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

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

11

Abstract

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

Author Summary

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

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 effectors 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   *EPI1*0 [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 *PiNPP*1.1 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 specializatio*n* 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. infetans* interactions with

141   potato and tomato using experimental evolution, and explores molecular mecanisms 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 concomittantly to the level of

144   host specialization. Our underlying hypotheses were that i) the original adapation 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 sucessful exploitation of the host.

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155

Results

Pathogenic fitness of *Phytophthora infestans*

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

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

Host specialization is a stable trait (at least over a short-term). After experimental evolution, the pathogenic fitness of both isolates was still similar on potato and still highly different on tomato (Fig 2B, compare to Fig 2A). As before experimental evolution, the difference in pathogenic fitness of the 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: $p < 0.01$ **, $p < 001$ ***.

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

Discussion

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

Our work clearly illustrates the absence of ongoing specific adaptation of *P. infestans* to potato and to tomato in controlled conditions: initial differences in pathogenic specialization were maintained throughout the experimental evolution. This may not be intuitive *a priori* for at least two reasons. First, pathogenicity changes during experimental evolution has been documented for various pathosystems, and in many cases the increase of fitness/virulence/growth was specific for the alternative host on which the parasite had been passed and associated with attenuation of these traits on the original host [53]. Depending on the pathosystem, as little as one [54] or only few passages [55, 56] on alternative hosts may be sufficient to obtain such patterns. Second, *P. infestans* is best described as a highly adapted pathogen as it has been reported to be locally adapted to the host species potato and tomato [15, 18] and generally adapted to particular potato cultivars as well [57-59]. There are even evidences that tomato isolates could evolve from a local potato population and acquire specialization to tomato [43, 52]. However, to our knowledge, there is only timid evidence for the possibility of ongoing specific adaptation of *P. infestans* to a particular host cultivar or host species in controlled conditions [60]. Results from our experimental evolution experiment do 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*, *Pi03192*). 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 'impecfect 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

Material and Methods

Plant material

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

Phytophthora infestans isolates

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

Experimental evolution experiment design

The experimental evolution experiment (S3 Fig) consisted in subculturing *P. infestans* isolates on detached leaflets: either on the original host (from which the isolate had been collected), on the alternative host (e.g. on tomato leaflets if the isolate had been collected on potato), or on alternating hosts (in turns on potato and on tomato). The same inoculum source was used to initiate these conditions for experimental evolution. For each condition, three replicated lineages were subcultured nine times at intervals of seven days. Inoculum (3.10^4 sporangia.mL^{-1}) was prepared in sterile water from sporangia produced on previously infected leaflets. New leaflets were inoculated 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 illumined 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 cm^2, and the assumed time available to exploit the leaf ($1/\mu$) 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-1α, 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*, *Pi03192*), 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 μL contained:

506   1.5 μL PCR-grade H_2O, 0.5 μL of the forward and 0.5 μL of the reverse primers at a concentration of

507   10 μM respectively, 5 μL Master Mix (2X conc.) and 2.5 μL 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

Acknowledgments

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 α = 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**

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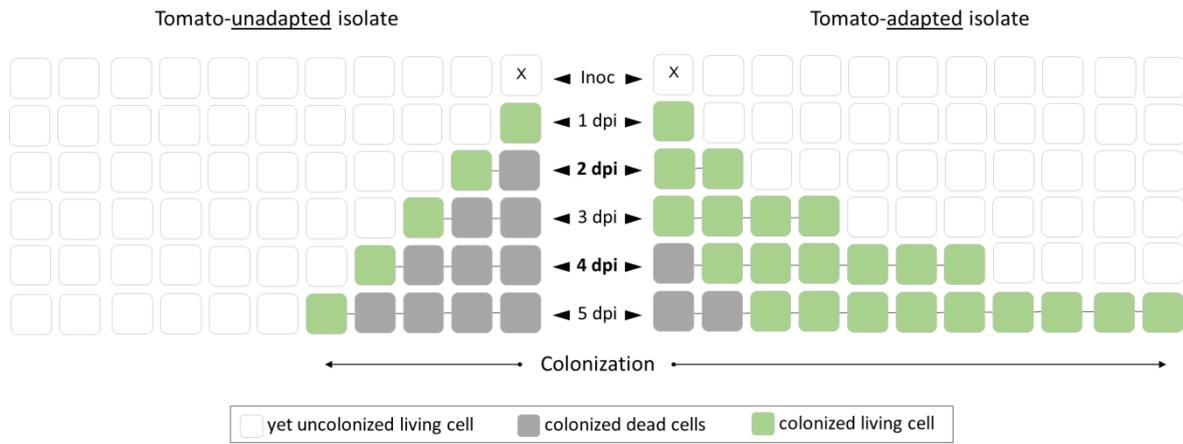
812

