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## Coinfection accelerates transmission to new hosts despite no effects on virulence and parasite growth

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1 **Title:** Coinfection acelbrates transmission to new hosts despite no effects on virulence and parasite  
2 growth

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10

11 **Abstract**

12 One of the fundamental aims of ecological, epidemiological and evolutionary studies of host-parasite  
13 interactions is to unravel which factors affect parasite virulence. Theory predicts that virulence and  
14 transmission are correlated by a trade-off, as too much virulence is expected to hamper transmission  
15 due to excessive host damage. Coinfections may affect each of these traits and/or their correlation.  
16 Here, we used inbred lines of the spider-mite *Tetranychus urticae* to test how coinfection with *T.*  
17 *evansi*, impacted virulence-transmission relationships, at different conspecific densities. The presence  
18 of *T. evansi* on a shared host did not change the relationship between virulence (leaf damage) and the  
19 number of transmitting stages (i.e., adult daughters ). The relationship between these traits was  
20 hump-shaped across densities, both in single and coinfections, which corresponds to a trade-off.  
21 Moreover, transmission to adjacent hosts increased in coinfection, but only at low *T. urticae* densities.  
22 Finally, we tested whether virulence and the number of daughters were correlated with measures of  
23 transmission to adjacent hosts, in single and coinfections at different conspecific densities. Traits were  
24 mostly independent, meaning interspecific competitors may increase transmission without affecting  
25 virulence. Thus, coinfections may impact epidemiology and parasite trait evolution, but not  
26 necessarily the virulence-transmission trade-off.

27

28 **Keywords:** trade-off hypothesis, coinfection, host-parasite interactions, multiple infections,  
29 herbivorous arthropods, interspecific competitors .

30 **Introduction:**

31 Studies on host-parasite interactions, be it from an evolutionary, ecological or disease perspective,  
32 generally evaluate the causes and consequences of parasite-induced fitness costs to hosts (i.e.  
33 virulence) and the spread of parasites among hosts (transmission). Coinfections, i.e. the presence of  
34 other parasites (strains or species) within the same host, are ubiquitous and a key factor affecting  
35 parasite life-history traits (1-3). Experimental work shows that coinfections can both increase or  
36 decrease within-host parasite growth (e.g. (4-7), often with consequences for virulence (4, 8, 9) and  
37 transmission (10), which are often related. Indeed, the virulence-transmission trade-off hypothesis  
38 posits that, despite virulence being a by-product of parasite growth, too high virulence leads to  
39 excessive host damage, curtailing transmission (11, 12). Evidence for the existence of a trade-off  
40 between these traits is limited (13), possibly due to environmental factors, such as host and/or  
41 parasite demography, interactions with the host immune system or coinfection, changing the  
42 selection environment and relationships between traits (1, 3, 12, 14, 15). Therefore, coinfection may  
43 be a factor affecting traits involved in the trade-off and/or modulating the interaction among them (1,  
44 16). However, most studies on coinfections focus on its effect on individual traits, not on their  
45 relationship.

46 One important environmental factor that may affect the outcome of coinfections is the relative  
47 density of each competitor in the within-host environment (5, 7, 17-19). Increasing densities of a  
48 competitor may increasingly reduce parasite growth (7, 18). Alternatively, the impact of a competitor  
49 may depend on parasite densities (5, 17, 19). For example, interspecific competition may only affect  
50 traits at lower intraspecific densities (17). Interactions among parasites may also impact transmission-  
51 related traits, independently of growth and virulence, such as triggering dispersal from hosts infected  
52 with competitors or impacting whether a new host becomes infected. Indeed, certain parasites avoid  
53 or choose a host, or a host tissue, depending on its infection status (20, 21). This means that multiple  
54 parasites in the environment have the potential to impact parasite life-history ecology and evolution  
55 at different scales, not restricted to the within-host environment.

56 A key aspect that may affect how parasite interactions in coinfections modify parasite traits is whether  
57 they are genetically correlated. Indeed, if that is the case, then any genetic change in one trait driven  
58 by the presence of competitors will affect the genetic value of other traits, which has major  
59 consequences for the evolutionary trajectories of populations. In contrast, if the correlation is purely  
60 environmental, then no direct evolutionary consequences are expected, but the ecological impact of  
61 the parasite, such as parasite severity or epidemic onset, may be modified.

62 This study used inbred lines of the spider mite *Tetranychus urticae* to investigate how an interspecific  
63 competitor, the closely related species *T. evansi*, impacts virulence, the number of adult daughters  
64 produced and transmission to a new host patch as well as the potential correlations, genetic or  
65 environmental, among these traits. In *T. urticae*, there is genetic variation for dispersal distance (22,  
66 23), which here we refer to, and is the same as transmission, and for host use (24), which may be  
67 correlated with virulence. Moreover, dispersal is a plastic trait, with individuals having higher dispersal  
68 at elevated intraspecific densities (22) and in the presence of kin (25). Moreover, selection for higher  
69 dispersal has been shown to be associated with higher diapause incidence and lower fecundity (23)  
70 and dispersing individuals had smaller eggs (26) and fewer offspring surviving to adulthood when  
71 laying eggs at higher densities (27). Further, in a recent companion study to this one conducted with  
72 the same inbred lines, we found a positive genetic correlation between virulence and the number of  
73 adult daughters produced when transmission was possible during the infectious period (28). Hence,  
74 there are both genetic and environmental relationships between transmission and other life-history  
75 traits. Finally, the outcome of competition between *T. evansi* and *T. urticae* can change due to  
76 variation across populations (29) but also depending on the sequence of arrival, as *T. evansi* excludes  
77 *T. urticae* except when the latter arrives first and occupies *T. evansi*'s preferred niche (30). In sum,  
78 competitive interactions between these mite species are strong and strongly impacted by both genetic  
79 and environmental factors. Therefore, the system composed of these two spider mite species is ideal  
80 to address how the presence of a competitor affects virulence, parasite growth (number of adult  
81 daughters) and transmission, as well as the potential (genetic) interactions among these traits.

