To further
18 understand the basis of host-related adaptation in *P. infestans*, we combined experimental evolution
19 and analysis of effectors involved in pathogenicity and cell death modulation. We aimed to check in
20 this way (i) if natural host adaptation of *P. infestans* is reversible during one growing season and (ii) if
21 this process is accompanied by changes in pathogenicity-related gene expression. Two isolates
22 differing substantially by their level of specialization were passaged for nine generations on
23 susceptible potato (cv. Bintje), tomato (cv. Marmande) or alternately on both hosts. Pathogenic
24 fitness and the expression of eight pathogen effectors with known host targets (*AVRblb2*, *EPIC2B*,
25 *EPI1*, *PexRD2*, *SNE1*, *PiNPP*, *INF1* and *Pi03192*) and the candidate effector carbonic anhydrase (*CA*)
26 were quantified before and after experimental evolution on these hosts. Fitness and gene expression
27 varied during the experimental evolution experiment, but independently of the subculturing host.
28 However, the level of host-related specialization of both isolates was stable over time and linked to
29 distinct expression patterns of antagonistic host cell death regulator genes, such as *SNE1* and *PiNPP*.
30 Relations between fitness and effector expression proved to be host- and/or isolate-dependent.
31 Altogether, our results demonstrate host adaptation of *P. infestans* to be a rather stable trait that is
32 not prone to fluctuate by transitory host changes. They further suggest that pathogenicity of *P.*
33 *infestans* strongly depends on its ability to establish a steady biotrophic interaction with its hosts by
34 regulating effector gene expression.

35

36 **Author Summary**

37 The infamous Irish potato famine pathogen *Phytophthora infestans* causes late blight on potato and
38 tomato, and extensive losses on both crops worldwide. Isolates causing tomato late blight markedly
39 differ in genotype and phenotype from isolates causing potato late blight: under controlled
40 conditions, isolates from tomato perform well on both hosts, while isolates highly pathogenic from
41 potato struggle to produce large lesions on tomato. Mechanisms explaining these differences are
42 unknown, but might provide clues to better understand the fundamental process of host
43 specialization in pathogens. *P. infestans* is known to secrete many effectors, modulating the outcome
44 of the interaction with its hosts. We thus coupled experimental evolution, by subculturing isolates
45 nine times on different hosts, and expression of host cell death regulating effectors to explain
46 pathogenic specialization. We showed that the level of pathogenic specialization depends on the
47 pathogen ability to maintain a biotrophic interaction with its host, and hence to suppress cell death.
48 Host specialization was not altered during serial passages, irrespective of the hosts, although overall
49 pathogenicity increased. These findings show that *P. infestans* is primarily a biotrophic pathogen,
50 feeding on living host tissue, and open ground for new breeding targets for improved resistance to
51 late blight.

52

53 Introduction

54 Parasitism is the relationship between two species in which one benefits at the expense of the other.
55 Relationships of parasites with plants are diverse, and their classification depends largely on
56 physiological/nutritional and ecological criteria [1-3]. According to the nutritional behavior of the
57 parasite, plant-parasite interactions may be biotrophic or necrotrophic. Biotrophy means that the
58 parasite keeps the host alive and derives energy from living cells (e.g. *Blumeria graminis*) [4],
59 whereas necrotrophy refers to relationships where the parasite kills the host cells to feed on dead
60 matter (e.g. *Botrytis cinerea*) [5, 6]. An initially biotrophic relationship may become necrotrophic (e.g.
61 *Colletotricum graminicola*, *Sclerotinia sclerotiorum*) [7, 8]. This situation is commonly referred to as
62 hemi-biotrophy, but there is some ambiguity due to divergent use of this term. For example,
63 interactions of the rice blast fungus *Magnaporthe oryzae* are called hemi-biotrophic [9], but there is
64 no single discrete switch from biotrophy to necrotrophy in infected tissue. Biotrophic invasion of host
65 cells is rather sequential and continues in neighboring rice cells while previously parasitized cells die.

66
67 Biotrophic hyphae growing ahead of dead tissue have also been reported in the late blight pathogen
68 *Phytophthora infestans* (Mont.) De Bary [10, 11], an oomycete [2] with outstanding scientific and
69 economic importance [12, 13]. Under natural conditions, *P. infestans* depends on living host tissue to
70 complete its life cycle and has low or no saprotrophic survival ability outside its hosts [see 14 and
71 literature cited therein]. However, as tissue damage (necrosis) occurs during later states of
72 colonization, its lifestyle has also been classified as hemi-biotrophic or even necrotrophic. As
73 schematized in Figure 1, the duration of biotrophy varies among *P. infestans* isolates and has been
74 related with the level of pathogenic fitness on tomato in laboratory conditions [15-18]. Biotrophy has
75 also been associated with distinct gene expression patterns on potato [19].

76

77 Plant pathogens face in their hosts a two-branched innate immune system [20]: pathogen-associated
78 molecular pattern triggered immunity (PTI) and effector triggered immunity (ETI). As outlined by the

79 authors of this seminal review, successful pathogens secrete effector proteins to suppress PTI and
80 lose/evolve effectors to overcome ETI. These principles also apply to oomycete-plant interactions
81 [21-23]. *P. infestans* is known to employ various effectors during the interaction with its hosts [24,
82 25] and gene expression polymorphisms among isolates have been reported on potato [19].
83 Interestingly, these polymorphisms can be associated with the duration of biotrophy [19, figure 7]. *P.*
84 *infestans* has a repeat-bloated 240 megabases (Mb) genome containing hundreds of fast-evolving
85 RxLR, CRN and other effector-coding genes that are expressed in a coordinated way during its
86 interaction with potato and tomato [24-26]. Secreted into the host apoplast or cytoplasm, they
87 disable host defense components and facilitate colonization: *P. infestans* effectors such as *EPI1* [27],
88 *EPI10* [28], *EPIC1/EPIC2B* [29, 30] and *AVRblb2* [31] are implicated in counter-defense against
89 apoplast-localized host proteases. Other effector genes manipulate host gene expression by
90 downregulating defense related genes [e.g. effector PI03192, 32] or upregulating susceptibility
91 factors [e.g. effector Pi04089, 33]. *P. infestans* also induces/suppresses host cell-death through the
92 coordinated action of cell death antagonistic effectors. As illustrated by Zuluaga et al. [24],
93 biotrophy-related suppressors of cell death such as *ipiO1* [34, 35] and *SNE1* [36] are secreted in the
94 early phases of the interaction, followed by the later secretion of synergistically interacting necrosis-
95 inducing effectors (NIE) such as *PiNPP1.1* and *INF1* [37]. As the timing of expression and biological
96 activity fit trophic phases of the interaction, effectors countering host cell-death may serve *P.*
97 *infestans* to fine tune the duration of biotrophy by taking control over the host plant cell death
98 machinery [e.g. *SNE1/PiNPP1.1*, 38]. Therefore, *P. infestans* effectors are thought to be involved in
99 host specialization [39]. This view is supported by the fact that *P. infestans* isolates are genetically
100 diverse and may carry different variants of an effector (e.g. *ipiO* family) [40].

101

102 Ecological specialization is a main concept in ecology, but its definitions are highly context-
103 dependent (see [41]). Historically developed as a species attribute (generalist and specialist species),
104 the concept of specialization is now largely extended in the ecological literature to any ecological

105 level (individual, population, species, community). Within-species variation of ecological
106 specialization is indeed widespread [42]: a species considered as ecological generalist may actually be
107 a heterogeneous collection of specialized individuals. Here, we will refer to ecological specialization
108 at the individual level, as the aim of our work is to explore concomitantly ecological and mechanistic
109 aspects of previously documented within-species variation of specialization in *P. infestans* [18, 43,
110 44]. Considering the important distinction between fundamental and realized specialization [45], it
111 should also be clarified that ecological specialization in the present study refers to Grinnellian
112 specialization assessed in controlled cross-inoculation experiments. Inferred here from direct
113 measures of species performance (i.e. pathogenic fitness) in multiple host environments (i.e. potato,
114 tomato) [46], it analyses the variance in performance across these hosts according to the Levins'
115 metric [47]. The terms 'generalist' and 'specialist' refer here to previously identified isolate types
116 (potato isolate, tomato isolate; [15]) showing distinct level of pathogenic specialization.

