



**HAL**  
open science

## Limited host availability disrupts the genetic correlation between virulence and transmission

Diogo P Godinho, Leonor R Rodrigues, Sophie Lefevre, Laurane Delteil, André F Mira, Inês R Fragata, Sara Magalhães, Alison B Duncan

### ► To cite this version:

Diogo P Godinho, Leonor R Rodrigues, Sophie Lefevre, Laurane Delteil, André F Mira, et al.. Limited host availability disrupts the genetic correlation between virulence and transmission. *Evolution Letters*, 2022, 7 (1), pp.58 - 66. 10.1093/evlett/qrac008 . hal-03446328v2

**HAL Id: hal-03446328**

**<https://hal.science/hal-03446328v2>**

Submitted on 20 Mar 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Limited host availability disrupts the genetic correlation between virulence and**  
2 **transmission**

3 **Authors**

4 Diogo P. Godinho\*<sup>1</sup>, Leonor R. Rodrigues<sup>1</sup>, Sophie Lefèvre<sup>2</sup>, Laurane Delteil<sup>2</sup>, André F. Mira<sup>1</sup>,  
5 Inês R. Fragata<sup>1,3</sup>, Sara Magalhães<sup>1,3</sup>† and Alison B. Duncan<sup>2</sup>†

6  
7 **Affiliations**

8 1. cE3c: Centre for Ecology, Evolution, and Environmental Changes, Faculty of Sciences,  
9 University of Lisbon, Edifício C2, 3<sup>o</sup> piso, 1749-016 Lisboa, Portugal

10 2. Institut des Sciences de l'Évolution, Université de Montpellier, CNRS, IRD, EPHE, CC065,  
11 Place Eugène Bataillon, 34095 Montpellier Cedex 05, France

12 3. Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa

13 \*Correspondence to: diogoprino@uport.ac.uk

14 †(equal last author)

15

16 **Keywords:** Host-parasite interactions; spider-mites; inbred lines; trade-off

17 **Type of article:** Letters

18

19 **Abstract**

20 Virulence is expected to be linked to parasite fitness via transmission. However, it is not clear  
21 whether this relationship is genetically determined, nor if it differs when transmission occurs  
22 continuously during, or only at the end of, the infection period. Here, we used inbred lines of the  
23 macro-parasitic spider mite *Tetranychus urticae* to disentangle genetic vs non-genetic  
24 correlations among traits, while varying parasite density and opportunities for transmission. A  
25 positive genetic correlation between virulence and the number of transmitting stages produced  
26 was found under continuous transmission. However, if transmission occurred only at the end of  
27 the infection period, this genetic correlation disappeared. Instead, we observed a negative  
28 relationship between virulence and the number of transmitting stages, driven by density  
29 dependence. Thus, within-host density dependence caused by reduced opportunities for  
30 transmission may hamper selection for higher virulence, providing a novel explanation as to why  
31 limited host availability leads to lower virulence.

32

33 **Lay summary**

34 Parasite fitness increases with parasite growth within hosts and subsequent transmission to  
35 uninfected hosts. However, within host parasite replication leads to virulence, that is, parasite-  
36 induced harm to hosts. Thus, parasite fitness increases with virulence. However, too much harm  
37 can lead to host death, which hampers transmission. Therefore, parasite fitness should be  
38 maximized at intermediate virulence levels. Despite the centrality of this virulence transmission  
39 trade-off to the study of host-parasite interactions, whether it is genetically determined or shaped  
40 by environmental factors, remains an open question. To address this, we experimentally infected  
41 bean plants with different densities of individuals from genetically-distinct lines of the herbivorous  
42 spider-mite *Tetranychus urticae*, a plant macroparasite, and measured replication rate, virulence  
43 and transmission. To test the impact of transmission timing on correlations among traits, we let  
44 parasites transmit to uninfected hosts continuously during the infection or only at the end of the  
45 infection period. We found a positive genetic correlation between virulence and transmission  
46 under continuous transmission, meaning that lines with high virulence transmitted more and lines  
47 with low virulence transmitted less. In contrast, when transmission was restricted to the end of the  
48 infection, no genetic correlation was found between virulence and transmission. There was,  
49 however, a strong effect of parasite density on the relationship between the two variables in this  
50 transmission scenario. Indeed, high parasite densities led to strong density dependence and thus  
51 a negative correlation between virulence and transmission. Thus, there was lower transmission  
52 from highly harmed hosts, independently of the line infecting it, and the optimal transmission was  
53 at intermediate levels of virulence, as expected. Therefore, both the cause of the correlation  
54 between virulence and transmission and its form was modulated by opportunities for transmission  
55 during infection. This finding has major consequences for the understanding of virulence in  
56 natural systems and its potential control.