82

## 83 **Materials and Methods**

### 84 Biological system

85 Spider mites are macroparasites of plants, including many economically important crops, with their  
86 complete life cycle occurring on their host plant (31). Both *T. urticae* and *T. evansi* females lay eggs on  
87 leaves, which take ~4 days to hatch. The juvenile stage comprises 1 nymph stage and 2 deuteronymph  
88 stages, with adults emerging after approximately 14 days in our laboratory (25°C, 16:8 L: D cycle). All  
89 stages feed by injecting their stylet into parenchyma cells and sucking out the cytoplasm, which leaves  
90 chlorotic damage on the leaf surface, our measure of virulence (32). *T. urticae* is a generalist species,  
91 feeding on more than 1000 different plant species (33), whereas *T. evansi* is a specialist species, mostly  
92 feeding on *Solanaceae* plants (34). In natural systems, co-occurrence of different spider mite species  
93 in the same geographical area is common, leading to co-infection of the same host plant (35).

94

95 Spider-mite populations

96 Inbred lines of *T. urticae* were created from an outbred population through 14 generations of sib  
97 mating at the University of Lisbon (35). A subset of 15 inbred lines was transferred to the University  
98 of Montpellier in January 2018 and maintained on bean leaves (*Phaseolus vulgaris*; variety Pongo) as  
99 described in Godinho et al. 2023. The *T. evansi* population was originally collected in October 2010 in  
100 the Alpes Maritimes (43.75313 N, 7.41977 E) on *Solanum nigrum*.

101

102 Prior to each experiment, cohorts of 40 mated female spider mites from each inbred line were  
103 isolated on a bean patch (2-3 leaves placed together). These females were allowed to lay eggs for 48h.  
104 Fourteen days later, the mated daughters of these females, of approximately the same age, were used  
105 in the experiments. The same procedure was used to create cohorts of *T. evansi*. All spider-mite  
106 populations, inbred lines and cohorts used in these experiments were maintained on bean leaves  
107 (*Phaseolus vulgaris*; variety Pongo) placed on water saturated cotton wool, in small plastic boxes (255  
108 mm length x 183 mm width x 77 mm height), at 25°C with a 16:8 L: D cycle, at 60% relative humidity.  
109 Not all inbred lines are represented in each experiment due to too few individuals available at the  
110 start of the experiment (N = between 14 to 16 lines).

111

112 *Experiment 1. Impact of interspecific competitors on within-host traits*

113 Females of each *T. urticae* inbred line were randomly assigned to one 'intraspecific density' treatment  
114 (5, 10 and 20 females), with or without 'interspecific competition' (10 *T. evansi* females) (Figure 1a).  
115 In all treatments, females were placed on a 2 x 2 cm bean leaf patch placed on wet cotton wool in  
116 plastic boxes. There were 3 to 13 replicates for each inbred line per treatment combination  
117 (intraspecific density x interspecific competition) distributed across 3 blocks. Variation in the number  
118 of replicates per line arose due to differences in the number of adult females produced in the  
119 synchronised cohorts. All females were allowed to feed and lay eggs on their leaf patches for 4 days.  
120 After this period, females were killed, the number of eggs was counted and a photograph of each  
121 patch was taken using a Canon EOS 70D camera. The damage inflicted by these adult female spider  
122 mites on each host patch, used as a measure of virulence, was determined using ImageJ and Ilastik  
123 1.3, as described in (28). Succinctly, the background from each photo was removed in ImageJ, then we  
124 used Ilastik to distinguish damaged area from healthy leaf and finally the damaged area was calculated  
125 via the colour contrast between damaged and undamaged leaf tissue in ImageJ. Because some leaf  
126 veins were incorrectly assigned as damage by Ilastik, uninfested bean leaf patches were left in the  
127 experimental boxes for the same period of time and photographed; these control patches were used

128 to establish an average baseline level of falsely assigned damage, which was subtracted from each  
129 measurement to estimate the actual damage (hereafter: 'damage'). After a period of 14 days, the  
130 female offspring surviving on each patch were counted. Only females were counted because the males  
131 of both species are not easily distinguishable, females are the main dispersers in these species and the  
132 number of females produced correlates with transmission (28). The data on damage inflicted and  
133 production of adult females, for the "intraspecific density" treatments in the absence of *T. evansi* are  
134 published elsewhere (28).