117

118 Overall, what determines the level of host specificity of *P. infestans* is not well understood. Besides
119 the potential effect of cultural practices [48], quantitative differences in pathogenic fitness of isolates
120 may be a particular strong explanatory factor [49-51]. The evolutionary process of pathogenic
121 specialization in *P. infestans* populations on potato and tomato is just as little understood as its
122 underlying determinants. Experimental evolution experiments are thus often used to explore those
123 evolutionary dynamics in real-time. There is evidence, from Mexico and Israel, that tomato isolates
124 could evolve from a local potato population and acquire specialization to tomato [43, 52]. In other
125 words, aggressiveness on tomato would have evolved in isolates already pathogenic on potato. This
126 could explain the lower host specificity of tomato isolates with respect to potato isolates. As
127 documented for various parasite-host systems [53], the observed increase of
128 fitness/virulence/growth on the new host on which the parasite has been passed is often associated
129 with attenuation of these traits on the original host. In the case of highly adaptive pathogens, as little
130 as one [54] or only several passages [55, 56] on the alternative hosts may be sufficient to alter or

131 revert adaptation patterns. *P. infestans* may be considered as a highly adaptive pathogen, as it has
132 been reported to be locally adapted to the host species potato and tomato [15, 18] and generally
133 adapted to particular potato cultivars as well [57-59]. However, to the best of our knowledge,
134 ongoing specific adaptation of *P. infestans* to a particular host cultivar or host species has not yet
135 been established in controlled conditions by experimental evolution experiments. Some evidence
136 arises from an early study of *P. infestans* maintained prior to experiments on chickpea agar [60]. The
137 authors suggest that *P. infestans* could recover initial virulence, but only on its original potato cultivar
138 after serial passages on that specific cultivar.

139

140 The present work investigates the lability of specialization in *P. infestans* interactions with
141 potato and tomato using experimental evolution, and explores molecular mechanisms potentially
142 involved in host specialisation. For this purpose, expression of nine effector genes – selected for their
143 established or presumed functions in pathogenicity – were assessed concomitantly to the level of
144 host specialization. Our underlying hypotheses were that i) the original adaptation patterns
145 (generalist or potato specialist) could be altered by serial passages through alternate hosts, ii)
146 alterations of pathogenicity towards less host specificity would lower the pathogenicity level on the
147 original host, and iii) each adaptation pattern involves specific combinations or kinetics in the
148 expression of pathogenicity-related effector genes. Our results proved these hypotheses to be
149 wrong, but highlighted the importance of the biotrophic stage in the determination of host specificity
150 in *P. infestans*. More specifically, they showed that the ability for the pathogen to maintain the
151 biotrophic stage for a long enough period is crucial in the successful exploitation of the host.

152

153

154

155

156 **Results**

157

158 **Pathogenic fitness of *Phytophthora infestans***

159

160 ***P. infestans* isolates differ in their level of host specialization.** Before experimental evolution, both
161 *P. infestans* isolates were equally fit on potato but their level of pathogenic fitness differed markedly
162 on tomato, the isolate collected on tomato (15.TII.02) being fitter than the isolate collected on
163 potato (15.P50.09) (Fig 2A). Each isolate appeared to be fitter on its host of origin than on the other
164 host (Fig 2A). This was most noticeable and statically significant for the potato isolate ($P = 0.005$), but
165 less pronounced and not statistically significant for the tomato isolate ($P = 0.08$). Considering potato
166 and tomato as different ecological niches, the Levin's measure of standardized niche breath B_A (scale
167 0-1) differed significantly between both isolates ($P = 0.03$), confirming isolate 15.TII.02 ($B_A = 0.88$; se =
168 0.06) to be more generalist than isolate 15.P50.09 ($B_A = 0.61$; se = 0.04).

169

170 **Subculturing hosts do not differentially impact pathogenic fitness.** Pathogenic fitness of replicate
171 lineages subcultured nine times on either potato, tomato or on alternating hosts did not significantly
172 differ (Fig 2B). This was true for both isolates and whatever the tested host (potato and tomato),
173 showing that subculturing habitats had no differential effect on the pathogenic fitness of *P. infestans*
174 isolates. However, there was a slight general increase of pathogenic fitness during experimental
175 evolution, that was statistically significant only for the tomato isolate.

176

177 **Host specialization is a stable trait (at least over a short-term).** After experimental evolution, the
178 pathogenic fitness of both isolates was still similar on potato and still highly different on tomato (Fig
179 2B, compare to Fig 2A). As before experimental evolution, the difference in pathogenic fitness of the
180 tomato isolate on potato and on tomato was still less obvious than for the potato isolate. However, it

181 could be shown here to be statistically significant ($P = 0.02$). Despite of this, the initial difference in
182 pathogenic specialization between both isolates was unchanged during experimental evolution, as
183 the evolved tomato isolate remained more generalist ($B_A = 0.95$; $se = 0.06$) than the evolved potato
184 isolate ($B_A = 0.62$; $se = 0.02$).

185

186 **Effector gene expression**

187

188 **State-specific markers and other genes are differentially expressed at 2 and 4 dpi.** Transcript
189 abundance differed between 2 and 4 dpi (Fig 3). As expected, transcript abundance of *SNE1* and
190 *PexRD2* genes, involved in biotrophy, was clearly higher at 2 than at 4 dpi (Fig 3A). The same was true
191 for the counter-defense genes *AVRblb2*, *EPIC2B*, *EPI1* and *Pi03192*. Transcripts of the stage-specific
192 marker for necrotrophy *PiNPP*, but also of *INF1* and *CA*, were more abundant at 4 dpi than at 2 dpi
193 (Fig 3B). Increased transcript abundance of these necrotrophy-related genes was associated with the
194 presence of macroscopically visible necrotic lesions at 4 dpi.

195

196 **Effector genes are differentially expressed between isolates and hosts.** Transcript abundance
197 differed significantly between isolates and host plants, revealing distinguishable patterns (Fig 4).
198 Transcript abundance of *EPI1*, *EPIC2B*, *CA* and *PiNPP* was significantly higher in the potato isolate
199 than in the tomato isolate, whatever the host (potato and tomato). Transcript abundance of *PiNPP*
200 was also higher in the potato isolate growing on its alternative host, *i.e.* tomato, than on potato (PT >
201 PP), contrasting with the similar transcript abundance found in the tomato isolate grown on each
202 host. Transcript abundance of *PexRD2* was generally higher on potato than on tomato (PP and TP >
203 PT and TT). Interestingly, transcript abundance of *SNE1* was high in the tomato isolate grown on
204 potato compared to other isolate-host combinations (TP > PP, PT and TT). On the opposite, transcript
205 abundance of *INF1* and *AVRblb2* was lower in the potato isolate grown on potato than in other

206 isolate-host combinations (PP < TP, PT and TT). Finally, transcript abundance of *Pi03192* did not
207 significantly differ among all tested isolate-host combinations.

208

209 **Gene expression patterns remain stable in experimental evolution on different hosts.** Overall,
210 similar levels of transcript abundance were measured in *P. infestans* lineages subcultured on potato,
211 tomato or on alternating hosts (Fig 4). However, it is noteworthy that after subculturing on tomato,
212 transcript abundance of the biotrophy related genes *PexRD2* and *SNE1* was slightly – but not
213 significantly – increased on potato relative to other subculturing hosts.

214

215 **Matching pathogenic fitness and effector gene expression**

216

217 **Subculturing on host tissue leads to isolate-specific phenotypic variation.** After nine passages on
218 their original host, the pathogenic fitness of the potato isolate was unchanged whereas that of the
219 tomato isolate significantly increased (Fig 5A). This isolate-specific increase of pathogenic fitness was
220 accompanied by a significantly lower transcript abundance of *AVRblb2* and *EPIC2B* but higher
221 transcript abundance of *Pi03192* and *PiNPP* (Fig 5B). No significant variation of these genes was
222 observed in the potato isolate, with unchanged pathogenic fitness. These isolate-specific effects of
223 subculturing were comparable on both tested hosts, and regardless of the host on which they had
224 been subcultured before. Transcript abundance of the other genes tested (*EPI1*, *SNE1*, *PexRD2*, *INF1*
225 and *CA*) was not significantly different before and after subculture (S1 Fig).

226

227 **Redundancy analysis points to isolate-specific reaction patterns on potato and tomato hosts.**

228 Variation of fitness and effector gene expression – generated by the study of two *P. infestans* isolates
229 on two hosts, before and after experimental evolution in different habitats – is summarized jointly by
230 redundancy analysis (RDA, Fig 6). The final RDA model explains 64.11 % of the total variance in the
231 dataset. The first and second constraint component axis represent 56.54 and 19.35 % of the

232 constrained variance (cumulative 75.89 %), respectively. A third constraint component axis (S2 Fig)
233 represents an additional 15.48 % of the constraint variance (cumulative 91.36 %). The first axis
234 separates isolates (Fig 6A; tomato isolate on the left, potato isolate on the right), the second axis
235 displays a separation according to the host on which the isolates were tested (individuals tested on
236 tomato on the top and individuals tested on potato below). The statistical significance of these
237 arrangements – suggesting the phenotype of isolates to be distinct and to depend on the host – was
238 confirmed by a permutation test for RDA ($P < 0.001$). However, the effect of the subculturing host is
239 not significant ($P = 0.17$) and thus not illustrated. Furthermore, significant interaction terms (isolate x
240 host, $P = 0.003$ and isolate x subculture, $P = 0.002$) point to isolate-specific reaction patterns. As
241 obvious from arrangements on the factorial map, the tomato isolate phenotype is distinct before and
242 after subculture (illustrated by open and filled boxes), but the potato isolate phenotype stayed
243 unaltered (isolate x subculture interaction). On the contrary, the model suggests phenotypic
244 variation of isolates on different hosts to be more pronounced for the potato isolate than for the
245 tomato isolate (isolate x host interaction), consistent with the clear host specificity in this isolate.