## 57 **Introduction**

58 Virulence, the harm inflicted by parasites on their hosts, is a trait with high relevance for human,  
59 animal, plant and ecosystem health. Parasites replicate within their host, exploiting their  
60 resources and damaging tissues, thus virulence is often positively correlated with replication rate  
61 (reviewed in Acevedo *et al.* 2019). Thus, it is expected that higher replication rates lead to more  
62 parasites emerging to infect novel hosts, with a positive impact on parasite fitness (i.e., the  
63 reproductive growth rate,  $R_0$ ; Anderson & May 1982). Therefore, selection is expected to also  
64 favour higher virulence. This holds as long as virulence is not so high as to induce host mortality,  
65 in which case higher virulence shortens the infection period, reducing the chances for  
66 transmission, or to cause host morbidity, decreasing the encounter rate with uninfected hosts,  
67 which will also hamper transmission. This reduction in transmission opportunities decreases  
68 parasite fitness, leading to the so-called virulence-transmission trade-off (Anderson & May, 1982).

69 This relationship between virulence and parasite fitness is an assumption of most theoretical  
70 models of virulence evolution (Anderson & May, 1982; Frank, 1996; Wild *et al.*, 2009). Because  
71 evolution rests on the existence of genetic variation, it is implicit that the underlying correlation  
72 between virulence and transmission is genetic. However, although such correlations have been  
73 found in several systems (reviewed in Acevedo *et al.* 2019), most studies used parasite isolates  
74 with different geographic origins and thus that differ not only genetically, but also in their recent  
75 ecological and evolutionary history (Ebert, 1994; Mackinnon & Read, 1999; de Roode *et al.*,  
76 2008; Doumayrou *et al.*, 2013). Hence, values for virulence and transmission may hinge upon  
77 different environments selecting for particular combinations of traits, and not on hardwired genetic  
78 correlations among traits (Hochberg, 1998). Disentangling between the environmental and  
79 genetic factors affecting the relationship between virulence and transmission is possible using  
80 inbred lines derived from the same genetically diverse parasite population (Godinho *et al.*, 2020).  
81 Using this approach is key to identify the conditions under which these parasite traits can evolve  
82 independently.

83 The evolution of virulence is also expected to be contingent upon other factors such as host  
84 population structure (Thrall & Burdon, 2003) and the timing of transmission (Day, 2003). Theory  
85 predicts that virulence should be reduced in viscous populations (in which individuals tend to  
86 remain in the patch where they were born) because in these populations relatedness among  
87 parasites increases and the benefits of producing dispersers are reduced (Boots & Sasaki, 1999;  
88 Wild *et al.*, 2009; Lion & Boots, 2010). Experimental evidence for this prediction has been shown  
89 in phages (Kerr *et al.*, 2006) and viruses (Boots & Meador, 2007). Selection on virulence is also  
90 predicted to be contingent upon the timing of transmission, with early transmission selecting for  
91 higher virulence (Day, 2003), which has been shown empirically in microcosm populations

92 (Berngruber et al 2013). Both these factors, population viscosity and the timing of transmission,  
93 may also change within-host parasite dynamics. Indeed, in viscous populations and in  
94 populations where individuals disperse at the end of infection only, individuals are expected to  
95 remain on their patch for longer periods, which may lead to density dependence. Density  
96 dependent parasite growth has been shown in several systems (Keymer, 1972; Ebert *et al.*, 2000;  
97 de Roode *et al.*, 2007; Pollitt *et al.*, 2013), and it may affect the relationship between virulence  
98 and transmission (Bonneaud *et al.*, 2020), a possibility that remains unexplored. Both population  
99 viscosity and the timing of transmission are expected to critically hinge upon host availability  
100 during the infection period, as individuals will only effectively disperse when other hosts are  
101 attainable. Therefore, host availability during infection is expected to affect virulence, as well as  
102 its correlation with transmission.

103           Here we tease apart the impact of genetic and environmental factors on the relationship  
104 between virulence and transmission, using inbred lines of the spider mite *Tetranychus urticae*, a  
105 plant macro-parasite (Godinho *et al.*, 2020). Spider mites spend their entire life-cycle on their host  
106 plants (Helle & Sabelis, 1985), with high intrinsic growth rates, their dispersal (i.e., transmission  
107 between hosts) and damage they inflict resulting in major economic losses in agriculture  
108 worldwide (Helle & Sabelis, 1985). This damage, presumably negatively correlated with plant  
109 fitness (Fineblum & Rausher, 1995) is visible as chlorotic lesions on the leaf surface. Thus, it can  
110 be easily quantified (Mira et al. 2022; Fig. S1), being a reliable measure of virulence (Noël *et al.*,  
111 2022). Spider mites can transmit to nearby hosts from early on in an infection, or over-exploit the  
112 host plant before transmission occurs (Smitley & Kennedy, 1985; Bitume *et al.*, 2013). Such  
113 transmission depends on many factors, like within-host parasite density, relatedness and/or the  
114 availability of suitable hosts (Bitume *et al.*, 2013; De Roissart *et al.*, 2015). Because spider mites  
115 are macroparasites, as a proxy for transmission we used the number of transmitting stages (i.e.,  
116 adult females (Anderson *et al.*, 1986)), a measure we validate here. We manipulated the  
117 opportunity for transmission and the strength of competition of inbred lines of the spider mite *T.*  
118 *urticae* and quantified how this affected the production of transmitting stages and virulence. We  
119 assessed whether a relationship between virulence and transmission was determined by genetic  
120 differences among lines, and/or by the build-up of density-dependence within the host.  
121 Additionally, we evaluated whether this relationship was affected by opportunities for transmission  
122 during the infection period.