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.
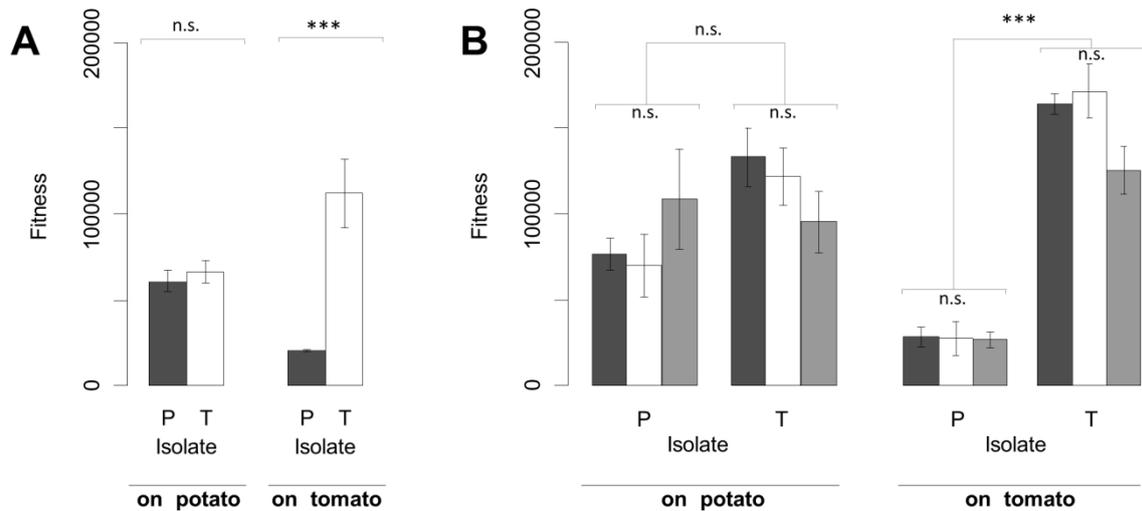
**Fig 2. Pathogenic fitness of *Phytophthora infestans* before and after experimental evolution.** Two *P. infestans* isolates collected on potato (P-isolate, 15.P50.09) and on tomato (T-isolate, 15.TII.02) were cross-inoculated on detached leaflets of the potato cv. Bintje and the tomato cv. Marmande. Pathogenic fitness at five dpi has been assessed two times: before experimental evolution **(A)** and after experimental evolution **(B).** Experimental evolution consisted of subculturing the initial inoculum nine times on potato (dark grey bars), tomato (white bars) and alternately on both hosts (light grey bars). Significance of differences was assessed by ANOVA followed by pairwise t-tests (significance level α = 0.05, *** *P* < 0.001, n.s. not significant).
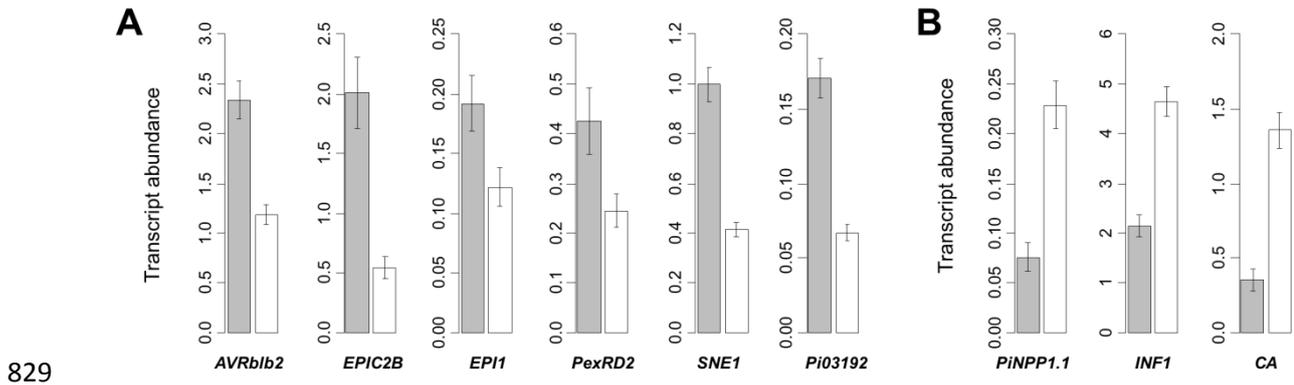
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830   **Fig 3. Effector gene expression in *Phytophthora infestans* at two infection stages.** Transcript