246

247 **Pathogenic fitness is correlated with effector gene expression.** Vector alignments in the loading plot
248 of RDA analysis (Fig 6B) indicate pathogenic fitness to be positively correlated with the expression of
249 *SNE1* and *PexRD2* (grouped left arrows), but negatively correlated with that of *PiNPP*, *CA*, *EPI1* and
250 *EPIC2B* (left arrow of fitness opposed to grouped right arrows of effector gene expression). Vectors
251 of fitness and *INF1* are almost orthogonally aligned, indicating no correlation between both variables.
252 The short vector length of *AVRblb2* and *Pi03192* illustrates their low contribution to the RDA model.
253 For verification purpose, RDA analysis was also performed separately on data obtained before and
254 after experimental evolution: vector alignments were consistent in both situations, with the
255 exception of *EPIC2B*. Pairwise correlation tests on unconstrained data (correlations ignoring factor
256 levels) confirmed the statistical significance of all correlative relationships mentioned above (Table
257 1).

258 **Table 1:** Pairwise correlations between fitness and effector gene expression of *P. infestans*.

259 Pearson product-moment correlation coefficient *r* and *p*-values were calculated on unconstrained

260 data (*n* = 12, grouped factor combinations). Asterisks indicate significance: *p* < 0.01 **, *p* < 0.001 ***.

gene	r	p-value	
<i>EPI1</i>	-0,65	> 0,0001	***
<i>PiNPP</i>	-0,59	> 0,0001	***
<i>CA</i>	-0,58	> 0,0001	***
<i>EPIC2B</i>	-0,45	0,0013	**
<i>PexRD2</i>	0,40	0,0048	**
<i>SNE1</i>	0,39	0,0068	**
<i>Pi03192</i>	0,15	0,2932	
<i>AVRblb2</i>	0,06	0,7018	
<i>INF1</i>	-0,02	0,8730	

261

262 **Correlations between fitness and effectors are host- and isolate-specific.** Pairwise correlation tests

263 constrained to one host (potato and tomato) or to one isolate (15.P50.09 collected on potato and

264 15.TII.02 collected on tomato) were performed to explore within-group correlations (Fig 7).

265 Correlations between fitness and effector gene expression were statistically significant on tomato

266 but not on potato (except for *EPIC2B*, Fig 7A). Because a visual inspection of correlative data revealed

267 the presence of split data clouds on tomato (two data groups with different means for each variable),

268 each corresponding to data from one isolate, correlation tests performed separately on data from

269 each isolate revealed isolate-specific correlations between pathogenic fitness and transcript

270 abundance (Fig 7B). *PiNPP* and *INF1* were each positively correlated with pathogenic fitness in isolate

271 15.TII.02 (collected on tomato), but negatively correlated with fitness in isolate 15.P50.09 (collected

272 on potato). *PexRD2* was not significantly correlated with pathogenic fitness in isolate 15.TII.02, but

273 positively correlated in isolate 15.P50.09.

274 Discussion

275 In the present work, quantitative differences in host specialization of *Phytophthora infestans* to
276 potato and tomato were analyzed in terms of stability over serial passages through different hosts
277 and in relation to effector gene expression. The two *P. infestans* isolates included in this study were
278 chosen to display distinct levels of host specialization [15]: the isolate from tomato performed
279 equally well on both hosts (therefore called a generalist), but the isolate from potato, highly
280 pathogenic on its original host, struggled on tomato (therefore called a potato-specialist). As such a
281 pattern has also been consistently reported from other studies [17, 18, 44, 61], the quantitative
282 differences in terms of specialization on potato and on tomato of the isolates studied here may be
283 considered to be representative for *P. infestans* populations on these hosts.

284

285 Our work clearly illustrates the absence of ongoing specific adaptation of *P. infestans* to potato and
286 to tomato in controlled conditions: initial differences in pathogenic specialization were maintained
287 throughout the experimental evolution. This may not be intuitive *a priori* for at least two reasons.
288 First, pathogenicity changes during experimental evolution has been documented for various
289 pathosystems, and in many cases the increase of fitness/virulence/growth was specific for the
290 alternative host on which the parasite had been passed and associated with attenuation of these
291 traits on the original host [53]. Depending on the pathosystem, as little as one [54] or only few
292 passages [55, 56] on alternative hosts may be sufficient to obtain such patterns. Second, *P. infestans*
293 is best described as a highly adapted pathogen as it has been reported to be locally adapted to the
294 host species potato and tomato [15, 18] and generally adapted to particular potato cultivars as well
295 [57-59]. There are even evidences that tomato isolates could evolve from a local potato population
296 and acquire specialization to tomato [43, 52]. However, to our knowledge, there is only timid
297 evidence for the possibility of ongoing specific adaptation of *P. infestans* to a particular host cultivar
298 or host species in controlled conditions [60]. Results from our experimental evolution experiment do
299 not contribute on this, but are rather in line with other studies on *P. infestans* stating the absence of

300 ongoing specific adaptation to a particular host cultivar [59, 62] or host species [61, 63] in controlled
301 conditions. We therefore conclude that the levels of quantitative adaptation of *P. infestans* to
302 different host – and therefore the level of host specialization – is a stable trait, at least on the short
303 term and in the conditions of the test. If the here realized number of nine serial passages equals as
304 believed the number of pathogen generations during a growing season in the field, we may speculate
305 that the observed isolate-specific levels of host specialization are not inverted in the field. Most
306 obviously, potato isolates struggling on tomato are not expected to get adapted to tomato during
307 one growing season. We cannot exclude the existence of additional evolutionary forces in natural
308 environments that could accelerate the process of adaptation, but the durable population separation
309 in the field [48, 64] and results from a competitive experiment in field plots [65] are in favor to our
310 view that host specialization is a stable trait. Interestingly, stability of specific adaptation of *P.*
311 *infestans* to a particular host during the experimental evolution experiment was related to unaltered
312 patterns of concomitantly assessed expression of effector genes.

313

314 The comparison of effector gene expression among host-isolate combinations also points to isolate-
315 and host-specific patterns. First, the effectors *EPI1*, *EPIC2B*, *CA* and *PiNPP1.1* provided a highly similar
316 profile: their expression was rather similar on both hosts, but differed greatly between both isolates.
317 Second, *PexRD2* and *INF1* were rather similarly expressed in both isolates but their expression level
318 differed between both hosts. These patterns of effector gene expression may reflect isolate and host
319 properties and possibly contributes to variability of pathogenic fitness among host-isolate
320 combinations. Following a validation step on more than two isolates, the observed isolate-specific
321 pattern of *EPI1*, *EPIC2B*, *CA* and *PiNPP1.1* could also be used as markers to distinguish both isolate
322 types (isolates from potato, isolates from tomato).

323

324 Despite the absence of specific adaptation of *P. infestans* to a particular host, we observed
325 pathogenic fitness after the experimental evolution experiment to be generally increased compared
326 to fitness of the same isolates before experimental evolution. Increasing fitness due to serial
327 passages on a given host has also been observed elsewhere for *P. infestans* on different potato
328 cultivars [66] and from other fungal pathosystems [67, 68]. The general increase of pathogenic
329 fitness could be due to the fact that *P. infestans* was maintained as axenic culture before
330 experiments. It has been established that *P. infestans* loses pathogenicity when maintained on
331 artificial culture medium [60]. The reason for the loss of pathogenicity during axenic culture has not
332 yet been established, but it may be linked to the absence of living host tissue. In fact, in natural
333 conditions, *P. infestans* has a biotrophic lifestyle and no or only limited saprophytic survival ability in
334 the absence of a living host [14, 69]. The increase of pathogenic fitness was only significant for the
335 isolate from tomato. We could thus speculate that the tomato isolate, highly biotrophic on tomato,
336 has lost more pathogenicity than the potato isolate during axenic culture. This could explain the
337 stronger rate of recovery during serial passages on living host tissue. Increased fitness of the isolate
338 at the end of the experimental evolution experiment was related to an altered expression of some of
339 the tested effector genes (*AVRblb2*, *EPIC2B*, *PiNPP1.1*, *PiO3192*). Among these genes, the expression
340 of the protease inhibitor *EPIC2B* was most noticeably altered: it was strongly reduced after
341 experimental evolution in the fitter tomato isolate. Interestingly, no change in effector gene
342 expression was observed for the potato isolate whose fitness was unaltered by experimental
343 evolution. These results show that – even in absence of specific adaptation of *P. infestans* to a
344 particular host – fitness may vary over time and is accompanied by variation of effector gene
345 expression.