135

### 136 *Experiment 2. Impact of interspecific competition on transmission*

137 We measured differences in dispersal traits (transmission) for the different *T. urticae* inbred lines  
138 assigned to the same 'intraspecific density' and 'interspecific competition' treatments as in  
139 Experiment 1. Adult *T. urticae* females were placed in groups of 5, 10 or 20 on a 2cm<sup>2</sup> bean leaf patch  
140 on wet cotton wool alone or with 10 *T. evansi* females. This first host patch was connected, in a row,  
141 to 2 other bean patches via 3 x 1 cm Parafilm bridges from day 1 of the experiment (Figure 1b). This  
142 experimental setup was replicated across several boxes. Females were allowed to feed and disperse  
143 across patches, and the number of mites on each patch was counted on days 1, 2, 3, 6, and 9 after the  
144 beginning of the experiment. There were 3 to 9 replicates for each inbred line per treatment  
145 combination (intraspecific density x interspecific competition) distributed across 2 blocks. Variation in  
146 the number of replicates is due to the number of offspring emerging as adult females among lines.

147

### 148 Statistical analysis

149 Analyses were performed using the software JMP SAS version 17 and SAS OnDemand for Academics  
150 (36).

### 151 *Impact of interspecific competitors on within-host traits*

152 In Experiment 1 General Linear Mixed Models (GLMM) were used to investigate how intraspecific  
153 density, interspecific competition and their interaction affected virulence and the number of female  
154 offspring becoming adult. These analyses included intraspecific density as a covariate and interspecific  
155 competition as a fixed factor. Next, we used a GLMM to test whether the relationship between  
156 virulence and the production of adult daughters (transmitting stages) changed with interspecific  
157 competition and if this effect varied across intraspecific densities. In this model, the number of adult  
158 daughters remained the response variable, with the linear, quadratic and saturating terms for

159 virulence, intraspecific density, interspecific competition and their interactions, up to and including 3-  
160 way interactions, included as explanatory variables. Full models were simplified by removing non-  
161 significant terms in a stepwise fashion. Inbred line and block were included in these models as random  
162 factors.

163

#### 164 *Impact of interspecific competitors on transmission*

165 In Experiment 2, different measures were taken to assess dispersal across host patches. We used a  
166 dispersal score to evaluate the spread of mites across the 3 host patch system. This was calculated  
167 each day as the (number of mites on host patch 2 + the number of mites on host patch 3\*2)/total  
168 number of mites (22). This score weights greater distances more, as they represent higher  
169 dispersal propensity, and corrects for the differences in the initial density of mites (22). The dispersal  
170 score was analysed in a GLMM with interspecific competition as a fixed factor, intraspecific density  
171 and time as covariates, and their interactions. The linear and quadratic terms for time were included  
172 in the model to account for saturation in transmission through time. As there was a significant  
173 interaction between competition and density, we separately tested the effect of interspecific  
174 competition on the dispersal score at each of the different densities. We also investigated, in separate  
175 GLMMs, including intraspecific density as a covariate and interspecific competition as a fixed factor,  
176 how interspecific competition affected the time for mites to reach, and the maximum number of *T.*  
177 *urticae* on host patches 2 and 3. Full models included interactions between explanatory variables that  
178 were simplified by removing non-significant terms in a stepwise fashion. All the above models included  
179 inbred line and block as random factors.

180

#### 181 *Genetic variance for within-host traits and transmission*

182 Broad-sense heritability,  $H^2 = \frac{Var(G)}{Var(G)+Var(E)}$  (37), for each trait in each experiment was determined  
183 by extracting the proportion of total variance in models explained by inbred line (among inbred line  
184 variance) by re-running models for within-host and transmission related life-history traits including all  
185 terms as random (competition, line, density, block and patch nested within block for dispersal score)  
186 to obtain all variance components. Note that these models did not include interaction terms. Traits  
187 were divided by the total number of adult females placed on a patch (e.g. traits per capita) since they  
188 are passed from parents to offspring at the level of an individual, not in groups of individuals. The  
189 significance of each model was assessed by comparing the Akaike's Information Criterion (AIC) of a  
190 model including inbred line with a model excluding it. A significant  $H^2$  indicates that trait variance is



191 significantly explained by differences in the additive or dominance genetic variance across  
192 individuals and/or by differences in maternal effects.

193

#### 194 *Correlations between within-host traits and transmission*

195 We assessed genetic correlations between traits measured in the 2 experimental set-ups separately  
196 for each combination of density and competition treatments. If correlations between traits are  
197 genetic, this can provide predictions for how they might evolve, given that selection on one trait will  
198 also affect the expression of the other. We only included traits for which there was significant genetic  
199 variance among inbred lines (Table S1).

200 First, we reported the Pearson's correlation coefficient across mean trait values for each of the inbred  
201 lines. Next, we extracted the standard errors for each correlation coefficient and associated p-values  
202 from a PROC MIXED COVTEST model as described in (38) using SAS Studio. As measures were taken in  
203 different experimental set-ups we bootstrapped (with replacement) the mean value for each inbred line  
204 at each density and interspecific competition treatment 20 times and randomly paired the different  
205 values. Trait values were standardised across all lines for each density and competition treatment,  
206 such that each variable had a mean of zero and standard deviation of one. This was so that values for  
207 pairs of traits were of a similar scale as required for the PROC MIXED COVTEST in SAS Studio.

208 All p-values < 0.05 were corrected for multiple testing (within each pair of traits) using the  
209 Bonferroni correction method.