## 123 **Materials and Methods**

### 124 Spider mite inbred lines

125 *Tetranychus urticae* was collected on different host plants, in Portugal in 2013 (Zélé *et al.*,  
126 2018a), and has since been reared on bean plants (*Phaseolus vulgaris*, variety Prelude), at the  
127 University of Lisbon. In October 2015, 50 individuals from six different field populations (total:

128 300) were collected and mixed to form an outbred population maintained at high densities (>1000  
129 mites). In October 2016, inbred lines were created from this population by sib mating. This  
130 procedure was repeated for 14 generations, ensuring an inbreeding coefficient above 94%  
131 (Godinho *et al.*, 2020). Inbred lines allow simultaneous measurement of many individuals of the  
132 same (nearly) homozygous genotype, increasing the accuracy of genetic estimates (Godinho *et al.*  
133 *et al.*, 2020). Additionally, given that the original population was outbred, genetic variation across  
134 lines is expected to be high and because they were derived from the same population, all lines  
135 share the same evolutionary and environmental history (Godinho *et al.*, 2020).

136 Lines were maintained separately on bean leaf patches in Petri dishes. A subset of 15  
137 inbred lines were transferred to the University of Montpellier in January 2018 and maintained on  
138 bean leaves (variety Pongo) in small plastic boxes (255 mm length x 183 mm width x 77 mm  
139 height) at optimal conditions (25°C with a 16:8 L: D cycle, at 60% relative humidity). Under these  
140 conditions, egg to adult development takes ca. 11 to 13 days. These same conditions were kept  
141 throughout all experiments.

142 Prior to the experiments, cohorts of spider mites from each inbred line were created by  
143 isolating 40 to 50 mated females of each line during 48h. After 14 days, the resulting mated  
144 females (daughters) were used in the experiments. In this species, mated females are the main  
145 dispersers (i.e., transmitting stages) and, additionally, the damage inflicted by males is negligible,  
146 thus, does not significantly contribute to virulence (Helle & Sabelis, 1985). Not all inbred lines are  
147 represented in each experiment due to too few individuals available at the start of the experiment  
148 (12 to 14 lines analysed).

149

#### 150 Continuous transmission during the infection period

151 Adult females were randomly assigned to two density treatments (10 or 20 females, respectively),  
152 on a four cm<sup>2</sup> bean leaf patch, where they fed and laid eggs for four days. Then, adult females  
153 were removed, and a photograph of each patch was taken using a Canon EOS 70D camera. The  
154 amount of damage inflicted by spider mites was measured using ImageJ and Ilastik 1.3, using a  
155 method developed specifically for spider mites ((Mira *et al.*, 2022); Fig. S1). Host patches  
156 unexposed to spider mites were used as a control. The number of eggs laid was registered, as a  
157 measure of parasite replication. On day four, a second leaf patch, uninfected by spider mites, was  
158 placed beside the first and connected to it by a 3 x 1 cm Parafilm bridge, thus allowing dispersal  
159 of the emerging adult female offspring (Fig. S2). The number of adult daughters on the new host  
160 patches was checked on days 11, 12 and 13 in blocks 2 to 4 and on days 12 and 13 in block 1.  
161 When there were more than 15 offspring on the new patch, the latter was replaced by a new one,  
162 such that uninfected patches were always available. On day 14, we counted the number of adult  
163 daughters on the original host patch and on each of the new patches, i.e., the number of  
164 transmitting stages produced (potential transmission). Effective transmission was inferred by the

165 cumulative number of females that infected a new host patch. This set-up mimics the life cycle of  
166 a parasite with continuous transmission during the infection period. There were 5 to 16 replicates  
167 for each inbred line per density treatment, distributed across four blocks.

