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Facilitative priority effects drive parasite assembly under coinfection

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Author contributions: RMP and A-LL designed and implemented the experiments. BB conducted the wild host survey. JLE compiled the wild host survey data. EN resolved the parasite multi-locus genotypes. FWH analyzed the data and wrote the first draft. All authors contributed substantially to revising the manuscript.

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1 **Abstract**

2 Host individuals are often coinfected with diverse parasite assemblages, resulting in
3 complex interactions among parasites within hosts. Within hosts, priority effects occur when the
4 infection sequence alters the outcome of interactions among parasites. Yet, the role of host
5 immunity in this process remains poorly understood. We hypothesized that the host response to
6 first infection could generate priority effects among parasites, altering the assembly of later
7 arriving strains during epidemics. We tested this by infecting sentinel host genotypes of *Plantago*
8 *lanceolata* with strains of the fungal parasite, *Podosphaera plantaginis,* and measuring
9 susceptibility to subsequent infection during experimental and natural epidemics. In these
10 experiments, prior infection by one strain often increased susceptibility to other strains, and these
11 facilitative priority effects altered the structure of parasite assemblages, but this effect depended
12 on host genotype, host population, and parasite genotype. Thus, host genotype, spatial structure,
13 and priority effects among strains all independently altered parasite assembly. Then, using a fine-
14 scale survey and sampling of infections on wild hosts in several populations, we identified a
15 signal of facilitative priority effects, which altered parasite assembly during natural epidemics.
16 Together, these results provide evidence that within host priority effects by early arriving strains
17 can drive parasite assembly, with implications for how strain diversity is spatially and temporally
18 distributed during epidemics.

19

20 **Keywords:** disease ecology; priming; priority effects; biotic interactions; coinfection;
21 competition; immunity

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22 **Introduction**

23 The diversity of parasites – organisms that live in and on hosts, potentially causing
24 disease – may rival the diversity of all other organisms on earth (Dobson *et al.* 2008). In light of
25 this diversity, it is not surprising that host individuals are often infected with diverse parasite
26 assemblages, composed of multiple parasite species or multiple genetic variants ('strains') of the
27 same species (Mideo 2009; Greischar *et al.* 2020). Within hosts, interactions among coinfecting
28 parasite strains can influence the dynamics of drug resistance (Wale *et al.* 2017), evolution of
29 virulence (Bhattacharya *et al.* 2019), and the magnitude of parasite epidemics (Susi *et al.* 2015),
30 with implications for host health (Read & Taylor 2001). Thus, understanding how parasite
31 strains interact in shared host individuals may be important for predicting the spread of infectious
32 diseases and ameliorating their impact on host populations. Yet, measuring how interactions
33 among parasites influence natural epidemics is notoriously difficult, as this requires manipulating
34 focal mechanisms of interactions and documenting the structure of parasite assemblages as
35 epidemics unfold (Mideo 2009; Hawley & Altizer 2011; Hoverman *et al.* 2013; Zhan &
36 McDonald 2013; Hellard *et al.* 2015; Tollenaere *et al.* 2015; Budischak *et al.* 2018). Using a
37 parasitic fungus that infects a wild host plant, this study experimentally tests whether parasite
38 interactions that are mediated by the host response to initial infection alter the structure of
39 parasite assemblages within hosts under field conditions, and then leverages the results of these
40 experiments to explain how parasite strains assemble during a natural epidemic.
41 Multiple parasites that encounter the same host individual can interact during
42 simultaneous infections, known as coinfections (Griffiths *et al.* 2014; Tollenaere *et al.* 2015;
43 Ezenwa 2016). One potential mechanism of interaction among coinfecting parasites occurs when
44 host immune responses to one parasite alter host susceptibility to secondary infections of another

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45 parasite (Lello *et al.* 2004; Mideo 2009; Chung *et al.* 2012; Halliday *et al.* 2018). This
46 mechanism can result in either antagonism or facilitation among coinfecting parasites, and
47 ultimately can alter parasite epidemics (Eswarappa *et al.* 2012; Tollenaere *et al.* 2015; Zélé *et al.*
48 2018). The immune response to initial infection can suppress coinfection when infection by one
49 parasite activates immune signaling pathways that induce resistance to subsequent infections, in
50 a process known by a variety of terms including immune priming, cross protection, induced
51 resistance, or cross-immunity (Jenner 1923; Fulton 1986; Van Loon 1997; Conrath *et al.* 2006;
52 Pieterse *et al.* 2014). Alternatively, an early arriving parasite can facilitate coinfection by
53 inactivating immune signaling pathways that protect hosts from multiple parasites (Spoel *et al.*
54 2007; Kliebenstein & Rowe 2008). These effects can be temporary and spatially restricted within
55 hosts (Koornneef *et al.* 2008), or systemic and persistent long after initial infection (Pieterse *et*
56 *al.* 2014). Both mechanisms of immune-mediated interactions among parasites have been
57 reported in plant and animal hosts (Glazebrook 2005; Ezenwa *et al.* 2010; Pieterse *et al.* 2014).
58 These effects, which have been predominantly tested in laboratory environments (but see
59 Halliday *et al.* 2018), indicate that the sequence and timing of infections may influence the
60 structure of parasite assemblages.

61 The field of community ecology provides a framework for understanding how the
62 sequence of infection on host individuals might alter parasite assemblages as epidemics unfold
63 (Hoverman *et al.* 2013; Vannette & Fukami 2014; Fukami 2015; Johnson *et al.* 2015; Halliday *et*
64 *al.* 2017; Clay *et al.* 2019; Karvonen *et al.* 2019). Specifically, interactions among parasites that
65 are contingent on the sequence of past events can be a consequence of priority effects within
66 hosts. Within hosts, priority effects occur when the per-capita strength of antagonism or
67 facilitation among parasites is altered by their sequence of arrival (Hoverman *et al.* 2013;

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68 Mordecai *et al.* 2016). Priority effects, in turn can drive community assembly, thereby altering
69 the structure of parasite communities during natural epidemics (Halliday *et al.* 2017; Clay *et al.*
70 2020). Priority effects are expected to occur most commonly when species exhibit high niche
71 overlap and when early arriving species have large impacts on the availability of that niche
72 (Vannette & Fukami 2014). A host comprises the entire niche available to parasites during
73 infection (Kuris *et al.* 1980; Rynkiewicz *et al.* 2015), and thus coinfecting parasites often exhibit
74 high niche overlap (Sousa 1992; Graham 2008; Seabloom *et al.* 2015), particularly when parasite
75 assemblages are comprised of coinfecting strains of the same parasite species (e.g., Wale *et al.*
76 2017). Although priority effects have been predominantly used to describe community assembly
77 in multi-species parasite assemblages (reviewed in Clay *et al.* 2019), these same principles may
78 apply to parasite assemblages comprised of multiple strains (Greischar *et al.* 2020). By activating
79 immune responses that alter host susceptibility, early arriving strains can therefore determine the
80 availability of the shared host niche (Cobey & Lipsitch 2013); thus, the immune response to
81 initial infection may drive priority effects among parasite strains within hosts, thereby altering
82 the structure of parasite assemblages within hosts.

83 The degree to which the sequence and timing of infection influences parasite assemblages
84 might depend on the history of interactions between host and parasite populations (Tollenaere *et*
85 *al.* 2015). This history of interactions between host and parasite populations, which is typically
86 measured through local adaptation assays (Greischar & Koskella 2007; Hoeksema & Forde
87 2008), is commonly reflected by differences in the susceptibility of certain host genotypes to
88 certain parasite genotypes (Burdon & Laine 2019). Interactions among sequentially arriving
89 parasites could also depend on host or parasite genotypes if a given host genotype is more or less
90 sensitive to infection by the first or second arriving parasite genotype (Lambrechts *et al.* 2006),

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91 or if the response triggered by the first arriving parasite is genotype specific (Ferro *et al.* 2019;
92 Westman *et al.* 2019). Thus, whether or not within-host priority effects alter parasite epidemics
93 might depend on complex interactions among host and parasite genotypes. Consequently, it is
94 essential to incorporate genotypic variation into studies of sequential infection among parasite
95 strains.