346

347 Correlation analysis of data for pathogenic fitness and effector gene expression showed
348 biotrophy to be a major clue to explain quantitative differences in specialization of *P. infestans* to
349 potato and tomato. Fitness was found to be positively related with the expression of *SNE1* and

350 *PEXRD2*, two effector genes that have been previously associated with biotrophy in regard to their
351 timely expression and their ability to oppose cell death [36, 70]. As expected by its documented
352 necrosis-triggering activity [37], effector *PiNPP1.1* was also negatively related with fitness in the
353 present study. The antagonistic activity on cell death of *SNE1* and *PiNPP1.1*, supported by our data,
354 has actually been proposed as a potential mechanism for *P. infestans* to control the duration of
355 biotrophy [38, illustrated model, 71-76]. Comparing gene expression in the two isolates included in
356 this study on their respective original and alternative hosts also suggests a similar antagonistic
357 interaction between *PiNPP1.1* and *PexRD2*. Consistent with this view, the potato-specialized isolate
358 coordinately increased expression of *PiNPP1.1* and reduced expression of *PexRD2* on tomato, relative
359 to the expression of both genes on its original host. The resulting stronger host cell death and shorter
360 biotrophic period were associated with a drastically lower pathogenic fitness. This relationship
361 between reduced biotrophy and reduced pathogenic fitness is consistent with the view that *P.*
362 *infestans* is a biotrophic pathogen that requires living cells to feed on.

363

364 Why would potato-specialized isolates of *P. infestans* trigger early host cell death on their
365 alternative tomato host by increased secretion of apoptosis-related effectors (i.e. *PiNPP1.1*) if this is
366 negatively related with fitness? A biological explanation refers to the “increasing plant defense”
367 theory [reviewed by 7], which claims that (hemi-)biotrophic pathogens eventually kill the host cells
368 they infected to protect themselves as a last resort against steadily increasing host defenses.
369 Particularly strong evidence for this theory is provided by work on the fungal pathogens
370 *Magnaporthe oryzae* [77] and *Colletotrichum graminicola* [78]: the authors found that the duration
371 of the biotrophic stage fits the speed of increasing host defense. Our results, as well as literature
372 reports on *P. infestans* [37, 79], suggest that highly expressed host defenses also force this otherwise
373 biotrophic pathogen to actively trigger host cell death (Fig. 8). Literature reports on steadily
374 increasing host defense during *P. infestans* infectious process [80, 81], and their more or less
375 successful effector-mediated suppression [72, 82] strengthen this view.

376

377 In addition to the cell death antagonistic effectors *PiNPP1.1 - SNE1/PexRD2*, we also studied
378 the expression of the counter-defense protease inhibitors *EPI1* and *EPIC2B* [27, 30], that were both
379 found to be negatively related to pathogenic fitness of *P. infestans* on tomato. This relation may be
380 explained through the programmed cell death in hypersensitive reactions (HR PCD), a pathogen-
381 triggered host response to infection that is associated with strong immune responses [83]. The direct
382 implication of *EPI1* and *EPIC2B* in host cell death has not yet been conclusively established, but we
383 argue – based on evidence from literature – that *EPI1* and *EPIC2B* protease inhibitor activity of these
384 effectors could modulate *P. infestans* interactions by blocking immunity related programmed cell
385 death in the host (Fig. 9 and S1 Appendix). This hypothesis is based on two main facts: (i) *EPI1* and
386 *EPIC2B* jointly inhibit protease activity in the tomato apoplast [30] and (ii) at least one of these
387 inhibited proteases (*PIP1*) is required for immunity-related PCD in tomato [84]. We thus hypothesize
388 that *P. infestans* could address PCD during the HR by secreting proteases inhibitors that target cell
389 death-related host proteases. On that condition, expression of proteases inhibitors by *P. infestans*
390 may reflect the intensity of host immune signaling for HR PCD encountered in the tested host-isolate
391 combinations. There is indeed literature evidence that *P. infestans* protease inhibitors are specifically
392 up-regulated in host tissue compared to culture medium [25], and are expressed concomitantly to
393 their respective host targets [27, 30]. We can thus expect a strong host immune signaling for HR PCD
394 in situations where *EPI1* and *EPIC2B* expression is increased, and only a low host immune signaling
395 for HR PCD in situations with low expression of these protease inhibitors. The negative correlation
396 with fitness may be explained by the continuous increase of other immune responses lowering
397 pathogenic fitness. It is now well established that HR is not limited to PCD, but also involves the
398 strong accumulation of various defense compounds and the rapid expression of defense-related
399 genes [85, 86-88]. This apparently also applies to *P. infestans*, where HR PCD is ubiquitous [10, 11,
400 71, 72, 89] and positively related to timing and/or intensity of defense [71-76]. We therefore
401 speculate that the short duration of biotrophy in the potato isolate-tomato interaction results from a

402 strong host immune response. In the absence of experimental evidence, the severe and fast cell
403 death observed may be either plant-controlled HR PCD and/or pathogen-controlled cell death in
404 response to overwhelming defenses (increasing defense theory, Fig. 8). In both cases, necrosis would
405 be associated with low pathogenic fitness of *P. infestans*.

406

407 To conclude, the set of results reported here clearly shows that the level of pathogenic
408 specialization of *P. infestans* to potato and tomato is a biotrophy-related trait, and that is unaltered
409 by the different tested conditions of our experimental evolution. Our data strongly support the idea
410 that pathogenic fitness of *P. infestans* is positively related with the duration of biotrophy, itself finely
411 regulated by the balance in the expression of antagonistic necrosis-inducing (PiNPP) and necrosis-
412 opposing (SNE1, PexRD2) effectors, and that this balance itself depends on the time required for host
413 defenses to reach a threshold level. Future research may address this hypothesis, by studying
414 inducible host defenses in response to a range of *P. infestans* isolates collected from different hosts.
415 As they stand, these conclusions and hypotheses however carry wide-ranging consequences for the
416 biology and management of late blight, and possibly of similar pathogens (downy mildews in the first
417 place). Indeed, they show that *P. infestans*, often called a hemibiotroph [15-18], should rather be
418 seen as an ‘imperfect biotroph’, since its basic infectious process and trophic mode is biotrophic, but
419 eventually fails to keep its host alive like ‘perfect biotrophs’ (rust or powdery mildews, for instance)
420 do for extended periods of time. The negative association between host damage and parasitic
421 fitness, clearly evident from our data, tend to revert the commonly held equation that increased
422 aggressiveness (*i.e.* faster and more extensive necrosis of host tissue) equates with greater fitness
423 and invasion potential in *P. infestans* ([see e.g. 19]. This, together with the stability of adaptation
424 patterns over serial passages though different hosts, open new and important ways to better control
425 late blight through host genetic resistance.

426

427 **Material and Methods**

428

429 **Plant material**

430 The potato cultivar Bintje and the tomato cultivar Marmande – both highly susceptible to late blight
431 – were grown in a glasshouse as described by Kröner et al. [15]. Fully developed young leaflets were
432 collected from seven week-old plants for experiments.

433

434 ***Phytophthora infestans* isolates**

435 The *P. infestans* isolates 15.P50.09 (from potato, EU_13_A2_86 clonal lineage) and 15.TII.09 (from
436 tomato, EU_23_A1 clonal lineage) were collected in 2015 in France from naturally infected potato
437 and tomato crops. They were selected for this study on the basis of previously available genotypic
438 and phenotypic data [15]: they belong to distinct clonal lineages that are – at least in France –
439 typically separated by the host (potato/tomato) and provide the usual pattern of pathogenic fitness
440 *in vitro* on these hosts (tomato isolates perform overall well, but potato isolates struggle on tomato).
441 During six months, both isolates were maintained by serial transfers on pea broth agar medium in
442 darkness at 15°C [90].

443

444 **Experimental evolution experiment design**

445 The experimental evolution experiment (S3 Fig) consisted in subculturing *P. infestans* isolates on
446 detached leaflets: either on the original host (from which the isolate had been collected), on the
447 alternative host (e.g. on tomato leaflets if the isolate had been collected on potato), or on alternating
448 hosts (in turns on potato and on tomato). The same inoculum source was used to initiate these
449 conditions for experimental evolution. For each condition, three replicated lineages were
450 subcultured nine times at intervals of seven days. Inoculum ($3 \cdot 10^4$ sporangia.mL⁻¹) was prepared in
451 sterile water from sporangia produced on previously infected leaflets. New leaflets were inoculated
452 on the abaxial side with four drops (each 20 µL) of this suspension. A detailed description of

453 inoculum preparation is available in Kröner et al. [15]. During the experimental evolution experiment,
454 temperature was maintained at 18/15°C (day/night) and the incubation chamber was illuminated to
455 obtain 16 h day length. Fitness and effector gene expression of *P. infestans* were assessed
456 simultaneously, before and after experimental evolution.