168

#### 169 Transmission at the end of the infection period

170 Females of each inbred line were randomly assigned to three density treatments, (5, 10 or 20  
171 founding females), on a four cm<sup>2</sup> bean leaf patch, where they laid eggs for four days. They were  
172 then removed, a photograph was taken of each leaf to measure the damage inflicted and the  
173 number of eggs laid was counted. Fourteen days later, the adult daughters on each patch were  
174 counted, i.e. the number of transmitting stages produced (potential transmission). In this set-up,  
175 transmission would only be possible after this measurement, i.e., at the end of the infection period  
176 (Fig. S2). There were 3-13 replicates for each inbred line per density, distributed across three  
177 blocks.

178

#### 179 Statistical analysis

180 We present correlations between different traits (Table 1): virulence (measured as the damage  
181 inflicted) and parasite replication (the number of eggs laid), virulence and potential transmission  
182 (the number of adult daughters, i.e., transmitting stages). Additionally, to test whether density-  
183 dependence rises from the negative effects of intraspecific competition on the survival of juvenile  
184 stages, we check the correlation between virulence and juvenile mortality (i.e., proportion of eggs  
185 that did not develop into adults). We also validate our measure of transmission by assessing the  
186 correlation between the number of transmitting stages (potential transmission) and the number of  
187 females colonising novel host patches (effective transmission). To test genetic correlations, we  
188 consider total values per host patch, as theoretical predictions are tailored to such population  
189 values (Anderson & May, 1982; Anderson *et al.*, 1986) and most experimental studies on this  
190 topic have used them (Mackinnon & Read, 1999; de Roode *et al.*, 2008; Doumayrou *et al.*, 2013).  
191 Note however that using per capita data would not modify conclusions on correlations, as density  
192 would be present in both numerator and denominator.

193 Genetic and environmental correlations were measured using a multi-response generalized  
194 linear mixed model fitted with an MCMCglmm (package MCMCglmm; (Hadfield, 2010)). Genetic  
195 correlations were determined by including the identity of the line as a random factor in each  
196 model and assessing the highest posterior density interval (HPDI) of the genetic (G) structure of  
197 the model, which represents the (co)variances between the two traits evaluated across inbred  
198 lines (Hadfield, 2010). Environmental correlations were obtained by assessing the HPDI of the  
199 residual (R) structure in the same model (Hadfield, 2010). Effects were considered significant  
200 when the HPDI did not include zero (Hadfield, 2010). The effect of initial density on correlations  
201 was assessed by comparing the deviance information criterion (DIC) of a model not including



202 density with that of a model including density as a covariate (i.e. making it a function-value trait;  
203 (Gomulkiewicz *et al.* 2018)). Models were considered different when the difference in DIC was  
204 higher than 2. Additionally, we report the genetic and environmental correlations for each density  
205 level separately (Table 1). We also tested if the relationship between virulence and transmission  
206 might be quadratic or saturating, as predicted by theoretical models (Anderson & May, 1982;  
207 Alizon *et al.*, 2009), by comparing the DIC of models with the number of transmitting stages  
208 produced as the response variable with virulence included as a linear term, with models that also  
209 included the quadratic and/or square-rooted terms for virulence, respectively.

210 To measure genetic variance for traits (variance among inbred lines) and the effect of  
211 density on this variance we used per capita values, by dividing the value for each host patch by  
212 the initial density of adult females (Fig. S3, Table S1). Note that here we did not use population  
213 values because heritability measures concern individual, not population traits. We then applied  
214 generalized linear mixed models fitted with a Markov Monte Carlo Chain approach (Hadfield,  
215 2010) with per capita trait values as the response variable and density included in the model as a  
216 fixed factor and inbred line as a random factor. Broad-sense heritability,  $H^2 = \frac{Var(G)}{Var(G)+Var(E)}$   
217 (Falconer & Mackay, 1996) and the corresponding confidence intervals were extracted from the  
218 abovementioned models for each trait. Because heritability is a proportion between 0 and 1, and  
219 thus the HPDI is bounded to fluctuate above 0, instead, we determined if there was significant  
220 genetic variation by a) assessing the 95% HPDI of the genetic component of each model (i.e.  
221 variance among inbred lines) and b) comparing, for each trait, the DIC of a model including inbred  
222 line as a random factor with that of a model without inbred line as a random factor (Henriques *et*  
223 *al.*, 2021).