96 The host response to infection may alter parasite interactions and epidemics (Lello *et al.*
97 2004; Graham 2008; Tollenaere *et al.* 2015), but we lack studies that experimentally manipulate
98 prior parasite exposure and measure the consequences for parasites outside of the lab (Hellard *et*
99 *al.* 2015; Pedersen & Fenton 2015; Budischak *et al.* 2018), hampering our understanding of the
100 general processes through which within-host parasite interactions alter parasite assemblages in
101 nature. This study addresses this research gap experimentally by first infecting host plants with
102 parasitic fungi, physically restricting those parasites from interacting directly within hosts, and
103 then testing whether the host response to initial infection alters the structure of parasite
104 assemblages. We then leverage the experimental results to explain how parasite strains assemble
105 within hosts during a natural epidemic. We find that parasites exhibit facilitative priority effects
106 driven by the host response to initial infection, and that these facilitative priority effects can alter
107 the structure of parasite assemblages during a natural epidemic. These results indicate that the
108 sequence of infection can determine the probability of coinfection, altering the trajectory of
109 parasite assembly, and leading to pronounced differences in the structure of parasite assemblages
110 among hosts.

111

112 **Results & Discussion**

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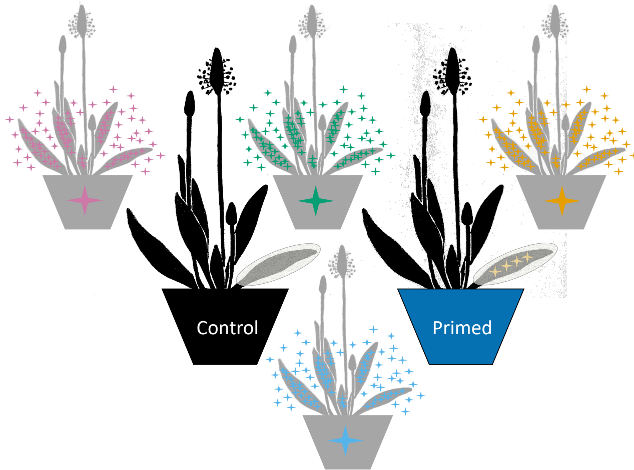
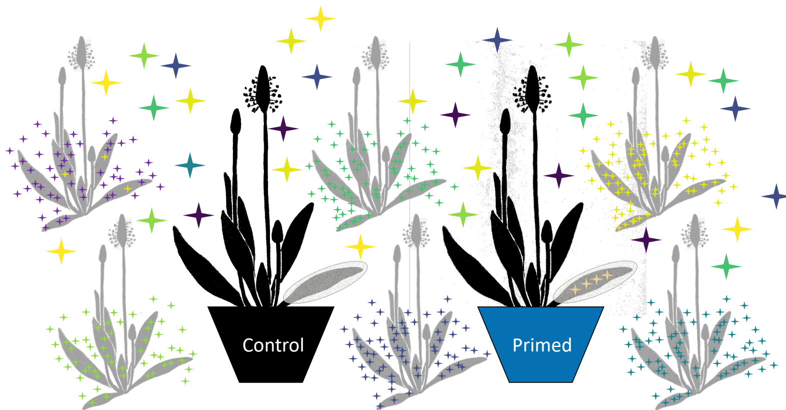
113 In order to examine the role of priority effects among parasites that are mediated by the
114 host (i.e., plant) in response to prior infection and the influence of priority effects on the structure
115 of parasite assemblages, we carried out two experiments, referred to as the "manipulated
116 epidemic experiment" and the "natural epidemic experiment", and a fine-scale survey and
117 sampling of infections in the wild, referred to as the "wild host survey", using the focal host
118 *Plantago lanceolata*, and the obligate parasite *Podosphaera plantaginis* (Fig 1).

A) **Manipulated epidemic experiment:**
Can the host response to prior infection drive parasite assembly via priority effects?



B) Natural epidemic experiment:
Can host-mediated priority effects influence parasite assembly during a natural epidemic?



C) Wild host survey:
Can host-mediated facilitative priority effects be detected in natural populations?

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121 **Figure 1.** Illustration of key differences and similarities among the three field studies presented in this

122 manuscript. Pot color represents different priming treatments (black = control; blue = primed). Star color

123 represents different parasite genotypes (i.e., strains). A) To test whether host (i.e., plant) responses to

124 prior infection can drive parasite assembly via priority effects, focal potted hosts (black) were either

125 inoculated or mock-inoculated with one of four priming strains. Then hosts were exposed to all four

126 priming strains by placing heavily infected potted hosts (grey) adjacent to the focal hosts under field

127 conditions. This experiment included four different host genotypes not depicted in this figure. B) To test

128 whether host-mediated priority effects among parasites can influence parasite assembly during a natural

129 epidemic, focal potted hosts (black) were either inoculated or mock inoculated with a priming strain

130 associated with a given host population and then embedded in a wild host population (grey) during a

131 natural epidemic. This experiment included four different host genotypes, two infection timing

132 treatments, and three host populations that are not depicted in this figure. C) To test whether a signal of

133 host-mediated facilitative priority effects among parasites could be detected in natural populations, focal

134 wild hosts (black) occurring in wild host populations (grey) were repeatedly surveyed over time. This

135 experiment included 13 host populations that are not depicted in this figure.

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139

140 *Can host responses to prior infection drive parasite assembly via priority effects?*

141 We carried out the manipulated epidemic experiment in a common garden at the Lammi
142 Biological Station to test whether parasite strains exhibit priority effects that are mediated by the
143 host response to initial infection (Fig. 1). Both the host and parasite species naturally occur in
144 this location. In the manipulated epidemic experiment, four host genotypes were either
145 inoculated or mock-inoculated with one of four parasite strains, which were sealed inside mesh
146 pollination bags to prevent direct strain interactions, and then exposed to all four priming strains
147 for four days. To be consistent with previously published literature (Laine 2011; Conrath *et al.*
148 2015; Douma *et al.* 2017; Mauch-Mani *et al.* 2017), we refer to the experimental treatment as the
149 "priming treatment" and the experimentally inoculated strains as "priming strains" (Fig. 1).

150 One challenge of predicting how within-host interactions will alter infection outcomes
151 during epidemics is the difficulty of isolating host-mediated interactions from other interactions
152 among parasites, such as resource or interference competition (Mideo 2009; Budischak *et al.*
153 2015, 2018). We overcame this limitation experimentally by leveraging the modular growth form
154 of plant hosts. Specifically, for foliar parasites in plant hosts, resource and interference
155 competition are expected to be strongest within individual host leaves (Tollenaere *et al.* 2015;
156 Borer *et al.* 2016; Halliday *et al.* 2017). Because powdery mildews only feed within individual
157 host leaves (Bushnell 2002) and the priming strain was restricted from spreading beyond the
158 inoculated host leaf onto the rest of the host plant, any response to experimental inoculation can
159 be interpreted as an effect that is mediated by the host response to initial infection. Thus, the
160 inoculation treatment was intended to test whether initial infection by one parasite could "prime"
161 the host to respond differently upon subsequent exposure, generating priority effects mediated by
162 the host.