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

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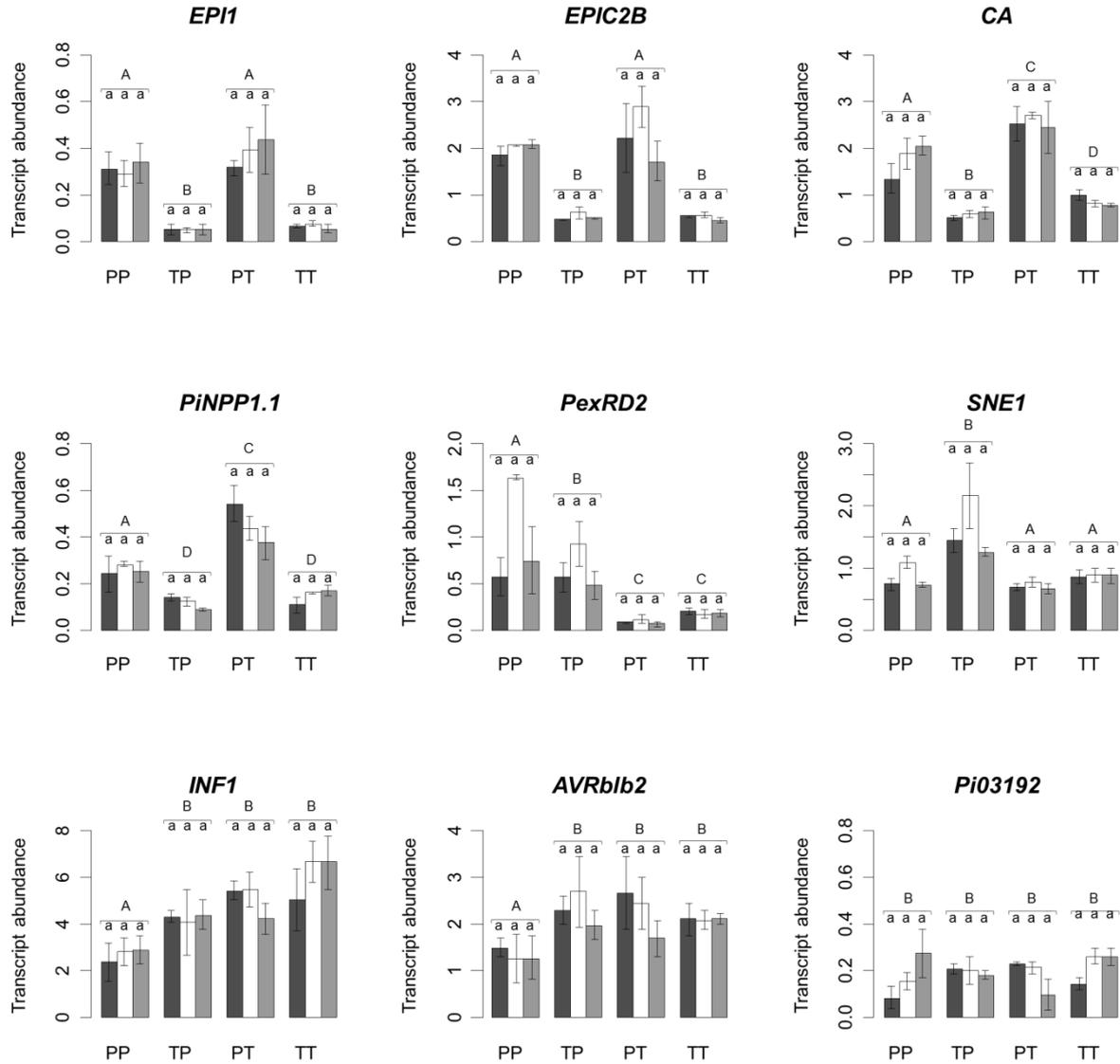
Fig 4. Effector gene expression of *Phytophthora infestans* after experimental evolution. Two *P. infestans* isolates collected on potato (P-isolate 15.P50.09) and on tomato (T-isolate 15.TII.02) have been subcultured nine times on potato (dark grey bars, n=3), on tomato (white bars, n=3) and alternately on both hosts (light grey bars, n=3). Transcript abundance of these experimentally evolved isolates was assessed on the potato cv. Bintje and the tomato cv. Marmande at two dpi (*INF1*, *CA* and *PiNPP* at four dpi), resulting in the following isolate-host combinations: P-isolate on potato (PP), T-isolate on potato (TP), P-isolate on tomato (PT) and T-isolate on tomato (TT). Different capital letters above bars indicate significant mean differences between isolate-host combinations