224 All models initially included 300000 iterations, with a burn-in of 10000 iterations, thinning of 100  
225 and a flat prior: For GLMMs (to assess genetic variance),  $V = 1$  and  $\nu = 0.002$ , assigning a weak  
226 informative variance ( $V$ ) and a low degree of belief ( $\nu$ ) (Gelman, 2006); For Multi-response  
227 GLMMs (to assess trait correlations),  $V = \text{matrix}(c(1,0,0,1), \text{ncol} = 2, \text{nrow} = 2)$  and  $\nu = 0.002$ ,  
228 meaning no covariance between traits, in accordance with the null hypothesis of no correlation.  
229 Additionally, we ran the models using an uninformative prior based on an inverse-Gamma  
230 distribution ( $V = \text{diag}(2) * (0.002/1.002)$ ,  $\nu = 1.002$ ), which gave similar results (not shown)  
231 (Gelman, 2006). These priors were used to allow the hyper-parameter values to reflect a  
232 reasonable range of values for the traits in question, without any previous information about them  
233 or their co-variance. All models were checked for convergence with a stationary test using the  
234 `heidel.diag` function and for autocorrelations in the Markov chain within fixed and random terms  
235 using the `autocorr.diag` function (package `coda`, (Plummer *et al.*, 2006)). When models failed one  
236 of these tests, the number of iterations was increased to 500000 or 700000 and the burn-in to

237 20000 or 50000. All figures were produced with the ggplot2 package in R and the regressions  
238 fitted with the geom\_smooth function (Wickham & Winston, 2016).

## 239 **Results**

### 240 Validation of our measure of transmission

241 We found a positive correlation, both environmental (i.e., among residuals of the two variables,  $r_e$   
242 = 0.99) and genetic (i.e., among the factor 'inbred line' for the two variables,  $r_g = 0.99$ ), between  
243 the number of transmitting stages produced (i.e., adult daughters) and transmission to uninfected  
244 hosts (Table 1a; Fig. 1), indicating that the former is a good proxy for the latter.

### 245 Genetic variation for replication rate, virulence, and transmission

246 To observe genetic correlations among parasite traits, genetic variance for these traits must be  
247 present in the population. Thus, we quantified genetic variance for virulence (% of damage  
248 inflicted), parasite replication (number of eggs) and transmission (the number of transmitting  
249 stages) among the *T. urticae* inbred lines (Fig. S3), using per capita measurements, which allows  
250 the determination of broad sense heritability,  $H^2$  (Table S1, Fig. S4 and S5). We found significant  
251 genetic variation for all traits, as the 95% HPDI of the genetic component in the models did not  
252 include 0 (Table S1) and the DIC of models including the inbred line as a random factor were  
253 lower than those excluding it (Table S2). Additionally, all traits were affected by the initial density  
254 of founding females on the host patch (Table S3), but not in a similar way. Indeed, transmission  
255 of all lines decreased with density, whereas for parasite replication and virulence the pattern was  
256 line-specific (Fig. S3).

257

### 258 Correlation between virulence and parasite replication

259 We found positive genetic and environmental correlations between virulence (% of damage  
260 inflicted) and parasite replication (number of eggs laid) both when transmission occurred  
261 continuously during the infection period and when transmission was restricted to the end of the  
262 infection period (Table 1b, Fig. 2). These relationships were not affected by the initial parasite  
263 density (Table 1b).

264

### 265 Correlation between virulence and transmission

266 When transmission was continuous, there was no overall environmental or genetic correlation  
267 between virulence and transmission across densities. However, at each density (intermediate and  
268 high), we found positive environmental and genetic correlations between virulence and  
269 transmission (Table 1c; Fig. 3a). In this scenario, selection on virulence will directly affect  
270 transmission, therefore parasite fitness is expected to increase with virulence. Accordingly, we did

271 not find any evidence for a quadratic or saturating relationship between virulence and  
272 transmission (DIC 2026.7 and 2026.6, respectively, as compared with the linear model 2026.4).  
273 In contrast, when transmission was restricted to the end of the infection, we found a negative  
274 environmental correlation between virulence and transmission, but no genetic correlation  
275 between these traits (Table 1c, Fig. 3b). Furthermore, although there was no quadratic nor  
276 saturating relationship between these traits (DIC 2691.1 and 2691.1, respectively, as compared  
277 with the linear model 2690.9), the direction of the correlation between virulence and transmission  
278 changes from positive at low density, to absent at intermediate density and negative at high  
279 density (Table 1c). This density dependence pertains from the negative effects of intraspecific  
280 competition at high densities, which is confirmed by a positive environmental correlation between  
281 virulence and juvenile mortality at all densities, which was not the case in the scenario with  
282 continuous transmission (Table 1d, Fig. 4). In this scenario, with transmission at the end of the  
283 infection period, virulence is not genetically correlated with transmission, but high virulence will  
284 lead to strong density dependence within hosts, independently of the genotype considered.