457

458 **Estimation of pathogenic fitness**

459 A single fitness estimate, representing the mean fitness of *P. infestans* from three lineages (biological
460 replicates), was calculated as proposed by Montarry et al. [91]. Relevant life-history traits of *P.*
461 *infestans* (latency, lesion size and sporangia production) were measured on potato and tomato
462 leaflets. Experimental conditions equal those encountered during the experimental evolution
463 experiment, with the exception that only one drop of inoculum (instead of four) was placed next to
464 the center of a detached leaflet. To determine latency period, six replicate leaflets per replicated
465 lineage (6 leaflets x 3 lineages = 18 leaflets in total) were inspected for sporangia formation at 24 h
466 intervals, beginning two days post inoculation (dpi). At five dpi, lesion size and sporangia production
467 were assessed on the same leaflets, as described by Montarry et al. [90]. The two constants of the
468 fitness model were fixed as follows: the underlying leaflet size of potato and tomato (X) was
469 determined experimentally to 20.3 cm², and the assumed time available to exploit the leaf (1/μ) was
470 set to five days. Fitness data (S1 Table) was used to calculate the Levin's measure of standardized
471 niche breath B_A on a scale from zero to one [15, 92]. For these calculations, potato and tomato were
472 considered as possible hosts of *P. infestans*. A low niche breath points to a high level of specialization
473 on one of these hosts, and *vice versa*.

474

475 **Effector gene expression**

476 **Sample preparation.** Effector gene expression was assessed at the same time as fitness in order to
477 assure identical conditions: same batch of leaflets, sporangia suspension and inoculation conditions.
478 Leaf discs (22 mm in diameter, centered on the site of inoculation) were sampled at 2 dpi and 4 dpi.

479 For each replicated lineage (three biological replicates), six leaf discs (one leaf disc per infected
480 leaflet) were grouped, shock frozen in liquid nitrogen and lyophilized. The sample preparation system
481 FastPrep-24 (MPbio) was used to obtain a homogenous powder. This was achieved by three
482 successive 30-second runs at speed 4. The homogenous powder was aliquoted to obtain two 5 mg
483 samples (two technical replicates of crushed tissue) that were stored at -80°C prior to RNA
484 extraction.

485

486 **RNA extraction, quality control and cDNA synthesis.** For each replicated lineage (three biological
487 replicates), total RNA was extracted separately from two 5 mg crushed-tissue samples by using the
488 SV Total RNA Isolation System (Promega), according to the manufacturer's instructions. The
489 suitability of this protocol for high quality RNA extraction – in the present experimental conditions –
490 was initially confirmed by calculating the RNA Integrity Number (RIN) from random samples by using
491 the 2100 Bioanalyzer (Agilent Technologies). During routine operation, total RNA was quantified by
492 the NanoDrop 1000 spectrophotometer (Thermo Scientific) and RNA quality was confirmed by
493 agarose gel electrophoresis. Synthesis of cDNA was performed with 1 μg of total RNA by using the
494 GoScriptTM Reverse Transcription System (Promega), according to the manufacturer's
495 recommendations. The absence of genomic DNA contamination was confirmed by amplification of an
496 intron-spanning region of the elongation factor 1 alpha (EF-1 α , GenBank: DQ284495), as described by
497 Rosati et al. [93]. The cDNA samples were stored at -80°C .

498

499 **qPCR primers, amplification conditions and calculations.** qPCR primers for eight *P. infestans* effector
500 genes (*AVRblb2*, *EPIC2B*, *EPI1*, *PexRD2*, *SNE1*, *PiNPP1.1*, *INF1*, *PiO3192*), the candidate effector gene
501 coding Carbonic Anhydrase (*CA*) and three reference genes (*ACTA*, *BTUB*, *EF2A*) have been designed
502 and optimized for this study (Table S2). Specificity of these primers was checked by sequencing
503 amplicons in both directions. Real-Time qPCR was performed twice on each cDNA sample (two
504 technical replicates of analysis) by using Lightcycler 480 SYBR Green I Master (ROCHE) chemistry in

505 combination with the Lightcycler 480 II system (ROCHE). PCR reaction volumes of 10 μ L contained:
506 1.5 μ L PCR-grade H₂O, 0.5 μ L of the forward and 0.5 μ L of the reverse primers at a concentration of
507 10 μ M respectively, 5 μ L Master Mix (2X conc.) and 2.5 μ L of cDNA template. Amplifications were
508 performed in 384-well plates under the following cycling conditions: 15 min at 95°C; 39 cycles of 15 s
509 at 95°C, 30 s at 62°C (but 64°C for *EPI1*, *ACTA* and *BTUB*) and 30 s at 72 °C. Melting curve analysis
510 was performed from 62°C to 96°C. Transcript abundance of effector genes was calculated by using
511 the relative quantification method described by Pfaffl [94]. To take into account for possible
512 variations of PCR efficiency, separate standard curves from template dilution series were included for
513 each PCR run x primer set x isolate combination (e.g. PCR run 1 x *AVRblb2* primer x isolate
514 15.P50.09). To calculate transcript abundance, Ct (threshold cycle) values were first transformed to
515 linear scale expression quantities. Expression of the target gene was then normalized in respect to
516 the geometric mean of the three reference genes. These normalized transcript abundance data (S3
517 Table) are expressed in this article as the means from three replicated lineages of *P. infestans* (three
518 biological replicates).

519

520 **Statistical analyses**

521 Statistical analyses were performed using the statistical software R version 3.1.1 [95]. The means of
522 fitness and transcript abundance of effector coding genes were calculated from three replicated
523 lineages of *P. infestans* (three biological replicates), after having averaged – for each replicate lineage
524 – results from technical replicates (for fitness: life-history traits measured on six leaflets; for
525 transcript abundance: two crushed-tissue samples from six leaflets x two PCR analysis).

526

527 Null hypotheses were rejected if $P < 0.05$. Data were transformed to logarithms or square roots when
528 necessary before performing analysis of variance (ANOVA), analysis of covariance (ANCOVA),
529 multiple comparisons of means with adjusted P -values (Student's t-tests), tests for association
530 between paired samples (Pearson's product moment correlation coefficient) and Redundancy

531 Analysis (RDA, data prepared for multivariate analysis is available in S4 Table). A permutation test for
532 constrained multivariate analyses was performed to test the significance of discrimination between
533 factors included in the RDA full model (experiment, isolate, subculturing host, and tested host). The
534 full model was then simplified by excluding the factor “subculturing host”, as model comparison
535 revealed no significant difference.

536

537

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547

548

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773

774 **Supporting information captions**

775

776 **S1 Appendix. Hypothesis - The proteases inhibitors EPIC2B/EPI1 block immunity-related**
777 **programmed cell death.**

778

779 **S1 Fig. Effector genes equally expressed before and after experimental evolution.** Two *P. infestans*
780 isolates collected on potato (P-isolate 15.P50.09) and on tomato (T-isolate 15.TII.02) had been
781 subcultured nine times on their original host: the P-isolate on the potato cv. Bintje and the T-isolate
782 on the tomato cv. Marmande. Effector gene expression was assessed before (dark grey bars) and
783 after subculturing (white bars). Both measurements were performed on the potato cv. Bintje and on
784 the tomato cv. Marmande, but results were averaged as the effects of subculturing were similar on

785 both tested hosts. For effector genes shown here, pairwise t-tests revealed no significant differences
786 in transcript abundance before and after nine times subculture (n=6, significance level $\alpha = 0.05$, n.s. =
787 not significant).

788

789 **S2 Fig. Loading plot of redundancy analysis.** Correlations among effector genes and fitness are
790 displayed on the first and the third constrained component axis, representing respectively 56.54 and
791 15.48 % (cumulative 72.02 %) of the constraint variance.

792

793 **S3 Fig. Experimental evolution experiment design.** *Phytophthora infestans* inoculum was prepared
794 on the host from which the isolate had been collected. This base inoculum served to start
795 experimental evolution, consisting in nine times subculture on detached leaflets: either on the
796 original host (from which the isolate had been collected), on the alternative host (e.g. on tomato
797 leaflets if the isolate had been collected on potato), or on alternating hosts (in turns on potato and
798 tomato). Each of these lineages was replicated three times. Fitness and effector gene expression of *P.*
799 *infestans* on potato and tomato were assessed simultaneously, before and after experimental
800 evolution.