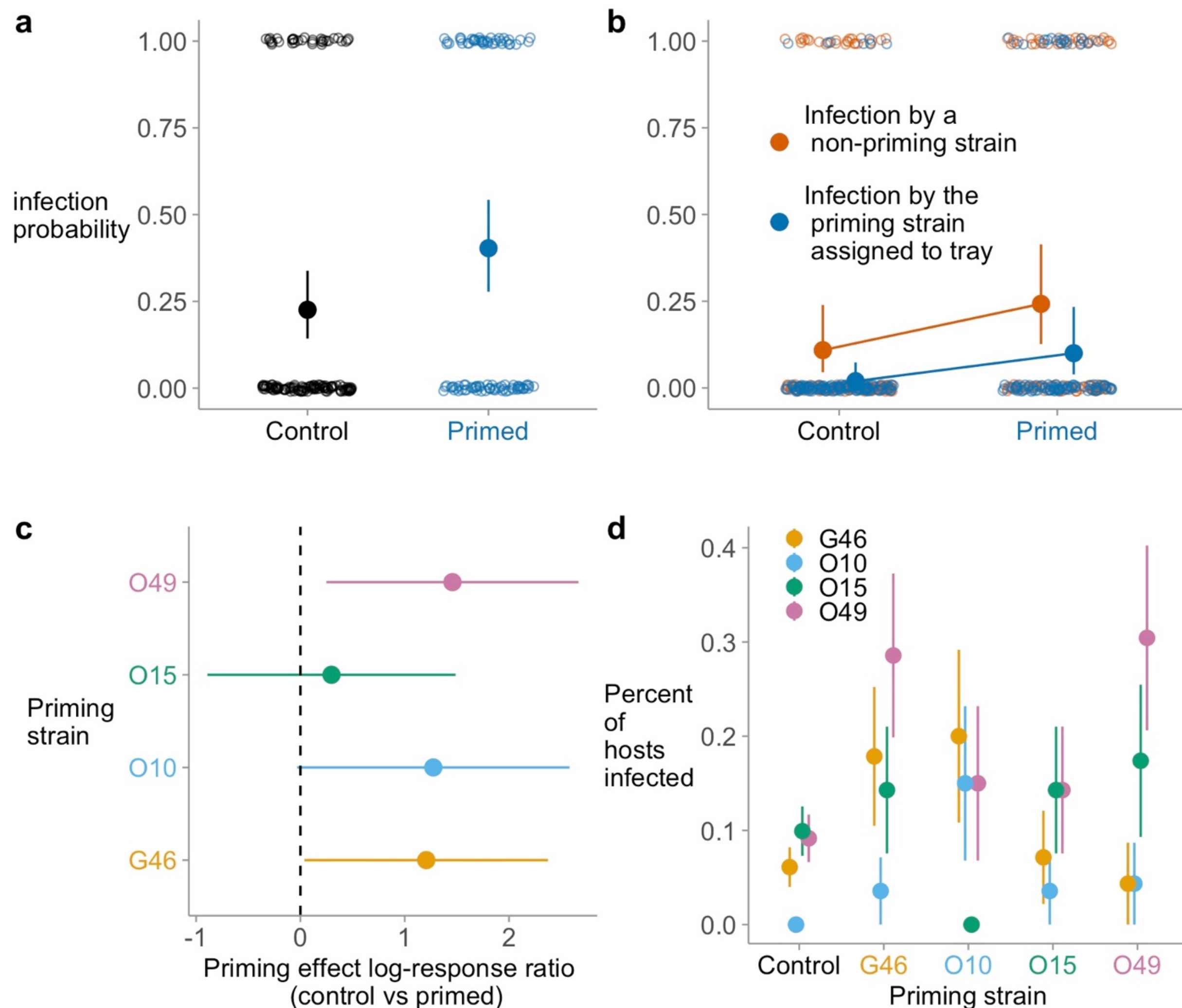
163 We tested whether the priming treatment altered the probability of a host becoming
164 infected in the manipulated epidemic using a logistic mixed model. As predicted, hosts that were
165 experimentally inoculated were more likely to become subsequently infected during the
166 experimental epidemic ($p = 0.0088$; Fig. 2a; Table S1a). This effect was qualitatively similar
167 using the (logit-transformed) proportion of leaves infected as a response measure representing
168 infection severity ($p = 0.019$; Table S1b). Although host susceptibility to infection and the
169 severity of infection were positively influenced by the priming treatment, this effect disappeared
170 when we evaluated infection severity among infected hosts only ($p = 0.82$; Table S1c),
171 suggesting that priority effects may act qualitatively (e.g., by altering susceptibility to infection)
172 rather than quantitatively (e.g., by altering infection severity). This result is consistent with
173 ecological theory, which suggests that priority effects should primarily function to prevent or
174 facilitate establishment or persistence rather than population growth, *per se* (Fukami *et al.* 2016).
175 This result therefore suggests that increased susceptibility to infection following early exposure
176 to a pathogen strain can influence subsequent infection outcomes in the field.
177 We next tested whether the facilitative effect of early exposure on susceptibility to
178 infection during the experimental epidemic differed among host genotypes and priming strains.
179 Consistent with theory grounded in the history of interactions between host and parasite
180 populations (e.g., Tollenaere *et al.* 2015), the facilitative effect of early infection depended on
181 the priming strain ($p = 0.050$) and host plant genotype ($p = 0.024$), though there was no
182 interaction between host plant genotype and the priming treatment ($p = 0.86$; Table S2a, Fig. 2c).
183 We therefore dropped the non-significant interaction, resulting in a reduced model, and estimated
184 the coefficients from the reduced model. Consistent with facilitative priority effects, the priming
185 strains G46 and O49 significantly increased the probability of infection under field conditions (p

186 = 0.039 and p = 0.016, respectively), while strain O10 marginally significantly increased the
187 probability of infection (p = 0.051) and strain O15 did not (p = 0.62). This result suggests that
188 only some early arrivers strongly influenced the availability of the niche for later arrivers,
189 providing a possible mechanism for facilitative priority effects within hosts (e.g., Vannette &
190 Fukami 2014). These results were qualitatively similar using the proportion of leaves infected as
191 a metric of infection severity (Table S2b). Although the effect of the priming treatment on host
192 susceptibility to infection and the severity of infection were influenced by host and parasite
193 genotype, this effect disappeared when we evaluated infection severity among infected hosts
194 only (Priming strain p = 0.95; Host plant genotype p = 0.61; Table S2c), lending further support
195 to the idea that priority effects act qualitatively rather than quantitatively in this system.
196 Together, these results suggest that the host response to initial infection depended on
197 which parasite strain arrived first. However, for priority effects to alter the structure of parasite
198 assemblages, later arriving strains must also be sensitive to the plant response to initial infection
199 (Vannette & Fukami 2014). To explore this mechanism of within-host interactions, we next
200 genotyped infections on each individual host following exposure to all four strains under natural
201 conditions and then tested for interactions among the priming treatment, plant genotype, and
202 whether or not the later arriving strain was the same as the early arriving strain. For priority
203 effects to occur, facilitative effects should occur among different strains. In other words, a
204 priority effect could only occur if the early arriving strain facilitated other later arriving strains.
205 Across both treatments, infection by a non-priming strain was about 1.4 times more likely than
206 infection by the priming strain ($p < 0.001$; Fig 2b). Consistent with the hypothesis that parasite
207 strains can exhibit within-host priority effects, the effect of early infection on the probability of
208 subsequent infection was qualitatively similar between secondary infections caused by the

209 priming strain ($p = 0.004$) and secondary infections caused by a different strain from the priming
210 strain ($p = 0.033$). In other words, there was a significant main effect of the priming treatment (p
211 $= 0.003$), but no interaction between the priming treatment and whether or not the host became
212 infected with a strain other than the priming strain ($p = 0.24$; Table S3).

213 Finally, we tested whether within-host priority effects altered the structure of parasite
214 assemblages using a multivariate generalized linear model (Wang *et al.* 2012; Warton *et al.*
215 2012). As expected, different parasite assemblages formed on hosts that received different
216 priming treatments (LRT = 37; $p = 0.040$; Table S4; Fig. 2d), though there were no significant
217 differences among different host genotypes (LRT = 34.94; $p = 0.085$), and the effect of priming
218 treatments on the structure of parasite assemblages did not interact with host genotype (LRT =
219 57; $p = 0.077$). Thus, priority effects among strains altered parasite assembly, and the trajectory
220 of assembly depended on the identity of the early arriving strain, but not the genotype of the
221 host.
222

223



224

225 **Figure 2.** Results from the manipulated epidemic experiment. The effect of the priming
226 treatment on a) whether or not a host became infected with any strain; b) whether or not a host
227 became infected with the priming strain assigned to the experimental block (control hosts were
228 mock-inoculated with the priming strain associated with that block), or any non-priming strain;
229 c) whether or not a host became infected with any strain as a function of the priming strain,
230 shown as a log-response ratio; d) the proportion of hosts infected by each strain during the
231 manipulated epidemic experiment. In panels a-c, filled points are model-estimated means, error
232 bars are model-estimated 95% confidence intervals, and open points show the raw data. In panel
233 d, points show the proportion of hosts infected and the error bars show one standard error around
234 those points. These results highlight the differential effect of each strain on the priming response
235 of the plant as well as the differential sensitivity of each strain to that priming effect. Only strain
236 O15 was a uniformly poor primer of subsequent infection. All other parasite strains significantly
237 facilitated subsequent infection by at least one other strain. Together, these results suggest that
238 by differentially determining the plant response to infection and experiencing differential
239 sensitivity to that response, prior infection can strongly alter assembly of these pathogen strains.