285

## 286 **Discussion**

287 In this study we show that opportunities for transmission shape the relationship between virulence  
288 and transmission, a key component of parasite fitness. We found a positive genetic correlation  
289 between virulence and transmission when the latter was continuous during infection,  
290 independently of the initial parasite density. In contrast, when transmission was restricted to the  
291 end of the infection period the relationship between these traits no longer had a genetic basis, but  
292 instead, was modulated by density dependence: beyond a certain level of virulence, at high initial  
293 densities of mites, despite an increase in replication (i.e., number of eggs laid), within-host  
294 competition prevents most offspring from becoming adult, such that highly virulent genotypes  
295 produce fewer adult daughters. Evidence for density-dependence has been found in this (Rotem  
296 & Agrawal, 2003; De Roissart *et al.*, 2015), and other host-parasite systems, including  
297 microparasites (Ebert *et al.*, 2000; de Roode *et al.*, 2007; Pollitt *et al.*, 2013).

298         The positive genetic correlation between virulence and transmission, found when  
299 parasites could transmit during the infection period, suggests that there will be selection for high  
300 virulence. If this is the case, why then do we still find genetic variance for this trait? We propose  
301 three non-mutually exclusive hypotheses. First, *T. urticae* is a generalist parasite, hence it may  
302 frequently shift among hosts, and optimal virulence may vary with the host species (Gandon,  
303 2004; Magalhães *et al.*, 2014; Zélé *et al.*, 2018b). Furthermore, different host plant species, as  
304 well as different individuals of the same host plant, may harbour different heterospecific parasites  
305 (Wimp *et al.*, 2004; Dinnage *et al.*, 2012). Coinfections with other parasites are expected to select  
306 for different levels of virulence depending on the direction and mechanism of the interaction  
307 (Choisy & de Roode, 2010; Alizon *et al.*, 2013; Clay & Rudolf, 2019). Finally, transmission within

308 the infection period relies on the occurrence of hosts to which parasites can transmit. This may  
309 not always be possible, as uninfected hosts may be locally absent or become rapidly infected  
310 (Hochberg 1991; Crossan *et al.* 2007). Mites are known to base their decision to leave a host on  
311 the perception of cues (e.g., volatiles) from hosts in the environment, including their infection  
312 status (Pallini *et al.*, 1997; Kiedrowicz *et al.*, 2017). Therefore, in the absence of uninfected hosts,  
313 mites may remain on their host until they overexploit it, which will eventually result in them  
314 switching to a parasite life cycle with transmission occurring only at the end of the infection  
315 period.

316 In the latter scenario, when infection was restricted to the end of the infection, we found  
317 no genetic correlation between virulence and transmission. Instead, we found a positive  
318 environmental correlation at low density, which became negative at high density, leading to a  
319 peak of transmission at intermediate levels of virulence, as predicted by theory (Anderson & May  
320 1982). However, this relationship between virulence and transmission was not determined by  
321 differences among inbred lines, hence it was purely environmental. The fact that the negative  
322 correlation between virulence and transmission disappears when transmission occurs  
323 continuously during the infection further supports the hypothesis that density dependence  
324 modulates the peak of transmission at intermediate levels of virulence only when transmission  
325 occurs at the end of the infection period. This effect of the timing of transmission may also explain  
326 the mixed empirical evidence for the occurrence of the virulence transmission trade-off, as  
327 continuous transmission will only be relevant for some parasite life-cycles and/or experimental  
328 designs (Acevedo *et al.*, 2019).

329 Such modulation of epidemiological trade-offs by the environment has been found in host  
330 traits in another system (Hall *et al.*, 2012). Also, previous work has shown that limited  
331 opportunities for transmission can select for lower virulence, ensuring a reduction in kin  
332 competition and/or host depletion (Wild *et al.* 2009; Lion & Boots 2010). In line with this, parasite  
333 transmission in more structured populations has been shown to select for lower virulence (Kerr *et al.*  
334 *et al.*, 2006; Boots & Meador 2007; Berngruber *et al.*, 2013). We do not find this in our experiment. In  
335 fact, at all densities, virulence and parasite replication (i.e., number of eggs) were positively  
336 correlated. However, at high densities, we observed high juvenile mortality (Fig. 4). Therefore,  
337 genotypes that induce more harm to their host will not necessarily produce more transmitting  
338 stages, which explains the absence of a positive genetic correlation between virulence and  
339 transmission. The negative environmental correlation found between these traits at high densities  
340 may simply be a by-product of density dependence: fewer transmitting stages are produced in  
341 plant patches that are more damaged by parasites. Possibly, spider mites are not selected to  
342 thrive in highly competitive environments, hence they still produce as many eggs as possible (a  
343 trait selected in low-competition environments) but then these individuals die due to absence of  
344 resources. Alternatively, spider mites may have been selected in saturated environments, but in