210

## 211 **Results**

### 212 *Impact of interspecific competitors on within-host traits*

213 Interspecific competitors had no effect on the virulence of *T. urticae* at any intraspecific density  
214 (interspecific competition;  $F_{1, 637} = 0.34$ ,  $p = 0.5609$ , interspecific competition\*intraspecific density;  $F_{1, 636} = 0.36$ ,  $p = 0.5567$ ) nor on the number of adult daughters (interspecific competition;  $F_{1, 637} = 1.09$ ,  $p = 0.2959$ , interspecific competition\*intraspecific density;  $F_{1, 636} = 0.14$ ,  $p = 0.7107$ ; Figures 2  
217 and S1; Table S2). When virulence was included as a covariate, the presence of interspecific  
218 competitors did not change the relationship between virulence and the production of adult  
219 daughters, i.e. transmitting stages (interspecific competition\*virulence;  $F_{1, 635} = 2.17$ ,  $p = 0.1416$ ,  
220 interspecific competition\*virulence<sup>2</sup>;  $F_{1, 637} = 0.03$ ,  $p = 0.8532$ , Figure 2; Table S1).

221

222 A significant interaction between intraspecific density and virulence ( $F_{1, 643} = 16.07$ ,  $p < 0.0001$ )  
223 showed that the shape of the relationship between virulence and the production of adult daughters,  
224 i.e. transmitting stages, changed at different densities (positive at low densities, no relationship at  
225 intermediate densities, and negative at high densities). This was corroborated with a significant  
226 quadratic term for virulence in a second model investigating factors affecting the number of adult  
227 daughters (virulence;  $F_{1, 648} = 12.45$ ,  $p = 0.0004$ , and virulence<sup>2</sup>;  $F_{1, 643} = 9.36$ ,  $p = 0.0023$ ; Figure 2;  
228 Table S2). However, as these results were not influenced by interspecific competition they are not  
229 discussed further here, as they are presented elsewhere (28).

230

### 231 *Impact of interspecific competitors on transmission*

232 The dispersal score was affected by both interspecific competition ( $F_{1, 447} = 20.68$ ,  $p < 0.0001$ ) and  
233 intraspecific density ( $F_{1, 1177} = 16.69$ ,  $p < 0.0001$ ), with a significant interaction between these two  
234 factors ( $F_{1, 445} = 7.68$ ,  $p = 0.0058$ ; Figure 3, Table S3). Models investigating the effect of interspecific  
235 competition separately at each density showed that there was only a significant effect of competition  
236 in the low density treatment (Table S4). This meant that *T. urticae* females were more likely to leave  
237 the first host patch in the presence of interspecific competitors only at low densities. The interaction  
238 between *T. urticae* density and the quadratic term for time was also significant ( $F_{1, 1844} = 23.57$ ,  $p <$   
239  $0.0001$ ), with values of the dispersal score saturating through time for patches in the intermediate  
240 and high density treatments (Figure 3, Table S3). Note, the dispersal score captured time to arrive on,  
241 and maximum numbers on patches 2 and 3 (Figure S2 and Table S5). We observed an effect of  
242 intraspecific density on all these underlying traits and of interspecific competition on the time to arrive  
243 to patch 2 and 3, the latter depending on the density of intraspecific competitors, as there was a  
244 significant interaction between the two factors (Table S5).

245

### 246 *Genetic variance for within-host traits and transmission*

247 For the within-host traits, we found low but significant broad-sense heritability for the number of adult  
248 daughters ( $H^2 = 0.057$ ) and virulence ( $H^2 = 0.060$ ). For measures of transmission, inbred line explained  
249 a significant portion of the variance ( $H^2$ ) for time to reach host patches 2 ( $H^2 = 0.062$ ) and 3 ( $H^2 = 0.036$ )  
250 and the dispersal score ( $H^2 = 0.039$ ), but not the maximum number of individuals on host patch 2 or 3  
251 (Table S1).

252

253 *Correlations between within host traits and transmission*

254 We explored the genetic relationships between traits related with transmission between hosts (i.e.,  
255 day arriving on and maximum number on each patch) and traits measured in the within host-  
256 environment (i.e., virulence and number of adult daughters) for traits that showed significant among  
257 line variation (virulence, number of adult daughters, day arriving on patches 2 and 3; Table S1), giving  
258 a total of 24 correlations. Of these, 6 models did not converge. Of the remaining 18 models, only 1  
259 was significant (Table 1), showing a negative correlation between virulence and the time to arrive on  
260 host patch 2 at high *T. urticae* densities in the presence of interspecific competition (Table 1; Figure  
261 S3). Collectively these results show that virulence and the number of adult daughters measured in the  
262 within-host environment are mostly independent of traits measuring transmission between hosts.

263

264 **Discussion**

265 In this study, we found that interspecific competition did not modify virulence, the production of adult  
266 daughters (i.e., transmitting stages) or the relationship between these traits. However, the presence  
267 of interspecific competitors increased transmission of *T. urticae* to new host patches at low  
268 intraspecific densities, which may be a mechanism to escape interspecific competition (at higher  
269 intraspecific densities this effect may be masked by more intense intraspecific competition).  
270 Differences in transmission between hosts were mostly genetically unrelated to measures of virulence  
271 or the number of adult daughters produced in the within-host environment. Therefore, selection is  
272 expected to act on each trait independently, that is, selection for virulence or the number of adult  
273 daughters is mostly unlinked to that on traits that foster early transmission. This means that selection  
274 for faster spread across host patches is not necessarily associated with higher virulence.