Can host-mediated priority effects among parasites influence parasite assembly during a natural epidemic?

The manipulated epidemic experiment tested whether hosts could mediate priority effects among parasites, and whether such priority effects could influence parasite assembly during an experimental epidemic. We next carried out the natural epidemic experiment (Fig 1) to test whether host-mediated priority effects could be generalized to predict the outcome of natural epidemics by embedding sentinel hosts that were either primed or mock-inoculated into an ongoing epidemic in three wild host populations in the Åland archipelago. In addition to manipulating infection sequence (primed vs mock-inoculated), this experiment also manipulated the timing of prior infection by priming hosts either four or eight days prior to exposing hosts to the natural epidemic.

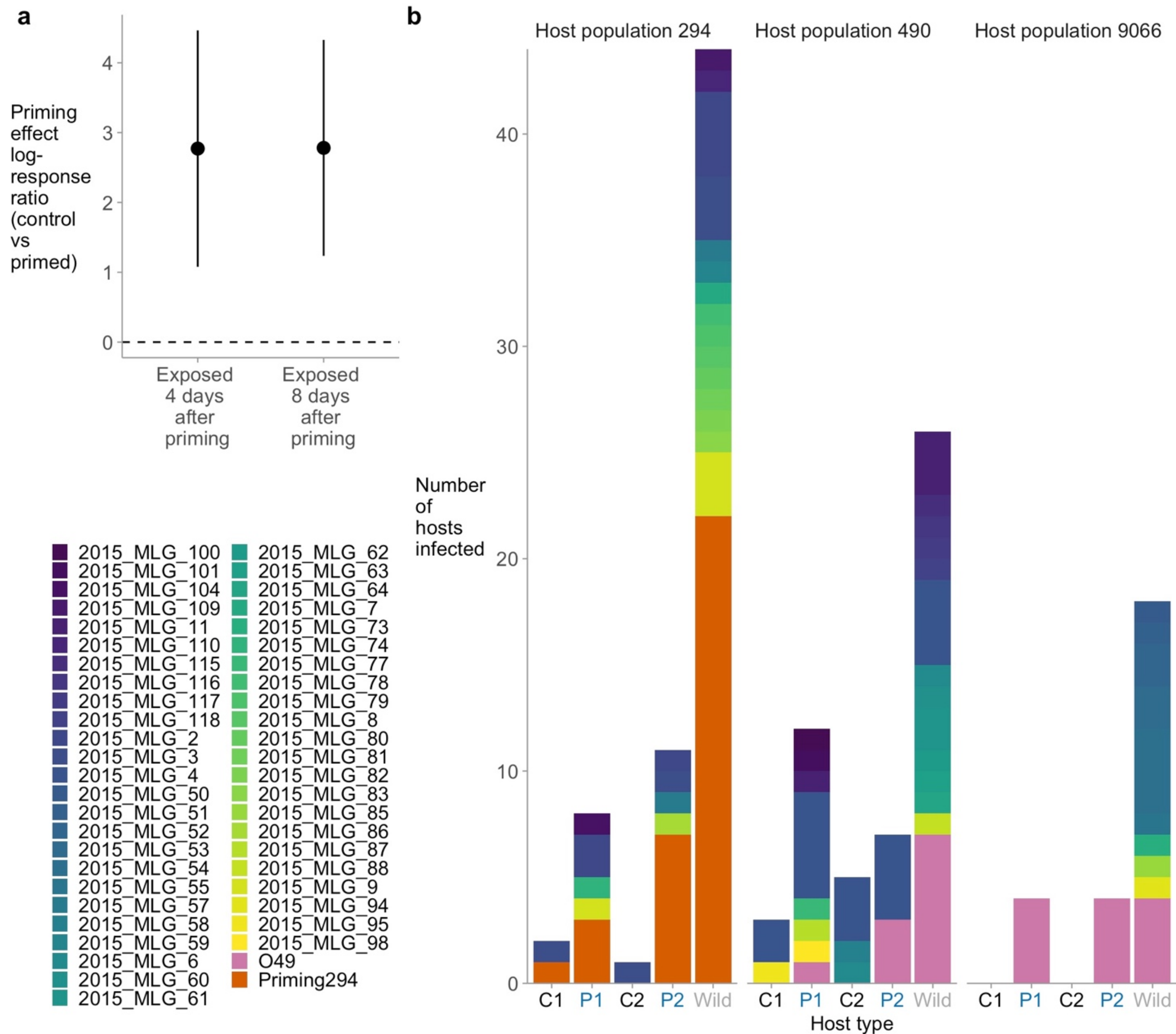
We first tested whether the host response to prior infection could generate priority effects using a logistic mixed model. Consistent with expectations from the manipulated epidemic experiment, the priming treatment significantly influenced the probability of a host becoming infected during the natural epidemic experiment ($p < 0.001$; Table S5). However, in contrast with the manipulated epidemic experiment, there was no significant effect of host genotype ($p = 0.34$). Consistent with expectations grounded in previous studies of this system (Penczykowski *et al.* 2018), the probability of infection differed among populations ($p = 0.002$). But, in contrast with expectations grounded in laboratory studies of plant immunity (Pieterse *et al.* 2012) and ecological theory (Fukami 2015), there was no difference in the magnitude of the priming effects between hosts that were primed eight days prior to experimental placement in the field and hosts that were primed four days prior to placement in the field (Fig 3a). The results were qualitatively

263 similar using the (logit-transformed) proportion of leaves infected as a response variable
264 representing infection severity. However, there was also a significant three-way interaction in the
265 model of infection severity, suggesting that priority effects occurred, that these priority effects
266 depended on infection sequence and timing, but only in certain populations, and only among
267 certain host genotypes (Fig S1a). The reduced model of infection severity among infected hosts
268 also included significant two-way interactions between population and host genotype ($p =$
269 0.033), and between host genotype and experimental treatment ($p = 0.019$; Fig S1b).

270 Our result that host-mediated interactions among parasites almost universally favored
271 coinfection is in contrast to prior studies suggesting that priming can commonly reduce the
272 probability of coinfection through cross resistance (Fulton 1986; Pieterse *et al.* 2014; Biere &
273 Goverse 2016), potentially raising concerns that these results might be system specific; however,
274 a prior study in this wild plant pathosystem suggests a different explanation for these contrasting
275 results. Specifically, using different *Plantago lanceolata* hosts and *Podosphaera plantaginis*
276 genotypes, Laine (2011) found that priming reduced spore production in the lab, but increased
277 infection severity in the field. Thus, we suggest that the difference between the results presented
278 here and commonly reported results of cross resistance in other systems might be attributable to
279 laboratory versus field environments, adding to a growing body of evidence that within-host
280 interactions studied in the laboratory might be poor predictors of infection outcomes during
281 natural epidemics (Seabloom *et al.* 2009; Leung *et al.* 2018; Clay *et al.* 2019).

282 Finally, we tested whether priority effects among parasite strains could lead to variation
283 in the structure of parasite assemblages among hosts during a natural epidemic using a
284 multivariate generalised linear model. This model did not include any significant interactions.
285 However, as expected, there were different parasite assemblages on hosts that received different

priming treatments (LRT = 111; $p < 0.001$), among different populations (LRT = 90; $p < 0.001$), and among different host genotypes (LRT = 44; $p = 0.048$; Table S6; Fig 3b). Thus, host genotype, spatial structure, and priority effects among strains all independently altered parasite assembly in the natural epidemic experiment.



293

294 **Figure 3.** Results from the natural epidemic experiment. The effect of the sequence and timing
295 of infection on a) whether or not a host became infected with any strain, shown as a log-response
296 ratio between each control and each priming treatment. (e.g., C1 vs P1 and C2 vs P2); and b) the
297 number of hosts infected by each strain grouped by whether a host was experimental (C1, C2,
298 P2, P2) or wild (shown in grey). Plants were primed either 8 days (treatment P1) or 4 days
299 (treatment P2) prior to being placed into the field. There were also C1 and C2 control plants set
300 up at the same time (but mock inoculated). Points are model-estimated means, and error bars are
301 model-estimated 95% confidence intervals. Colors represent different strains, with pink and
302 orange corresponding to the two priming strains used in this experiment (O49 and Priming294,
303 respectively). These results show that, consistent with the manipulated epidemic experiment,
304 experimental priming increased the probability of a host becoming infected. However, that effect
305 did not depend on infection timing (P1 vs P2). As a consequence of this effect, experimentally
306 primed hosts had more complex parasite communities that were more similar to wild hosts than
307 mock-inoculated control hosts.