345 this case we would expect them to maximize their competitive ability, rather than resource  
346 consumption, which would also not lead to more virulent genotypes producing more transmitting  
347 stages. In any case, under this scenario, given the breakdown of the genetic correlation between  
348 virulence and transmission, selection on virulence may not affect transmission. In other words,  
349 the evolution of virulence may not affect parasite fitness, given that parasite fitness includes both  
350 within-host (parasite replication) and between-host (transmission, here the number of transmitting  
351 stages) traits (Anderson & May, 1982; Alizon & Michalakis, 2015). Thus, variance for virulence in  
352 parasite populations, as observed in this (Fig. S3) and other systems (Dutta *et al.*, 2021; Little *et*  
353 *al.*, 2008; Mackinnon and Read, 1999a) may be maintained, instead, by the heterogeneity  
354 pertaining from host availability, variability and connectivity (Thrall & Burdon, 2003; Boots &  
355 Meador, 2007; Lion & Boots, 2010). Moreover, the (co)evolution of host traits, such as resistance,  
356 may create temporal heterogeneity for parasites, contributing to the maintenance of variance for  
357 virulence in parasite populations (Lambrechts *et al.*, 2006).

358 Our results support the prediction that high virulence is advantageous in the beginning of  
359 the infection period, when many uninfected hosts are available, but this advantage may be lost  
360 later on when fewer hosts are available (Day 2003). Indeed, in our study we find a positive  
361 correlation between virulence and transmission, both under continuous transmission and  
362 transmission at the end of the infection period, but in the latter case only when parasite density is  
363 low, which should typically occur at the beginning of epidemics. At high density, though, this  
364 correlation is negative, which suggests that reduced transmission opportunities could result in  
365 lower parasite virulence, if parasites respond plastically to the current environment, as has been  
366 shown in viruses and *Plasmodium* (Reece *et al.*, 2008; Leggett *et al.*, 2013). Besides affecting the  
367 relationship between virulence and transmission, host availability may also affect the length of the  
368 infection, the other key component of parasite fitness (Anderson & May, 1982), since early  
369 transmission may slow host exploitation. However, our design did not allow us to test this  
370 hypothesis.

371 In sum, we show that virulence is genetically correlated with transmission. However, this  
372 key assumption of most theoretical models of the evolution of virulence only holds under  
373 continuous transmission. Instead, selection for higher virulence in this system is expected to  
374 disappear under limited parasite transmission due to within-host density-dependence. This  
375 suggests that this fundamental difference in parasite life cycle, i.e., continuous transmission vs  
376 transmission at the end of the infection period, has key implications for virulence evolution.  
377 Additionally, it highlights that the effect of within-host density dependence may hinge upon the  
378 parasite life cycle. Indeed, whereas for parasites that transmit at the end of the infection period  
379 (e.g. obligate killers), within-host density dependence may underlie the predicted optimal  
380 transmission at intermediate virulence levels, for parasites that transmit continuously, it may only  
381 come into play later in the epidemic, when uninfected hosts become limited. In any case, our

382 results demonstrate that only by including the whole parasite life-cycle in experimental set-ups is  
383 it fully possible to understand the ecology and evolution of virulence (Alizon & Michalakis, 2015).

#### 384 **Acknowledgments**

385 We deeply thank Élio Sucena, Flore Zélé, Margarida Matos, Oliver Kaltz, Sylvain Gandon and  
386 Yannis Michalakis, for their very useful suggestions and comments.

#### 387 **Funding**

388 ERC (European Research Council) consolidator grant COMPCON, GA 725419 attributed to SM,  
389 PHC-PESSOA grant (38014YC) to ABD and SM, and FCT (Fundação para Ciência e Tecnologia)  
390 PhD scholarship (PD/BD/114010/2015) to DPG and with the Junior researcher contract  
391 (CEECIND/02616/2018) attributed to IF. This is ISEM contribution no ISEM 2022-317.

#### 392 **Competing interests**

393 Authors declare no competing interests.

#### 394 **Author contributions**

395 Conceptualisation and supervision by SM and ABD; Investigation by DPG, LRR, SL, LD, AFM  
396 and ABD; Formal analysis by DPG and IF; Visualisation by DG, LRR and IF; Writing the original  
397 draft by DPG, SM and ABD with reviewing and editing by LRR and IF.

#### 398 **Data and materials availability**

399 Data is deposited in Dryad: <https://doi.org/10.5061/dryad.4xgxd25dv>.

400

401

#### 402 **References**

403

404 Alizon, S., Hurford, A., Mideo, N. & Baalen, M. Van. (2009). Virulence evolution and the trade-off  
405 hypothesis: History, current state of affairs and the future. *J. Evol. Biol.* 22:245–259.

406 Alizon, S. & Michalakis, Y. (2015). Adaptive virulence evolution: The good old fitness-based  
407 approach. *Trends Ecol. Evol.* 30:248–254.

408 Alizon, S., Roode, J.C. de & Michalakis, Y. (2013). Multiple infections and the evolution of  
409 virulence. *Ecol. Lett.* 16:556–567.