275

276 *Impact of interspecific competitors on within-host traits*

277 We found no significant impact of *T. evansi* on virulence or the production of adult daughters in *T.*  
278 *urticae*. This may stem from the fact that *T. evansi* is a poor competitor on bean plants. These results  
279 may have been very different had this experiment been done on a host plant to which *T. evansi* is  
280 better adapted. For instance, *T. evansi* is generally found to be the superior competitor on tomato  
281 plants, often excluding *T. urticae* ((19, 39) but see (29, 30)). As interspecific competition did not modify  
282 these traits, it also did not lead to changes in their interaction. Our results contrast with other studies  
283 that show that the impact of interspecific competition on parasite growth and virulence can change  
284 in response to the relative densities of each parasite in coinfection (5, 7, 18). Instead, as previously

285 found in the absence of competitors (28), we here find a positive relationship between virulence and  
286 transmission at low densities, no relationship at intermediate densities and a negative relationship at  
287 high densities. This result is because of intense within-host intraspecific competition among juvenile  
288 *T. urticae* developing on the host patch: at higher densities, despite many more eggs being laid, fewer  
289 offspring become adults (28).

290

#### 291 *Impact of interspecific competitors on transmission*

292 Interspecific competition changed how *T. urticae* moved among host patches, but this depended on  
293 the intensity of intraspecific competition. At low intraspecific densities, the presence of *T. evansi*  
294 increased transmission of *T. urticae* females to the second and third host patch sooner and increased  
295 the density of mites on these host patches. At intermediate and high *T. urticae* densities, however,  
296 interspecific competitors did not affect transmission. This is probably because the density of *T. urticae*  
297 was so high that there was no additional effect of interspecific competition. It could be that *T. urticae*  
298 females just respond to the total number of spider mites on the patch. However, this is unlikely since  
299 the dispersal score in the absence of interspecific competition did not change across intraspecific  
300 densities (Figure 3).

301 The finding that interspecific competition causes *T. urticae* to move to a new host faster means that  
302 coinfection may be an important driver of epidemic spread. Coinfection can cause individuals to  
303 become superspreaders, when an infected host is responsible for a disproportionate number of  
304 transmission events (40). Here, we only measured the number of spider mites moving from one host  
305 patch to another, which is not the same as the number of new hosts infected. Nevertheless, it gives  
306 an idea of the number of transmission stages leaving an infected host, which is a measure of infection  
307 potential, similar to parasite shedding (10, 41-43). These different effects of parasite intraspecific  
308 densities and coinfection could be used to predict parasite spread in natural populations and to  
309 manage or control epidemics, for instance by identifying (and isolating or treating) the most infectious  
310 individuals (44).

311 Whereas some studies have shown that the intensity of interspecific parasite competition modulates  
312 the effect of intraspecific competition within the host (5, 7, 17, 19, 30), the effect on transmission is  
313 less clear. From the dispersal literature, it is clear that intra- and interspecific competition can interact  
314 to shape the movement of organisms at different scales (45). However, parasite studies are rare (e.g.  
315 (10) for an example with different parasite strains) and do not measure other traits (e.g., virulence),  
316 which are key to evaluate the impact of interspecific competition on disease epidemics. Thus, it is as

317 yet unclear how the relative impact of inter and intraspecific competition among parasites affects  
318 transmission. This is especially true because we here found that interspecific competition affects  
319 transmission differently depending on intraspecific density. New infections are often seeded by low  
320 densities of mites (a few adult females), but during the course of infection densities become very high  
321 until the host is completely overexploited (46). Whether coinfections with *T. evansi* will foster the  
322 spread of *T. urticae* through host populations may thus depend on how often coinfections involve  
323 early or late stages of infection. One possible scenario is that *T. evansi* promoting *T. urticae*  
324 transmission will result in the latter arriving first to a host plant, which will give a headstart to *T. urticae*  
325 and, as a consequence facilitate coexistence due to priority effects (30). In turn, this headstart may  
326 result in *T. urticae* reaching higher densities before the arrival of *T. evansi*, which may reduce the  
327 impact of *T. evansi* on transmission. This would then diminish the effect of interspecific competitors  
328 on transmission, thus creating a negative feedback loop, such that this effect would only be detected  
329 transiently.

330

### 331 *Correlations between within-host traits and transmission*

332 We showed that within-host traits are mostly genetically independent of measures of transmission  
333 between hosts. Indeed, there was no genetic relationship among traits in 17/18 possible tests across  
334 treatment combinations, despite these traits being genetically determined. These results contrast  
335 with previous a study in which we found a genetic correlation between adult daughters (i.e  
336 transmitting stages) and transmission (28). However, in that case, transmission was measured from  
337 hosts where virulence was inflicted, and, thus, highly dependent on within-host processes that lead  
338 to the production of transmitting stages. Here, by obtaining independent measures for virulence and  
339 transmission, we did not find that these traits are genetically linked and, by removing the connection  
340 mediated by within-host processes, we also do not observe an effect of intra and interspecific  
341 competition on this relationship. Only one genetic correlation was found to be significant, that  
342 between virulence and the time to arrive on host patch 2. Moreover, the sign of this correlation  
343 hinged on interspecific competition: it was negative in the presence of *T. evansi*, with no correlation  
344 between these traits in the absence of competition. This means that more virulent lines are  
345 responding to the presence of *T. evansi* as a trigger to leave the first host patch sooner.