308

309

310 *Can a signal of host-mediated facilitative priority effects among parasites be detected in natural*
311 *populations?*

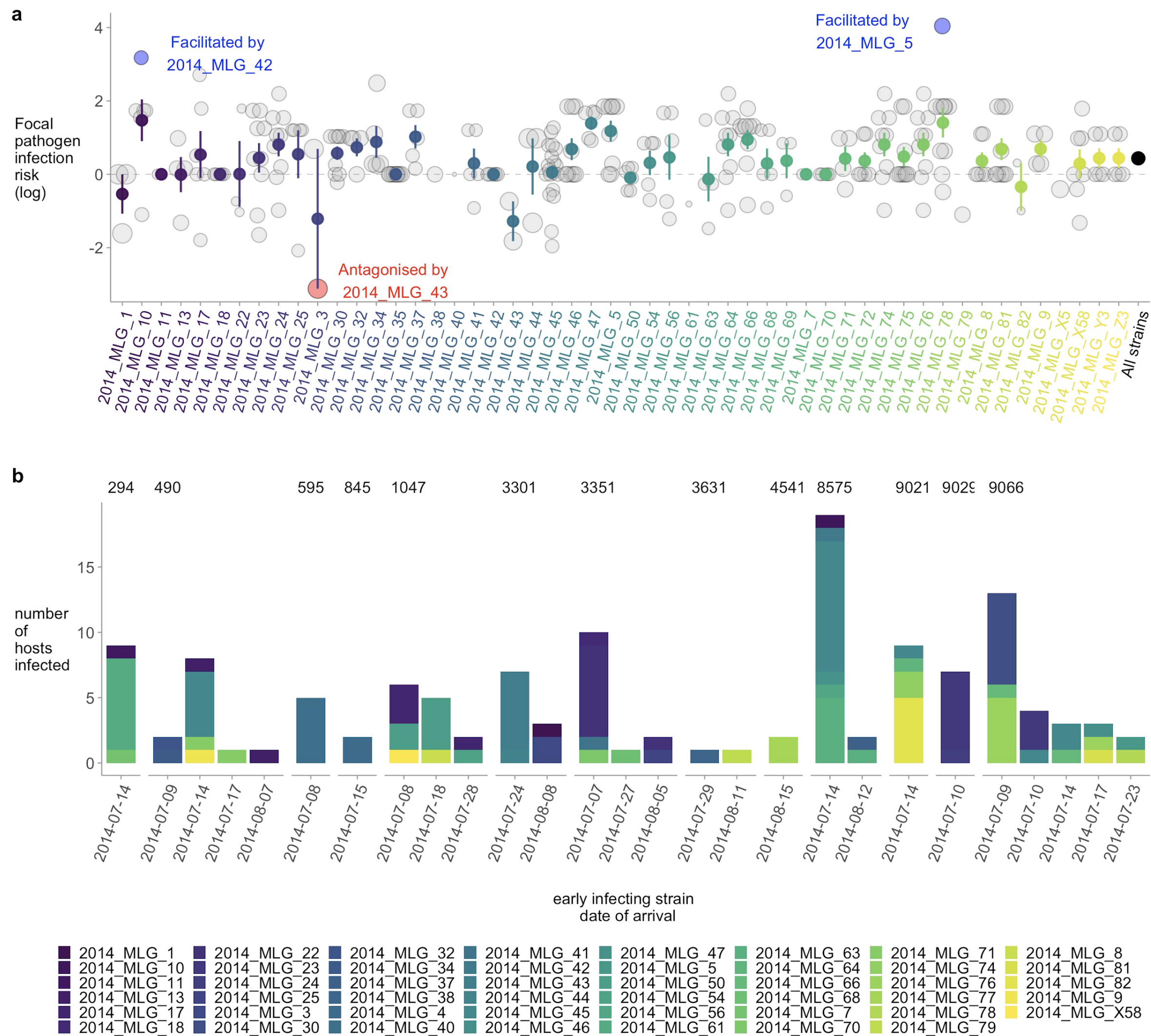
312 Our experimental results showed consistent host-mediated facilitative priority effects
313 among parasite strains. However, in addition to host-mediated interactions, parasites can also
314 interact via resource or interference competition during natural epidemics (Mideo 2009). In
315 theory, the host-mediated interactions can be either antagonistic or facilitative, whereas resource
316 and interference competition are generally expected to reduce the risk of coinfection (Mideo *et*
317 *al.* 2008; Pedersen & Greives 2008; Halliday *et al.* 2018). Thus, although both experiments
318 suggested that the host response to prior infection can facilitate subsequent infection via within-
319 host priority effects, the degree to which this process plays out to influence parasite assemblages
320 during natural epidemics remains unclear.

321 We tested whether host-mediated priority effects are sufficiently important to influence
322 the structure parasite assemblages in nature by analyzing the results of a longitudinal survey of
323 wild host individuals during a natural epidemic (i.e., the wild host survey; Fig 1). The wild host
324 survey was carried out in the Åland archipelago, and included 105 host individuals from 13
325 populations, sampled biweekly for infection starting on 7 July, 2014. Once a host became
326 infected, it entered the dataset as a focal host. To determine infection sequence among hosts, we
327 sampled lesions and genotyped infections twice on each focal host: first when more than one leaf
328 on a focal host was infected, and then again at the end of the season. These two genotyping
329 sessions provide data on the sequence and timing of infection among hosts, while biweekly
330 surveys of whole host populations provide information on parasite phenology.

331 We hypothesized that if the host response to prior infection was sufficiently strong to
332 alter parasite community assembly, then we would observe a signal of facilitation among co-
333 occurring parasite strains during a natural epidemic. To test whether parasite strains exhibit
334 priority effects within hosts, we first fit a series of cox proportional hazards models following
335 Halliday et al (2017, 2018). These models test whether the time until infection by each parasite
336 strain was influenced by whether or not a host had been previously infected by another strain. In
337 total this analysis tested for 286 potential pairwise interactions among parasite strains. However,
338 despite the large number of potentially interacting parasite strains, only three pairwise
339 interactions resulted in a significant priority effect as defined by Halliday et al (2017, 2018).
340 Specifically, 2014_MLG_78 and 2014_MLG_10 were significantly facilitated by prior infection
341 by 2014_MLG_5 and 2014_MLG_42 respectively ($p = 0.009$; $p = 0.035$), while 2014_MLG_3
342 was antagonized by prior infection by 2014_MLG_43 ($p=0.009$); importantly, given the
343 numerous comparisons per host individual, even these significant effects should be interpreted
344 with caution. These results therefore suggest that, on average, parasite strains were not exhibiting
345 measurable priority effects within hosts.
346 There are many potential reasons for the absence of significant interactions among
347 parasites, including the physiological state of wild host plants (Penczykowski *et al.* 2018), the
348 presence of counterbalancing direct interactions among parasites (Mideo *et al.* 2008), skewed
349 distribution of infections by the different strains in the field (Laine 2007), or the relatively
350 infrequent sampling of host individuals in the field. However, although within-host priority
351 effects were rarely significantly positive or negative, we still wanted to know whether a signal of
352 facilitation could be observed among potentially interacting parasite strains. Overall, early
353 infection tended to facilitate subsequent infection by other strains more commonly than

preventing subsequent infection by other strains ($p<0.001$; Fig 4a), consistent with priority effects being mediated by the host response to prior infection. These results support the hypothesis that facilitative interactions among parasite strains, mediated by the host response to prior infection, would result in a signal of facilitation among co-occurring parasite strains.

Finally, to test whether parasite phenology among strains altered parasite assembly within hosts, we fit a multivariate generalised linear model. We hypothesized, in accordance with ecological theory (e.g., Vannette & Fukami 2014) and our experimental results, that strains that emerged later in the growing season (i.e., strains with later phenology) would be more sensitive to facilitative priority effects, and that strains that emerged earlier in the growing season (i.e., strains with early phenology) would more strongly influence parasite assembly. Consistent with this hypothesis, the structure of parasite assemblages differed significantly among hosts with differing phenology of the early-arriving strains (LRT = 15.23; $p = 0.002$; Fig 4b; Table S7), even after accounting for survey date (LRT = 238; $p = 0.001$), and host different population (LRT = 189; $p = 0.001$).