801

802 **S1 Table. *Phytophthora infestans* pathogenic fitness.**

803

804 **S2 Table. RT-PCRq primer sequences for genes of interest and reference genes, amplicon length
805 and primer efficiency.**

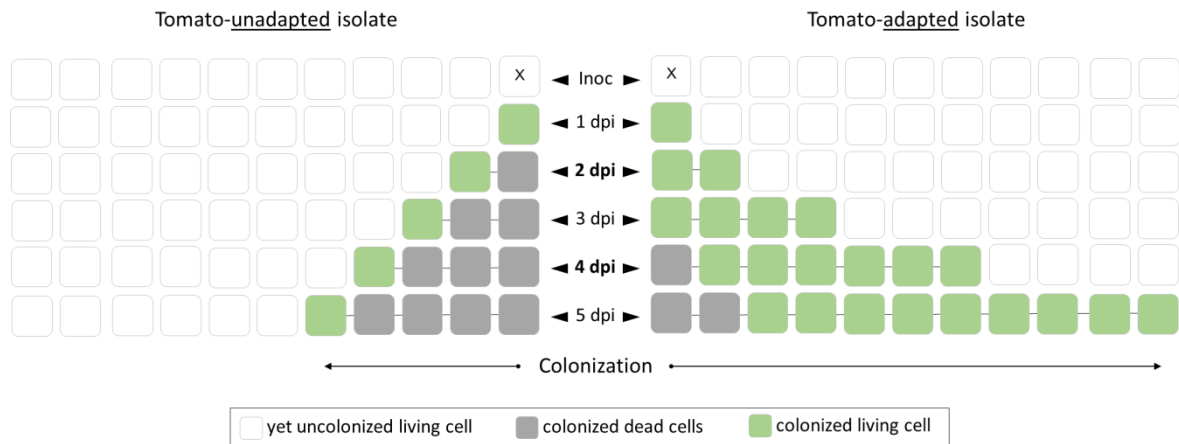
806 **S3 Table. *Phytophthora infestans* effector gene expression (normalized transcript abundance).**

807

808 **S4 Table. *Phytophthora infestans* mean fitness and effector gene expression (normalized transcript
809 abundance) for RDA analysis.**

810 **Figures**

811



812

813 **Fig 1. Colonization of tomato leaf tissue by adapted and unadapted *Phytophthora infestans***

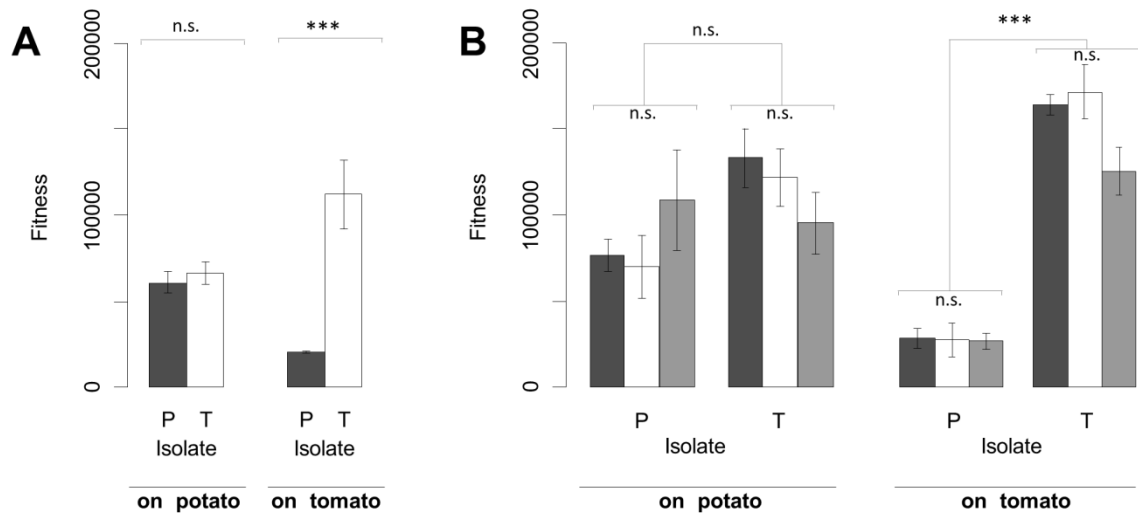
814 **isolates.** This schematic view illustrates spatio-temporal changes of host cell status

815 (uncolonized/colonized, living/dead) in tomato leaf tissue typically observed during colonization by

816 differentially adapted *P. infestans* isolates. Horizontal reading (line-by-line) illustrates the spatial

817 aspect of colonization at one to five dpi respectively. Vertical reading (column-by-column) illustrates

818 the temporal status of a given host cell.



819

820 **Fig 2. Pathogenic fitness of *Phytophthora infestans* before and after experimental evolution. Two *P.***

821 *infestans* isolates collected on potato (P-isolate, 15.P50.09) and on tomato (T-isolate, 15.TII.02) were

822 cross-inoculated on detached leaflets of the potato cv. Bintje and the tomato cv. Marmande.

823 Pathogenic fitness at five dpi has been assessed two times: before experimental evolution (A) and

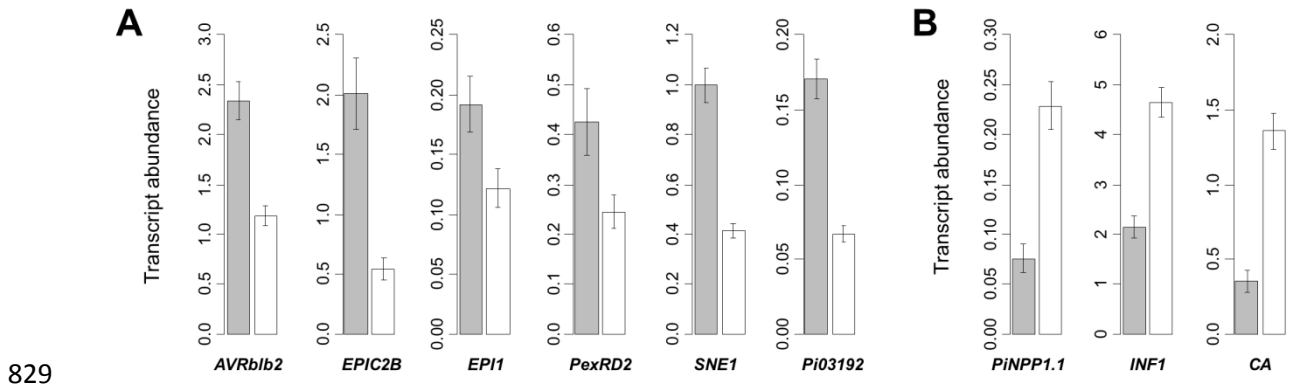
824 after experimental evolution (B). Experimental evolution consisted of subculturing the initial

825 inoculum nine times on potato (dark grey bars), tomato (white bars) and alternately on both hosts

826 (light grey bars). Significance of differences was assessed by ANOVA followed by pairwise t-tests

827 (significance level $\alpha = 0.05$, *** $P < 0.001$, n.s. not significant).

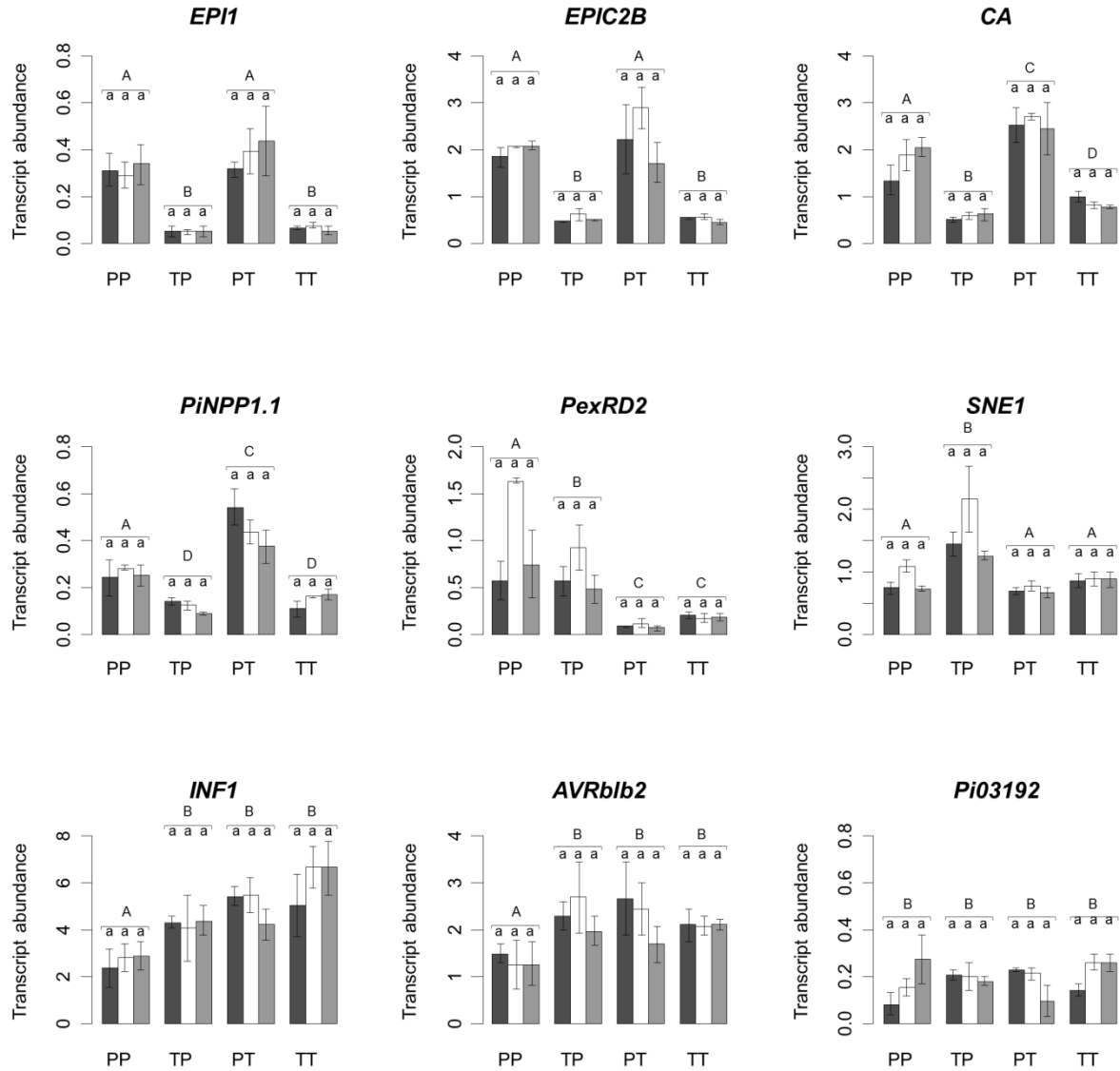
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830 **Fig 3. Effector gene expression in *Phytophthora infestans* at two infection stages.** Transcript
831 abundance has been assessed at the early biotrophic (2 dpi, grey bars) and at the later
832 transitory/necrotrophic stage of infection (4 dpi, white bars). Some genes were found to be more
833 expressed at 2 dpi (**A**), but others at 4 dpi (**B**). The relative timing of gene expression was the same
834 for both isolates (15-P50.09 and 15-TII-02) tested on both hosts (the potato cv. Bintje and the tomato
835 cv. Marmande), before and after nine times subculturing. Results are thus presented as the global
836 mean of these factors (n = 96).