346

347 How such direct measures of transmission, independent of within-host processes (e. g. virulence),  
348 actually scale up and affect the spread of *T. urticae* across a population of potential hosts is not  
349 straightforward and may well depend on the presence and relative densities of interspecific  
350 competitors (20). Moreover, different life-history strategies could co-exist in a parasite population,

351 some maximising fitness within hosts and others maximising the spread across hosts. If genetic  
352 variation for within-host traits and transmission are uncoupled, then contrasting selection pressures  
353 in each environment may maintain variation for both across scales.

354

### 355 *Conclusion*

356 Our results show that interspecific competition may increase the rate of parasite spread across hosts  
357 and that this trait is genetically independent of traits measured in the within-host environment, i.e.,  
358 virulence and the production of adult daughters (transmitting stages). Therefore, parasites selected  
359 for higher virulence locally are not those necessarily favoured in travelling wave epidemics, or those  
360 that spread far to seed infections in new host populations. In the future it would be interesting to  
361 explore how the traits measured in this study relate to those affecting the infection of a greater  
362 quantity of hosts or parasite spread over longer distances.

363

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368

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376

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482



483 Figure legends

484 Figure 1: Experimental set up in a) Experiment 1, in which adult female *T. urticae* (black and white  
485 spider mites) were placed in groups of 5, 10 or 20 on a 2cm<sup>2</sup> bean leaf patch with or without 10 *T.*  
486 *evansi* (red spider mites), b) Experiment 2, in which adult female *T. urticae* were placed in groups of  
487 5, 10 or 20 on a 3 x 2cm<sup>2</sup> bean leaf patches with or without 10 *T. evansi*. The figure only depicts the  
488 low density treatment.

489

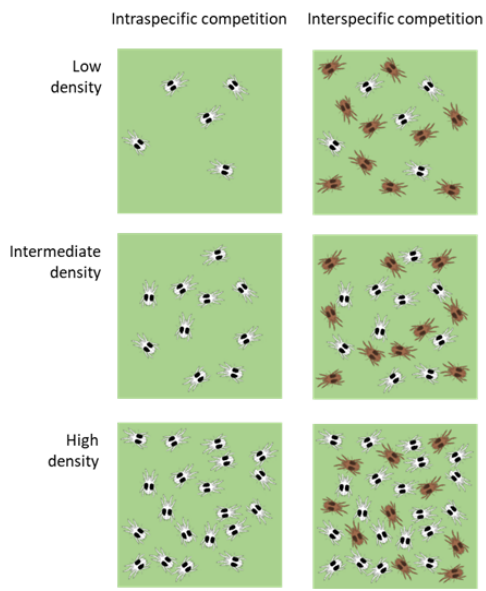
490 Figure 2: Relationship between virulence and the production of adult daughters (i.e., transmitting  
491 stages) in experiment 1 at low, intermediate and high intraspecific density in a) the absence (blue)  
492 and b) presence (red) of 10 *T. evansi* interspecific competitors. Values are given at low (5 females;  
493 lighter colour solid line and circles), intermediate (10 females; medium colour, dotted line, triangles)  
494 and high (20 females; darker colour, dashed line, squares) densities. Each dot is the mean value for an  
495 inbred line at each density (+ SE). The effect of intraspecific density creating a humped-shape  
496 relationship between virulence and adult daughters is not affected by coinfection.

497

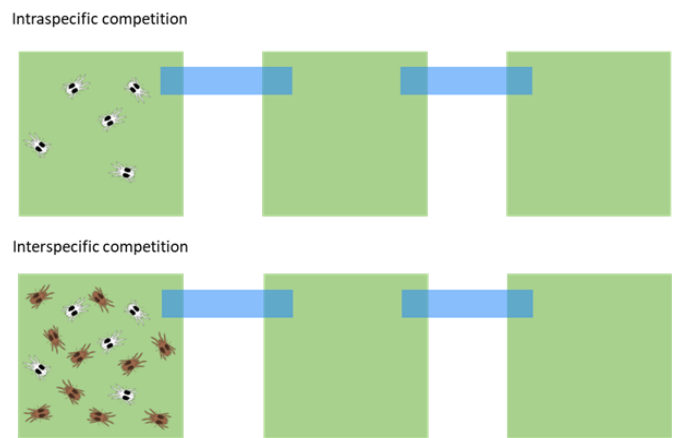
498 Figure 3: Mean dispersal score through time ( $\pm$  standard error) measured in Experiment 2 at each of  
499 the different *T. urticae* densities in the presence (red) or absence (blue) of *T. evansi*. The presence of  
500 the interspecific competitor leads to increased transmission to patches 2 and 3 at lower intraspecific  
501 densities.