369

370 **Figure 4.** Results from the wild host survey. a) The effect of infection sequence on the risk of a
371 host becoming infected with each parasite strain. The x-axis shows the focal (i.e., late arriving)
372 parasite strains from field-collected samples. The grey, blue, and red points are coefficient
373 estimates from cox proportional hazards models measuring the pairwise interaction between each
374 focal strain and each co-occurring other (i.e., early arriving) strain; point size represents the
375 average number of surveys per host individual; blue and red indicate significant facilitation and
376 antagonism ($p < 0.05$), respectively, and grey indicates insufficient evidence for significant
377 priority effect ($p > 0.05$). The colored points with error bars show the mean and one standard
378 error across all coefficients for each focal parasite strain. The black point is the model estimated
379 mean across all parasite strains, and the black error bar (which is small and largely obscured by
380 the black circle) is the 95% confidence interval from an intercept-only model, indicating that on
381 average, interactions tended to be positive. b) The number of hosts infected by each strain at the
382 end of the growing season grouped by the date that the early arriving strain was first observed in
383 the field. Panels represent different host populations. These results show that, across all parasite
384 strains, interactions were most commonly facilitative, and hosts that were first infected by strains
385 with early phenology were more likely to become infected by more complex communities at the
386 conclusion of the growing season.

387

388 **Conclusions**

389 The sequence and timing of infection can strongly influence parasite interactions and
390 epidemics (Halliday *et al.* 2017, 2018; Clay *et al.* 2019, 2020), yet the degree to which this
391 process is driven by the host response to infection versus other mechanisms of interaction among
392 parasites remains poorly understood. This study leveraged a model wild-plant pathosystem to fill
393 this gap (Penczykowski *et al.* 2016). Specifically, our study revealed three key findings: (1) by
394 manipulating infection sequence during an experimental epidemic, we showed that host-
395 mediated interactions among parasites almost universally favored coinfection; (2) by
396 manipulating infection sequence during a natural epidemic, we showed that this process could
397 alter how parasite strains assemble; and (3) by tracking wild host individuals during the course of
398 a different natural epidemic, we identified a signal of host-mediated facilitation among parasite
399 strains that could be linked to the structure of parasite assemblages. Our results therefore provide
400 comprehensive evidence that parasite interactions, mediated by the host response to initial
401 infection, can facilitate subsequent infection by different parasite strains, altering the trajectory
402 of parasite assembly during natural epidemics.

403 In addition to isolating host-mediated interactions from other interactions among
404 parasites, our experimental approach overcomes an additional key limitation of past
405 observational studies: the need to rely on counterfactual reasoning. Linking infection sequence
406 and within-host priority effects during natural epidemics is notoriously challenging (Budischak
407 *et al.* 2018; Rynkiewicz *et al.* 2019). Our experimental data showed clear evidence of host-
408 mediated facilitation among parasites. Pairing this experimental approach with a fine-scale
409 survey and sampling of infections in the wild during a natural epidemic thus allowed us to

410 interpret a signal of host-mediated facilitation that would be uninterpretable from observational
411 data alone. Our results suggest that, by differentially determining the plant response to infection
412 and experiencing differential sensitivity to that response, prior infection can strongly alter the
413 structure of parasite assemblages during epidemics.

414 Priority effects among strains favored coinfection and altered parasite assembly, and the
415 trajectory of assembly depended on the genotype of the early arriving strain. This result supports
416 the idea that species interactions – in this case host-parasite and parasite-parasite interactions –
417 can depend on intraspecific variation in characteristics of organisms (Bolnick et al. 2011;
418 Laughlin et al. 2012; Siefert 2012). Intraspecific diversity is ubiquitous in host and parasite
419 populations, and has prompted considerable research into local adaptation among hosts and
420 parasites (Greischar & Koskella 2007), parasite aggregation (Shaw & Dobson 1995), and disease
421 emergence (Lloyd-Smith et al. 2005). Yet, how this diversity impacts parasite assembly is not
422 known. Importantly, our results suggest that by influencing host susceptibility, intraspecific
423 variation can determine how strain diversity is spatially and temporally distributed during
424 epidemics.

425 In our experimental manipulation of a natural epidemic, we found evidence that infection
426 sequence, but not infection timing altered future host susceptibility. We also found strong
427 evidence that host genotype and spatial structure altered the structure of parasite assemblages
428 within hosts, but these effects did not alter the direction or magnitude of priority effects. Thus,
429 we conclude that differences among host genotypes and among populations may play a large role
430 in the assembly of parasite strains in natural populations, but these effects are independent of the
431 robust effect of facilitation by sequentially arriving parasite strains in this system.
432

433 **Methods**

434 *Study system*

435 The host plant, *Plantago lanceolata* L. (ribwort plantain), is a perennial rosette-forming
436 herb that reproduces either sexually, as an obligate outcrosser with wind-dispersed pollen, or via
437 vegetative propagation of side-rosettes (Sagar & Harper 1964; Ross 1973). This species has a
438 cosmopolitan distribution, and grows in fragmented populations on dry meadows and pastures in
439 the Åland archipelago (SW Finland) (Ojanen *et al.* 2013). Plantago lanceolata is host to the
440 obligate parasite *Podosphaera plantaginis* (Castagne; U. Braun and S. Takamatsu), a powdery
441 mildew fungus in the order Erysiphales within the Ascomycota. The fungus grows on the leaf
442 surface and extracts plant nutrients via haustoria that enter the epidermis (Bushnell 2002). On the
443 leaf surface, mycelia produce chains of asexual, wind-dispersed transmission spores (conidia).
444 The parasite reproduces clonally throughout the summer growing season, and then produces
445 resting structures (chasmothecia) via haploid selfing or outcrossing (Tollenaere & Laine 2013)
446 which enable the parasite to survive when the host plant has died back to rootstock in winter.
447 Within the chasmothecia, haploid ascospores develop, which re-initiate epidemics in spring
448 (Tack & Laine 2014).

449 The metapopulation dynamics of this host-parasite interaction in ca. 4000 populations in
450 Åland have been studied since the year 2001 (Laine & Hanski 2006; Jousimo *et al.* 2014).
451 Powdery mildew infection combined with stressful environmental conditions can cause high
452 mortality of *Pl. lanceolata* (Laine 2004), and infection reduces host population growth rates
453 (Laine 2004; Penczykowski *et al.* 2015).

454 Successful infection is the outcome of a high degree of specificity where a given *Pl.*
455 *lanceolata* genotype can be susceptible to some *Po. plantaginis* strains while able to block

456 infection by others (Laine 2004, 2007). Hosts that are qualitatively susceptible to a given parasite
457 strain may still vary in their ability to mitigate its sporulation once infected (i.e., quantitative
458 resistance). Evidence for diversity within and among host populations comes from laboratory
459 inoculation experiments showing variation in resistance to a given set of parasite strains among
460 clonal plant lines under controlled conditions (Laine 2004, 2007). The *Po. plantaginis*
461 populations in Åland are also diverse, comprised of genetically and phenotypically distinct
462 parasite strains (Tollenaere *et al.* 2012), with a high proportion of coinfection by different
463 multilocus genotypes (MLGs) (Susi *et al.* 2015).
464
465 *Study design*
466 The study consisted of two experiments and a fine-scale survey and sampling of
467 infections in the wild (Fig. 1). In the first experiment, which we refer to as the "manipulated
468 epidemic experiment", we tested whether parasite strains exhibit priority effects that are
469 mediated by the host plant response to prior infection. We addressed this question by first
470 inoculating or mock-inoculating hosts of four different genotypes with one of four parasite
471 strains and then later exposing the same hosts to all four strains in a common garden
472 environment. In the second experiment, which we refer to as the "natural epidemic experiment",
473 we tested whether host-plant mediated priority effects among parasite strains can influence
474 parasite assembly during a natural epidemic. We addressed this question by first inoculating or
475 mock-inoculating hosts with one of three different parasite strains, then embedding those hosts
476 into one of three wild host populations in order to expose the hosts to a natural epidemic, and
477 then compared the effect of these treatments on the structure of the resulting parasite assemblage
478 on each sentinel host plant as well as wild plants from the same populations. These experiments