837



838

839 **Fig 4. Effector gene expression of *Phytophthora infestans* after experimental evolution. Two *P.***

840 *infestans* isolates collected on potato (P-isolate 15.P50.09) and on tomato (T-isolate 15.TII.02) have

841 been subcultured nine times on potato (dark grey bars, n=3), on tomato (white bars, n=3) and

842 alternately on both hosts (light grey bars, n=3). Transcript abundance of these experimentally

843 evolved isolates was assessed on the potato cv. Bintje and the tomato cv. Marmande at two dpi

844 (*INF1*, *CA* and *PiNPP* at four dpi), resulting in the following isolate-host combinations: P-isolate on

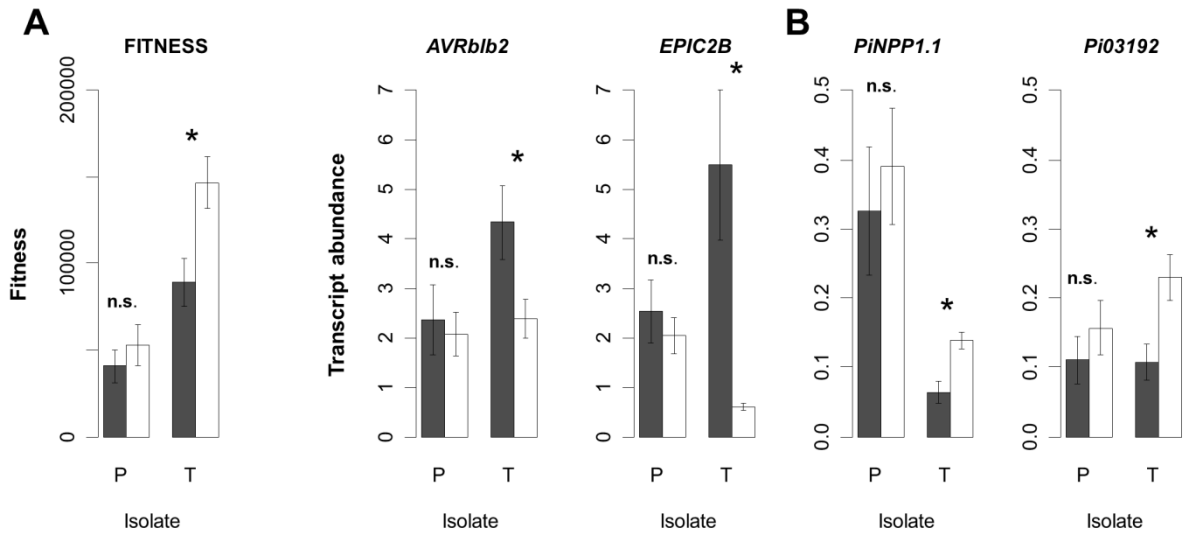
845 potato (PP), T-isolate on potato (TP), P-isolate on tomato (PT) and T-isolate on tomato (TT). Different

846 capital letters above bars indicate significant mean differences between isolate-host combinations

847 (pairwise t-test, n=9, significance level $\alpha = 0.05$). There were no significant differences in transcript
848 abundance among subculturing hosts (see minuscule letters above bars).

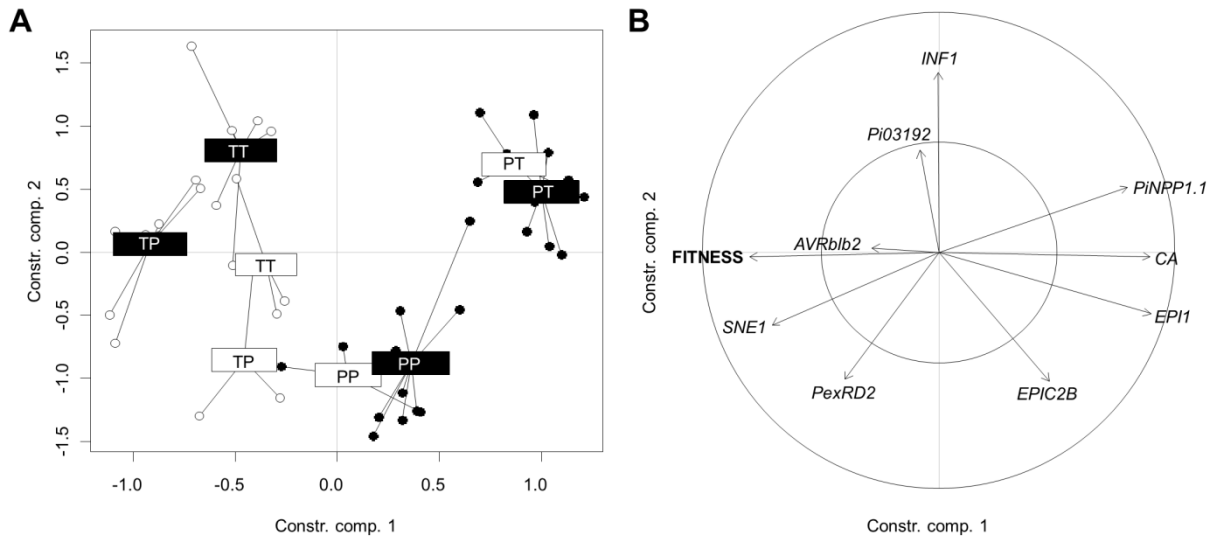
849

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851

852 **Fig 5. Isolate-specific effects of subculturing on fitness and effector gene expression.** Two *P.*
853 *infestans* isolates collected on potato (P-isolate 15.P50.09) and on tomato (T-isolate 15.TII.02) had
854 been subcultured nine times on their original host: the P-isolate on the potato cv. Bintje and the T-
855 isolate on the tomato cv. Marmande. Fitness (**A**) and effector gene expression (**B**) were assessed
856 before (dark grey bars) and after subculturing (white bars). Fitness was assessed at five dpi and gene
857 expression at two dpi (or at four dpi for *PiNPP*). Both measurements were performed on potato and
858 on tomato. Results were averaged as the effects of subculturing were similar on both tested hosts.
859 Significance of isolate-specific mean differences (t-test comparing before and after subculture, n=6,
860 significance level $\alpha = 0.05$) is indicated above bars (n.s. = not significant, * $P < 0.05$).