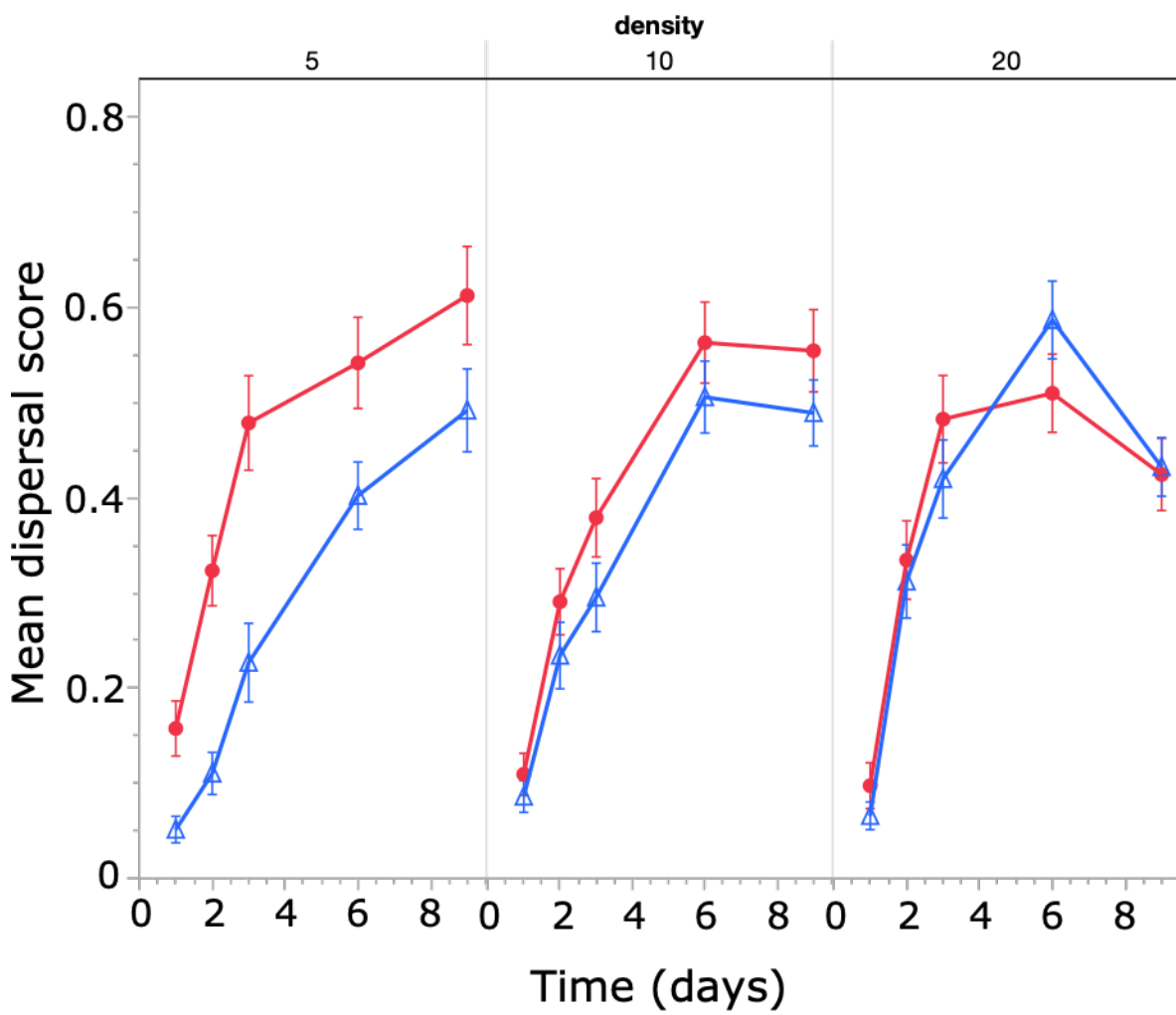
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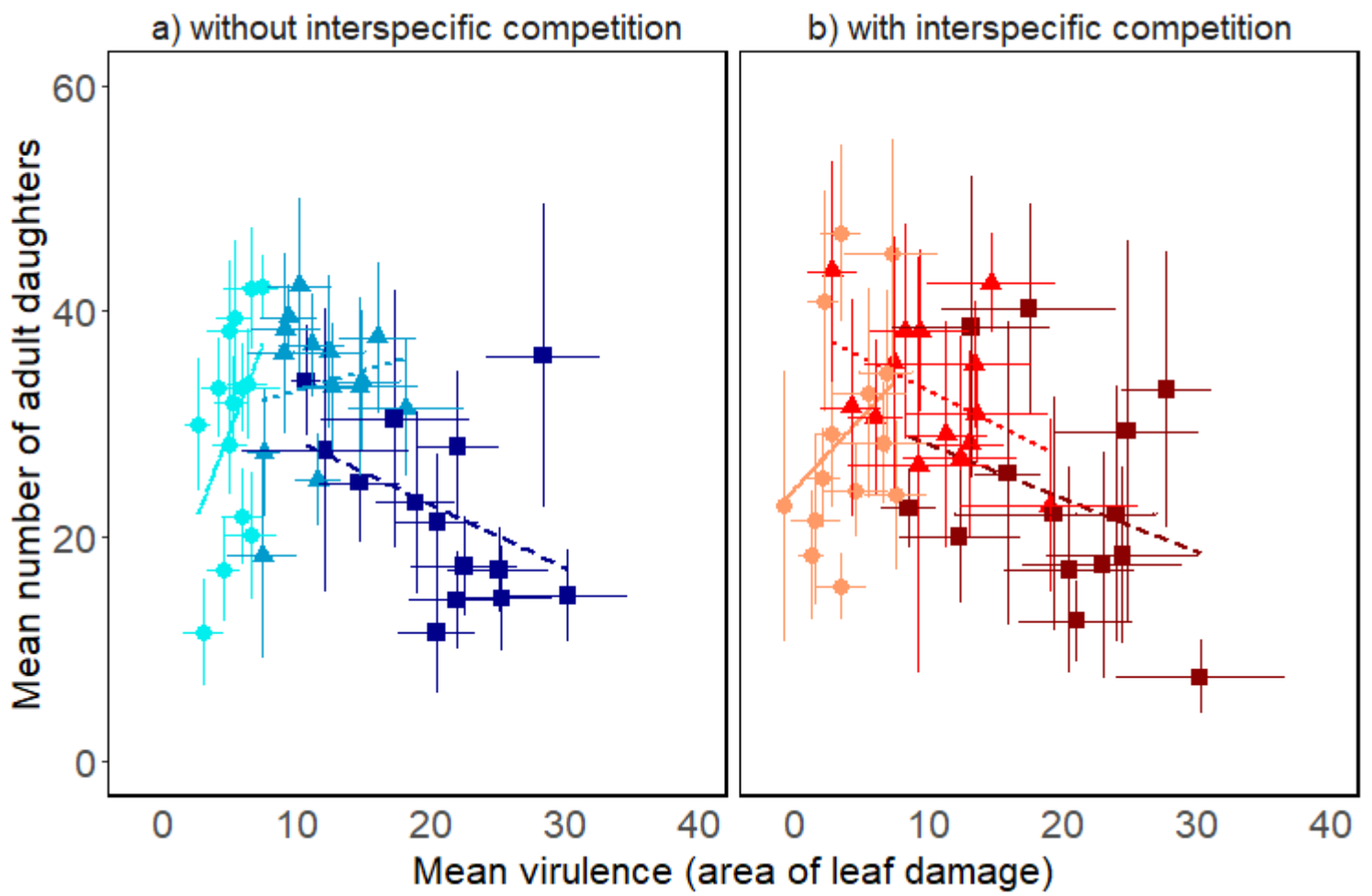
a)