479 revealed strong facilitative priority effects among parasite strains, and indicated that these
480 facilitative priority effects can alter parasite assembly during natural epidemics. Finally, we
481 analyzed the results of an observational study, which we refer to as the "wild host survey," to test
482 whether a signal of host-plant mediated facilitative priority effects is detectable among parasite
483 strains in natural populations. We addressed this question by tracking infections on wild host
484 plants over the course of a natural epidemic.
485
486 Experimental plant genotypes. To test whether the outcome of sequential infections
487 varied across different host plant genetic backgrounds, we used a set of four host plant maternal
488 lines in both experiments (Table S8). Each maternal line came from a single seed head from a
489 different mother plant in the Åland archipelago. Seeds were sown in 9 x 9 cm flower pots in a
490 mixture of 30% sand, and 70% potting soil. Plants were maintained in the greenhouse at +20 °C
491 until transport to the common garden location, where they were acclimated to outside conditions
492 for at least two weeks before the start of the experiments.
493
494 Experimental parasite strains. The parasite strains used for inoculating plants in both
495 experiments were collected from field populations in the Åland archipelago at the end of
496 epidemics in 2014 (Table S9). We collected and purified the powdery mildew isolates as follows.
497 Infected leaves were detached using forceps and placed into 9 cm Petri dishes containing moist
498 filter paper. Between every sampled leaf, the forceps were sanitized with DNA-Away (Molecular
499 Bio Products) to avoid cross-contamination. To ensure that each parasite isolate was a single
500 strain (multi-locus genotype, MLG), we purified the isolates through three successive single-
501 colony transfers of spores onto detached, greenhouse-grown leaves (Nicot *et al.* 2002).

502 Inoculated leaves were maintained on moist filter paper in Petri dishes in a growth chamber
503 under standard conditions of 21°C (± 2 °C) and 16L:8D photoperiod. We then amplified the
504 fungal isolates through 2-3 rounds of inoculations to generate enough spores for the experiments
505 described below.
506
507 Manipulated epidemic experiment. We performed the manipulated epidemic experiment
508 to test how inoculating a single leaf of the rosette with a single parasite strain ("priming")
509 affected susceptibility of the plant to later-arriving parasite strains. The common garden
510 experiment was performed in a 30 x 45 m fenced field at the University of Helsinki's Lammi
511 Biological Station (Lammi, Finland). The experiment consisted of a total of 320 plants placed in
512 groups of eight in plastic trays (0.5 x 0.3 m), with two plants from each of the four maternal plant
513 lines in each of 40 trays. Trays were equally spaced apart in the field along a 5 m x 5 m grid.
514 Each plant included two leaves from the same rosette spiral enclosed in separate sleeves made
515 from spore-proof polyester material (pollination bags from PBS International) and secured at the
516 leaf base, which were used for a separate study and are not discussed further. One plant from
517 each maternal line in the tray was assigned to the "primed" treatment, and the other plants were
518 assigned to the mock-inoculated "control" treatment. On 26 July 2015, primed plants were
519 enclosed in a plastic bag with a single leaf emerging through a small hole in the bag. A fine
520 paintbrush was used to inoculate that leaf with one of the four priming strains, depending on the
521 tray ID. Twenty-four hours after inoculation, the leaf was covered with a spore-proof sleeve, the
522 plastic bag was wiped with ethanol and removed. Control plants underwent the same procedure,
523 but no powdery mildew was inoculated.

524 The plants in the common garden were then bulk-exposed to all four powdery mildew
525 strains over the course of four days (30 July-2 August; days 4 to 8 post-priming). This was done
526 by rotating heavily infected source plants next to the trays, such that each tray was exposed to
527 each of the strains for 24 hours.
528 The plants in the common garden were screened for infection between 19-23 August. We
529 recorded the total number of uninfected and infected leaves for each plant. We collected several
530 infected leaves from each plant for genotyping. These infected leaves were stored in paper
531 envelopes at room temperature until DNA extraction (see Genetic analyses section below).
532
533 Natural epidemic experiment. This experiment tested whether the sequence and timing of
534 priming influenced subsequent infection among sentinel plants placed into three field
535 populations during natural epidemics. We used the same set of four host plant maternal lines as
536 in the common garden experiment, and plants in the priming treatment were inoculated with a
537 parasite strain that had been present in the field population the previous year (Table S8). Priming
538 of a single leaf was performed as in the common garden experiment. To manipulate infection
539 timing, plants were primed either 8 days (treatment P1) or 4 days (treatment P2) prior to being
540 placed into the three field populations on 4 August 2015. There were also C1 and C2 control
541 plants set up at the same time (but mock inoculated). For each of the four host plant maternal
542 lines in each of the three field populations, we had 10 replicates of P1, 10 replicates of P2, 5
543 replicates of C1, and 5 replicates of C2, for a total of 360 plants. Groups of paired primed and
544 control sentinel plants were placed on plastic trays throughout the field populations. The trays
545 were watered and moved to new locations every two days for 8 days, to standardize exposure to
546 powdery mildew spores. After the 8 days of exposure to natural epidemics, the plants were

547 covered individual spore-proof pollination bags and transported back to the Lammi Biological
548 Station, where infections were allowed to continue developing for another 10 days. Then we
549 counted infected and uninfected leaves in each size class as described for the common garden
550 experiment. We also saved infected leaves in individual paper envelopes to genotype and
551 determine which strains infected them.

552 While the sentinels were in the field populations, we surveyed wild plants from each
553 population for infection and tagged infected plants located at least 1.5 m apart. Up to 44 infected
554 plants per population were tagged. At the end of the epidemics, we collected samples of infected
555 leaves from the tagged plants for genotyping and parasite strain identification. Infected leaves
556 were stored in paper envelopes at room temperature until prepared as samples.

557

558 Wild host survey. The wild host survey was carried out in the Åland archipelago in 2014
559 and consisted of fifteen host populations that had been infected for at least three consecutive
560 years (Jousimo *et al.* 2014). Distances between pairs of populations ranged from ~ 1 km to ~ 40
561 km. Each population was surveyed biweekly for infection starting on 7 July, 2014, by visually
562 scanning plants for signs of the mildew. When host individuals became infected, the date of
563 infection was recorded and those hosts were physically tagged in order to be resurveyed as focal
564 hosts. Up to thirty focal hosts were tagged in each population. Focal plants were located at least
565 3 m from one another and their locations were recorded by GPS. To minimize the impact of
566 sampling on pathogen community assembly, we sampled lesions from focal hosts and genotyped
567 the infections only after more than one leaf of a focal host was infected. We sampled lesions in
568 such a manner that spores also remained on the plants, thus the infection was not removed from
569 the epidemic. All hosts that survived were then resampled at the end of the season (n = 105 hosts

570 across 13 populations). Sampling consisted of placing infected leaves in paper envelopes, which
571 were stored in a cool, dry place until the end of the field season, at which point the samples were
572 taken to Helsinki and stored at -20C. These two genotyping sessions were used to infer the
573 sequence of infection on host individuals, while the frequent surveys of whole host populations
574 were used to infer phenology of the parasite strains.
575
576 Genetic analyses. We genotyped infections in all three studies to determine which
577 powdery mildew strains successfully established on the host plants. Each sample consisted of a
578 lesion from an infected leaf, which we placed into a 1.5 mL tube that was stored at -20°C until
579 DNA was extracted using an E.Z.N.A. Plant Mini Kit (Omega Bio-Tek, Norcross, GA) at the
580 Institute of Biotechnology, University of Helsinki. The lesions consisted of both host tissue and
581 fungal material. Samples were genotyped at 19 single nucleotide polymorphism (SNP) loci with
582 the Sequenome iPlex platform at the Institute for Molecular Medicine Finland (See Tollenaere *et*
583 *al.* 2012; Parratt *et al.* 2017 for details). Because *Po. plantaginis* conidial spores are haploid,
584 samples were classified as coinfected if two different nucleotides were called at any locus
585 (Tollenaere *et al.* 2012). The observed coinfections were resolved into single infections with an
586 algorithm that compared each coinfection profile to the genotypes of all single infections in the
587 experiment (i.e., to the four strains in the manipulated epidemic experiment or to all single
588 infections from the same population in the natural epidemic experiment). When a match was
589 found, the genotype of the other coinfecting strain could be determined as having the
590 complementary alleles at the heterozygous loci. However, for samples with only a few
591 heterozygous loci and where multiple strains had the same nucleotides at those loci, we could
592 only unambiguously identify one of the two coinfecting strains. For samples from the