847   (pairwise t-test, n=9, significance level α = 0.05). There were no significant differences in transcript

848   abundance among subculturing hosts (see minuscule letters above bars).

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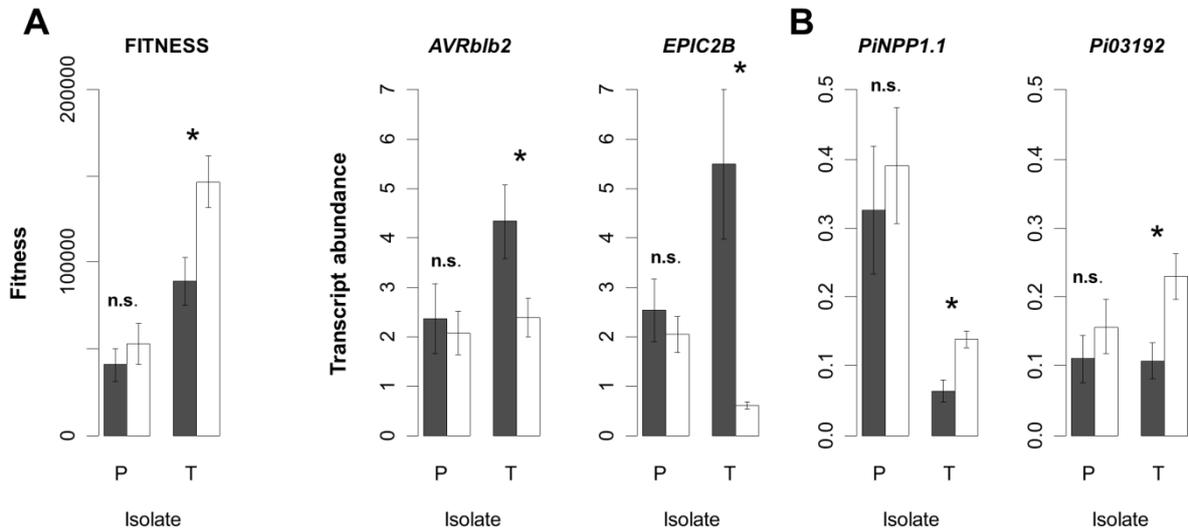
Fig 5. Isolate-specific effects of subculturing on fitness and effector gene expression. Two *P. infestans* isolates collected on potato (P-isolate 15.P50.09) and on tomato (T-isolate 15.TII.02) had been subcultured nine times on their original host: the P-isolate on the potato cv. Bintje and the T-isolate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

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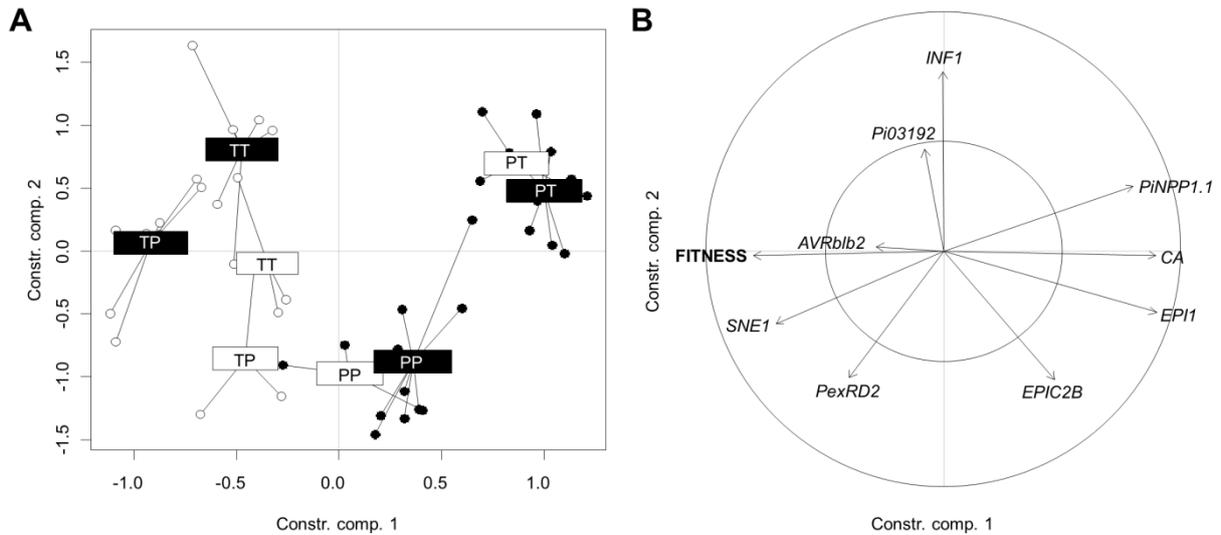
Fig 6. Redundancy analysis (RDA) for transcript abundance and fitness of *Phytophthora infestans*.