b)







503 **Table 1:** Summary of genetic correlations between transmission-related traits at each of the different intraspecific densities, in the presence or absence of  
 504 interspecific competition. The Pearson's correlation across mean trait values for the different inbred lines is presented for each pair of traits  $\pm$  the standard  
 505 error calculated from the PROC MIXED COVTEST on the bootstrapped data. The  $\chi^2$  and log likelihood test comparing models with and without the genetic  
 506 correlation are also shown. All values of  $p < 0.05$  were corrected using Bonferroni corrections (counting 6 tests per pair of traits). Significant correlations  
 507 are shown in bold.

508

		Density 5		Density 10		Density 20	
Trait measuring transmission between hosts	Trait in within-host environment	No competition	Competition	No competition	Competition	No competition	Competition
Day arriving on host patch 2	<b>Virulence</b>	$r_g = -0.10 \pm 0.28$ SE $\chi^2 = 0.6, p = 0.4386$	$r_g = 0.16 \pm 0.29$ SE $\chi^2 = 1.1, p = 0.2943$	$r_g = 0.11 \pm 0.28$ SE $\chi^2 = 0.3, p = 0.5839$	$r_g = -0.19 \pm 0.27$ SE $\chi^2 = 0.4, p = 0.5271$	$r_g = 0.49 \pm 0.24$ SE $\chi^2 = 2.7, p = 0.1003$	$r_g = -0.67 \pm 0.14$ SE $\chi^2 = 8.1, p = 0.0264$
	<b>No. adult daughters</b>	$r_g = -0.22 \pm 0.27$ SE $\chi^2 = 6, p = 0.0858$	$r_g = -0.05 \pm 0.29$ SE $\chi^2 = 0.1, p = 0.7518$	$r_g = 0.22 \pm 0.03$ SE $\chi^2 = 1.3, p = 0.2542$	$r_g = -0.07 \pm 0.29$ SE $\chi^2 = 0, p = 1.0$	$r_g = -0.14 \pm 0.31$ SE $\chi^2 = 0, p = 1.0$	$r_g = 0.40 \pm 0.27$ , $\chi^2 = 1.7, p = 0.1923$
Day arriving on host patch 3	<b>Virulence</b>	$r_g = -0.01 \pm 0.28$ SE $\chi^2 = 0.5, p = 0.4795$	$r_g = 0.19 \pm 0.28$ SE $\chi^2 = 0.3, p = 0.5839$	$r_g = 0.65 \pm 0.17$ SE $\chi^2 = 6.00, p = 0.0858$	Model does not converge	Model does not converge	Model does not converge
	<b>No. adult daughters</b>	$r_g = -0.07 \pm 0.03$ SE $\chi^2 = 0.3, p = 0.5839$	$r_g = 0.26 \pm 0.23$ SE $\chi^2 = 0.5, p = 0.4795$	$r_g = 0.003 \pm 0.18$ SE $\chi^2 = 6, p = 0.0858$	Model does not converge	Model does not converge	Model does not converge

509