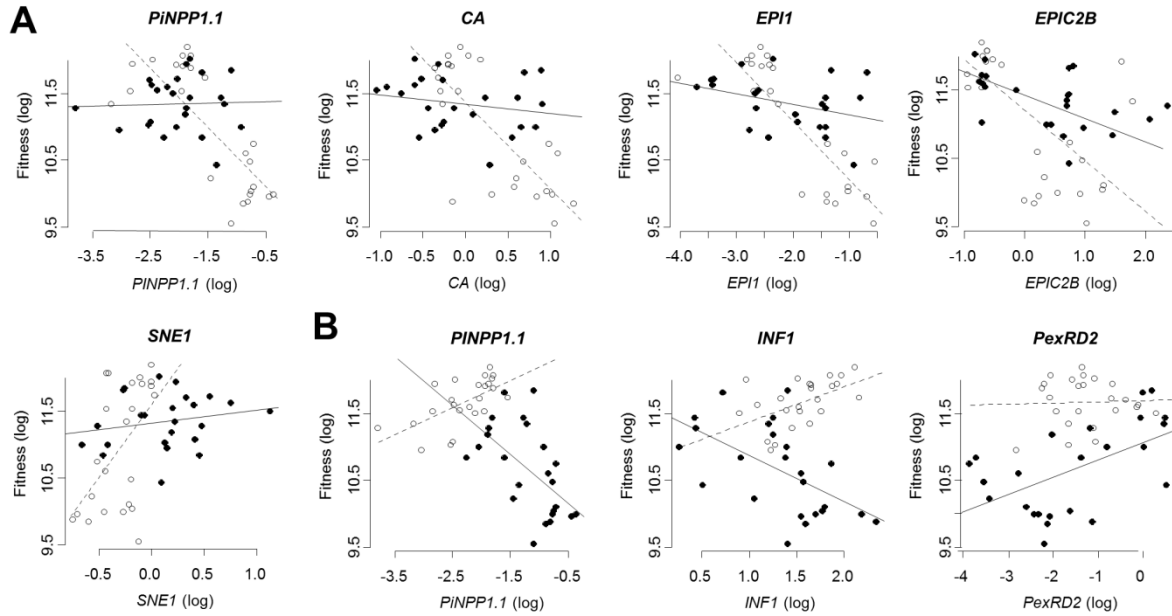


861

862 **Fig 6. Redundancy analysis (RDA) for transcript abundance and fitness of *Phytophthora infestans*.**

863 **(A)** A factorial map separating factor combinations. White boxes represent centroids before
864 experimental evolution and black boxes centroids after experimental evolution. Isolate-host
865 combinations are abbreviated as follows: P-isolate on Potato (PP), T-isolate on Potato (TP), P-isolate
866 on Tomato (PT) and T-isolate on Tomato (TT). Subculturing habitats (on potato, on tomato,
867 alternately on both hosts) are not tagged because model comparison with and without the
868 subculturing host variable revealed no significant difference ($P = 0.82$). **(B)** A loading plot showing
869 correlations among effector genes and fitness.

870



871

872 **Fig 7. Within-group correlations between fitness and effector genes of *Phytophthora infestans*. (A)**

873 Data were grouped by tested host. Black circles represent data obtained on potato (cv. Bintje,

874 correlations not significant at $\alpha = 0.05$, except for *EPIC2B*) and white circles represent data on tomato

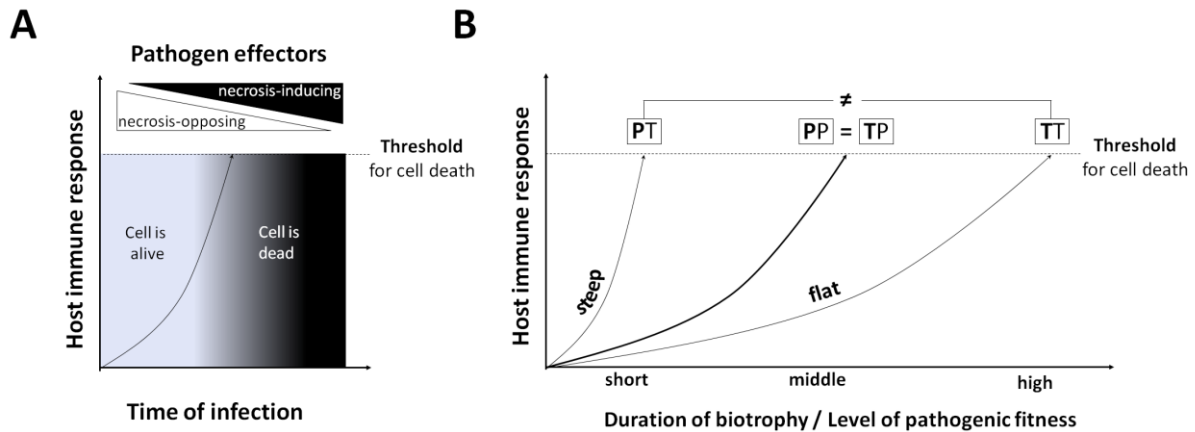
875 (cv. Marmande, correlations highly significant). (B) Data were grouped by isolate. Black circles

876 represent data obtained for isolate 15.P50.09 (collected from potato, significant negative

877 correlations for *PiNPP* and *INF1* but positive correlation for *PexRD2*) and white circles represent data

878 for isolate 15.TII.02 (collected from tomato, highly positive correlations except for *PexRD2*).

879



880

881 **Fig 8. Host immune responses could limit the duration of biotrophy in *Phytophthora infestans***

882 **interactions. (A)** Host immune responses force *P. infestans* to kill its host. It is well established that *P.*

883 *infestans* secretes effectors into the parasitized host cell and that secretion is timely regulated.

884 Biotrophy related effectors are secreted in the early state of the interaction to maintain the host cell

885 alive. With progressing time of infection, secretion of pro-life effectors decreases while necrotrophy

886 related effectors with cell-death inducing activity are increasingly secreted. As the parasitized cells

887 die concomitantly to this switch, a tightly controlled and timely secretion of necrosis-opposing and

888 necrosis-favoring effectors is regarded as a mean of *P. infestans* to regulate the duration of

889 biotrophy. However, the reasons why *P. infestans* – an almost obligate parasite – actively kills host

890 cells is not understood and may even appear counterintuitive. The here advocated explanatory

891 approach refers to the “increasing host defense” theory (see explanations in the text) suggesting

892 biotrophic pathogens to actively kill the host cell as a last resort against an intolerable level

893 (threshold) of host defenses. Literature reports about steadily increasing host defenses in *P. infestans*

894 interactions in conjunction with the here and previously observed timely regulated expression of pro-

895 life/pro-death effector genes (e.g. SNE1/PiNPP1.1) support this view. **(B)** Duration of biotrophy in *P.*

896 *infestans* interactions is positively related with host adaptation. It has been proposed that timing and

897 intensity of host immune responses impact on the outcome of interactions between *P. infestans* and

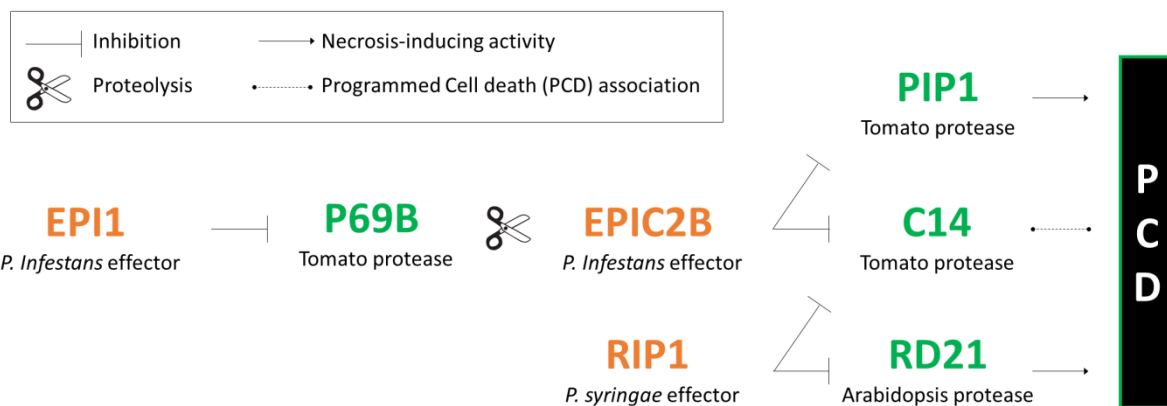
898 its hosts. Results from a range of studies show that immune responses have to be induced timely and

899 strongly to be effective against *P. infestans*. Considering these results and with regard to the theory

900 that strong host immune responses force *P. infestans* to rapidly kill its host (see A), we would expect
901 duration of biotrophy to be positively related with the level of host adaptation. Consistent to this
902 prediction, similar pathogenic fitness of both tested isolates on potato matched macroscopically
903 assessments of lesions. Furthermore, higher fitness of the tomato isolate on tomato in respect to the
904 potato isolate matched a visibly more biotrophic interaction. Isolate-host combinations are
905 abbreviated as follows: P-isolate on Potato (PP), T-isolate on Potato (TP), P-isolate on Tomato (PT)
906 and T-isolate on Tomato (TT).

907

908



909

910 **Fig 9. Protease inhibitors EPI1 and EPIC2B could block immunity-related programmed cell death.**

911 The *P. infestans* effector EPI1 prevents degradation of EPIC2B by inhibiting the tomato protease
912 P69B. The thereby guarded EPIC2B inhibits in turn the tomato protease C14 that has been reported
913 to be associated with stress-induced programmed cell death (PCD). EPIC2B also inhibits the tomato
914 protease PIP1 that has been shown to be required for HCD. Tomato C14 is furthermore inhibited by
915 the *Pseudomonas syringae* effector RIP1 that also inhibits in Arabidopsis the pro-death protease
916 RD21. Altogether, these literature findings show that *P. infestans* could take control about the
917 tomato host cell death machinery by secreting proteases inhibitors targeting cell death-related host
918 proteases (e.g. PIP1, C14).

919