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

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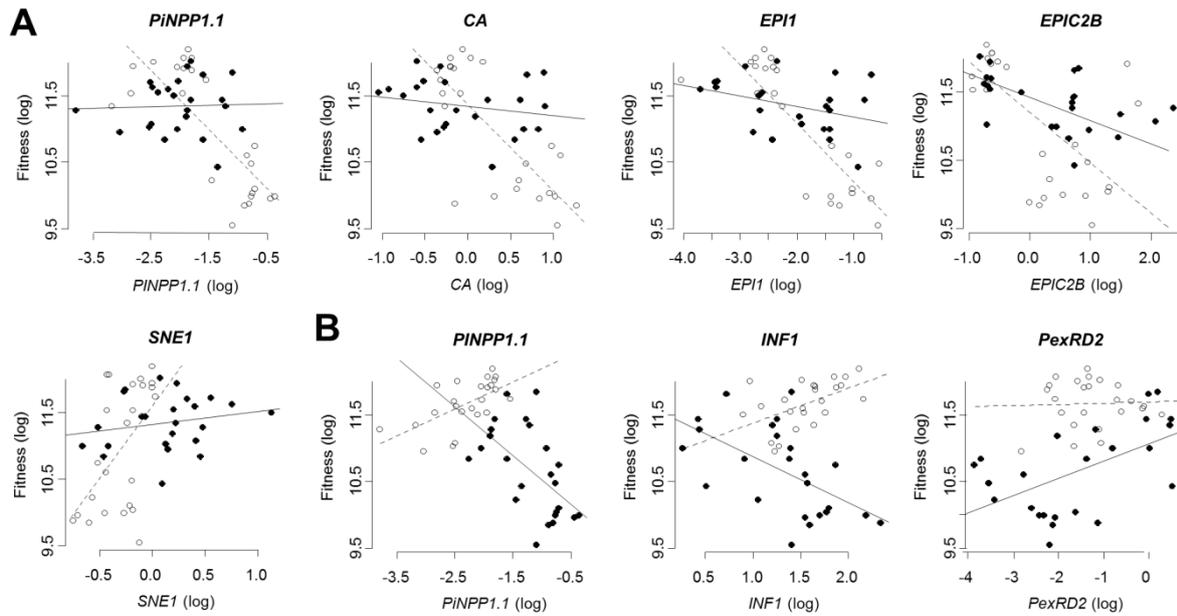
Fig 7. Within-group correlations between fitness and effector genes of *Phytophthora infestans*. (A) Data were grouped by tested host. Black circles represent data obtained on potato (cv. Bintje, correlations not significant at α = 0.05, except for *EPIC2B*) and white circles represent data on tomato (cv. Marmande, correlations highly significant). **(B)** Data were grouped by isolate. Black circles represent data obtained for isolate 15.P50.09 (collected from potato, significant negative correlations for *PiNPP* and *INF1* but positive correlation for *PexRD2*) and white circles represent data for isolate 15.TII.02 (collected from tomato, highly positive correlations except for *PexRD2*).