593 manipulated epidemic experiment that failed to call all 19 SNPs, we were still able to identify the
594 strain if the nucleotides at the successfully called SNPs were unique to one of the four strains in
595 that experiment. However, samples from the natural epidemic experiment or wild host surveys
596 that were missing genotype data from any of the 19 SNPs were excluded from the analysis.
597 From sentinel plants in the manipulated epidemic and natural epidemic experiments, we
598 randomly selected four infected leaves per plant for genotyping (if fewer than four leaves on the
599 plant were infected, then all infected leaves were sampled). In addition, we genotyped infections
600 from a subset of the primed leaves to verify that plants were primed with the correct parasite
601 strains. A large number of samples failed to call several of the 19 SNP loci during our first round
602 of genotyping in spring 2016. To replace those samples for which genotyping failed, we
603 extracted DNA from remaining infected leaves from the same plants and genotyped those
604 replacement samples in spring 2017.
605
606 *Analysis*
607 All analyses were conducted in R version 3.5.2 (R Core Team 2015). We omitted plants
608 from analyses that were inoculated, but never became infected or that were mock-inoculated but
609 became infected (85 in the manipulated epidemic experiment; 165 in the natural epidemic
610 experiment), as well as plants that died prior to data collection (4 in the manipulated epidemic
611 experiment; 2 in the natural epidemic experiment) resulting in a total sample size of n = 231 in
612 the manipulated epidemic experiment and n = 193 in the natural epidemic experiment.
613 Manipulated epidemic experiment. We first tested whether the priming treatment altered
614 infection during the manipulated epidemic by constructing three models using the R package
615 lme4 (Bates *et al.* 2014): (1) the probability of a plant becoming infected, using a logistic mixed

616 model, (2) the logit-transformed proportion of leaves infected as a response measure representing
617 infection severity, using a linear mixed model, and (3) the logit-transformed proportion of leaves
618 infected as a response measure representing infection severity, limited to infected hosts only,
619 using a linear mixed model. All three models included the experimental treatment (inoculated
620 vs. mock inoculated) as a fixed effect. We included the log-transformed number of leaves as a
621 fixed covariate in the model, because plants with more leaves have a higher probability of
622 intercepting infectious spores. Inoculation tray was included as a random effect in the model. To
623 test whether treatment effects differed among host genotypes and priming strains, we fit three
624 models with the same three response variables, this time including the full priming treatment
625 (five levels: mock-inoculated, and four priming strains), plant genotype, and their interactions as
626 fixed effects, the log-transformed total number of leaves as a fixed covariate, and priming tray as
627 a random effect. We evaluated differences among various model coefficients using the emmeans
628 package in R (Lenth *et al.* 2018).

629 To test whether the priming strain facilitated other later arriving strains at least as
630 strongly as it facilitated itself, we fit a model with a binomial response representing whether or
631 not the host became infected, and including the priming treatment as a binary factor (control vs
632 primed) and its interaction with whether or not the infecting strain was different from the
633 priming strain. The model also included log-transformed total number of leaves as a fixed
634 covariate and the tray and plant id as nested random intercepts. Finally, we tested whether
635 within-host priority effects altered the structure of parasite assemblages, using a multivariate
636 generalized linear model using the MVabun package in R (Wang *et al.* 2012). To measure the
637 effect of prior infection on the structure parasite assemblages, we constructed a model that tests
638 whether the distribution of infection by each strain was affected by the priming treatment (five

639 levels: control, and four priming genotypes), plant genotype, and their interactions, including the
640 log-transformed total number of leaves as a covariate. These models avoid some of the problems
641 associated with distance-based models such as permANOVA. However, because these models
642 cannot handle unbalanced grouping variables, experimental tray was included as a covariate
643 rather than a random effect in the model.

644 Natural epidemic experiment. We first tested whether the priming treatment altered
645 infection during the natural epidemic by constructing three models using the lme4 package in R:
646 (1) the probability of a plant becoming infected, using a logistic mixed model, (2) the logit-
647 transformed proportion of leaves infected as a response measure representing infection severity,
648 using a linear mixed model, and (3) the logit-transformed proportion of leaves infected as a
649 response measure representing infection severity, limited to infected hosts only, using a linear
650 mixed model. Each model included host population, plant genotype, and the experimental
651 treatment (C1: control for the first priming treatment, C2: control for the second priming
652 treatment, P1: first priming treatment at eight days prior to placement in the field, and P2: second
653 priming treatment at four days prior to placement in the field) as interactive fixed effects and the
654 log-transformed total number of leaves as a fixed covariate. Experimental tray was included as a
655 random intercept in the model, but this random effect did not explain any of the variance in some
656 of the models, leading to a computational singularity. Despite the computational singularity, we
657 opted to keep experimental tray in all models to account for non-independence among samples in
658 each patch. In the logistic regression model, there were no significant interactions ($p = 1$), and
659 owing to the binary response variable and high number of predictors, the model suffered from
660 complete separation. We therefore iteratively removed all non-significant interactions, yielding a

661 reduced model. We evaluated differences among various model coefficients in the reduced
662 models using the emmeans package.

663 We tested whether priority effects among parasite strains could lead to variation in the
664 structure of parasite assemblages among hosts during a natural epidemic with a multivariate
665 generalised linear model using the MVabun package in R, to test whether the distribution of
666 infection by each strain was affected by the priming treatment (four levels: C1, C2,P1, and 2),
667 patch, plant genotype, and their interactions, including the total number of leaves as a covariate.
668 To fit this model, we removed from the dataset any plant that had an infection that could not be
669 genotyped, resulting in a total of 181 plants for this analysis. Similar to previous analyses, we
670 reduced the model by removing non-significant interactions.

671 Wild host survey. To test whether parasite strains exhibit priority effects within hosts, we
672 first fit a series of cox proportional hazards models following Halliday et al (2017, 2018) using
673 the coxphf package in R (Ploner & Heinze 2015). To fit these models, we made one critical
674 assumption about the data: we assumed that whichever strain was first observed in genotyping
675 was the "early arriving strain". This assumption allows us to use temporal data from the first
676 observations of a host, regardless of the time between that survey and the genotyping date.
677 However, this assumption ignores the possibility of rare parasite strains being locally cleared
678 early during the epidemic. Furthermore, because we could not resolve the sequence of infection
679 on host individuals that are coinfected during the initial genotyping survey (occurring in 22/105
680 host individuals), both coinfecting parasites were assumed to have been present at the initial
681 infection of the host. We then fit a series of cox proportional hazards models of infection by each
682 parasite as a focal (i.e., late-arriving) parasite with prior infection by each other (i.e., early-
683 arriving) parasite as the only predictor in each model. These models tested whether the time until

684 infection by each strain was influenced by infection sequence on a host individual. Across the
685 Åland archipelago, distinct host populations often harbor distinct parasite assemblages. Parasite
686 strains were therefore only modeled among hosts that occurred in populations where those
687 Parasites had been observed. To explore whether the magnitude of priority effects among
688 parasite strains tended to be facilitative or antagonistic, we next fit an intercept only linear mixed
689 model with the coefficient from the cox proportional hazards models (i.e., the interaction
690 coefficient) as the response variable and the identity of the focal (i.e., late arriving) parasite in
691 the cox proportional hazards models as a random intercept, weighting the regression by the
692 number of surveys per host individual to give more explanatory power to host individuals that
693 were surveyed more times over the growing season. Finally, we tested whether parasite
694 phenology among strains altered the structure of parasite assemblages within hosts using a
695 multivariate generalised linear model on the distribution of infections by each strain at the end of
696 the epidemic, in the R package MVabund. To measure parasite phenology, we recorded the
697 earliest date that each strain was observed in the field during the 2014 epidemic. We then
698 modeled the presence or absence of each strain at the end of the season as a function of the
699 phenology of the early-infecting strains, with the sampling date of the final survey and host
700 population as covariates in the model.
701
702

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