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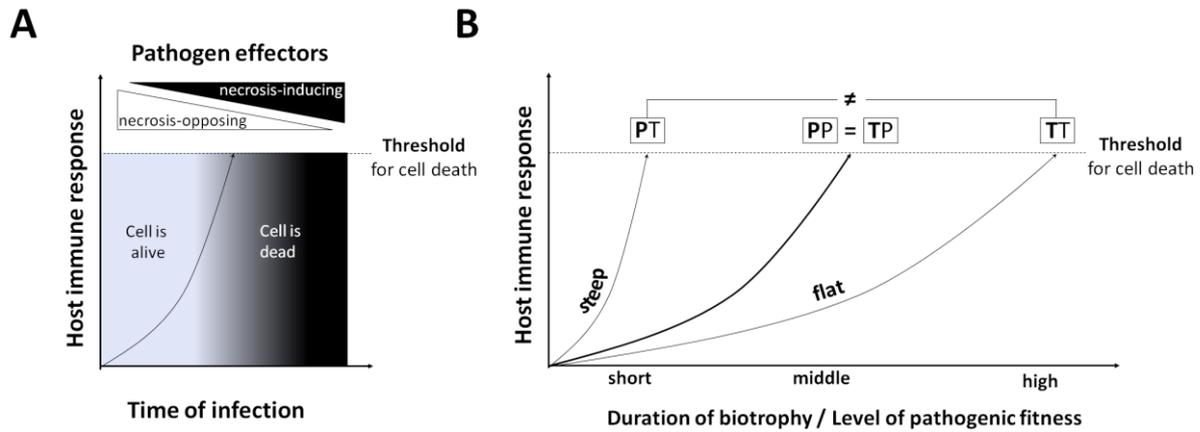
Fig 8. Host immune responses could limit the duration of biotrophy in *Phytophthora infestans* interactions. (A) Host immune responses force *P. infestans* to kill its host. It is well established that *P. infestans* secretes effectors into the parasitized host cell adnd that secretion is timely regulated. Biotrophy related effectors are secreted in the early state of the interaction to maintain the host cell alive. With progressing time of infection, secretion of pro-life effectors decreases while necrotrophy related effectors with cell-death inducing activity are increasingly secreted. As the parasitized cells die concomitantly to this switch, a tightly controlled and timely secretion of necrosis-opposing and necrosis-favoring effectors is regarded as a mean of *P. infestans* to regulate the duration of biotrophy. However, the reasons why *P. infestans* – an almost obligate parasite – actively kills host cells is not understood and may even appear counterintuitive. The here advocated explanatory approach refers to the "increasing host defense" theory (see explanations in the text) suggesting biotrophic pathogens to actively kill the host cell as a last resort against an intolerable level (threshold) of host defenses. Literature reports about steadily increasing host defenses in *P. infestans* interactions in conjunction with the here and previously observed timely regulated expression of pro-life/pro-death effector genes (e.g. SNE1/PiNPP1.1) support this view. **(B)** Duration of biotrophy in *P. infestans* interactions is positively related with host adaptation. It has been proposed that timing and intensity of host immune responses impact on the outcome of interactions between *P. infestans* and its hosts. Results from a range of studies show that immune responses have to be induced timely and 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).

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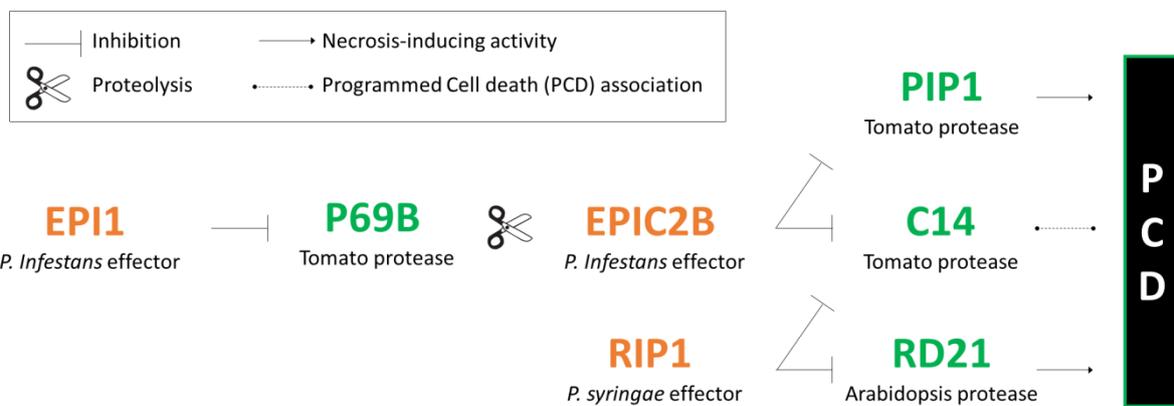
909

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