410 Anderson, R.M. & May, R.M. (1982). Coevolution of hosts and parasites. *Parasitol.* 85:411–426.

411 Anderson, R.M., May, R.M., Joysey, K., Mollison, D., Conway, G.R., Cartwell, R., *et al.* (1986).  
412 The invasion, persistence and spread of infectious diseases within animal and plant communities.  
413 *Phil. Trans. R. So. Lond. Ser. B-Biol. Sci.* 314:533–570.

414 Bitume, E. V., Bonte, D., Ronce, O., Bach, F., Flaven, E., Olivieri, I., *et al.* (2013). Density and  
415 genetic relatedness increase dispersal distance in a subsocial organism. *Ecol. Lett.* 16:430–437.

416 Bonneaud, C., Tardy, L., Hill, G.E., McGraw, K.J., Wilson, A.J. & Giraudeau, M. (2020).  
417 Experimental evidence for stabilizing selection on virulence in a bacterial pathogen. *Evol. Lett.* 4:

- 418 491–501.
- 419 Boots, M. & Meador, M. (2007). Local interactions select for lower pathogen infectivity. *Science*  
420 315:1284–1286.
- 421 Boots, M. & Sasaki, A. (1999). ‘Small worlds’ and the evolution of virulence: Infection occurs  
422 locally and at a distance. *Proc. R. Soc. B-Biol. Sci.* 266:1933–1938.
- 423 Choisy, M. & Roode, J.C. de. (2010). Mixed infections and the evolution of virulence: Effects of  
424 resource competition, parasite plasticity, and impaired host immunity. *Am. Nat.* 175:E105–E118.
- 425 Clay, P.A. & Rudolf, V.H.W. (2019). How parasite interaction strategies alter virulence evolution in  
426 multi-parasite communities. *Evolution* 73:2189–2203.
- 427 Day, T. (2003). Virulence evolution and the timing of disease life-history events. *Trends Ecol.*  
428 *Evol.* 18:113–118.
- 429 Dinnage, R., Cadotte, M.W., Haddad, N.M., Crutsinger, G.M. & Tilman, D. (2012). Diversity of  
430 plant evolutionary lineages promotes arthropod diversity. *Ecol. Lett.* 15:1308–1317.
- 431 Doumayrou, J., Avellan, A., Froissart, R. & Michalakakis, Y. (2013). An experimental test of the  
432 transmission-virulence trade-off hypothesis in a plant virus. *Evolution* 67:477–486.
- 433 Ebert, D. (1994). Virulence and local adaptation of a horizontally transmitted parasite. *Science*  
434 265:1084–1086.
- 435 Ebert, D., Zschokke-Rohringer, C.D. & Carius, H.J. (2000). Dose effects and density-dependent  
436 regulation of two microparasites of *Daphnia magna*. *Oecologia* 122:200–209.
- 437 Falconer, D.S. & Mackay, T.F.C. (1996). *Introduction to Quantitative Genetics*. Longman, Harlow.
- 438 Fineblum, W. & Rausher, M. (1995) Tradeoff between resistance and tolerance to herbivore  
439 damage in a morning glory. *Nature* 377:517–520.
- 440 Frank, S.A. (1996). Models of parasite virulence. *Q. Rev. Biol.* 71:37–78.
- 441 Gandon, S. (2004). Evolution of multihost parasites. *Evolution* 58:455–469.
- 442 Gelman, A. (2006). Prior distributions for variance parameters in hierarchical models. *Bayesian*  
443 *Analysis* 1:515–534.
- 444 Godinho, D.P., Cruz, M.A., Charlery de la Masselière, M., Teodoro-Paulo, J., Eira, C., Fragata, I.,  
445 *et al.* (2020). Creating outbred and inbred populations in haplodiploids to measure adaptive  
446 responses in the laboratory. *Ecol. Evol.* 10:7291–7305.
- 447 Gomulkiewicz, R., Kingsolver, J.G., Carter, P.A. & Heckman, N. (2018). Variation and evolution of  
448 function-valued traits. *Annu. Rev. Ecol. Evol. Syst.* 49:139–164.
- 449 Hadfield, J.D. (2010). MCMC methods for multi-response generalized linear mixed models: The  
450 MCMCglmm R package. *J. Stat. Soft.* 33:1–21.
- 451 Hall, S.R., Becker, C.R., Duffy, M.A. & Cáceres, C.E. (2012). A power-efficiency trade-off in  
452 resource use alters epidemiological relationships. *Ecology* 93:645–656.
- 453 Helle, W. & Sabelis, M.W. (1985). *Spider mites: Their biology, natural enemies and control.*  
454 *Control*. Elsevier, Amsterdam.
- 455 Henriques, J. F., Lacava, M., Guzmán, C., Gavín-Centol, M. P., Ruiz-Lupián, D., De Mas, E., *et*

- 456 *al.* (2021). The sources of variation for individual prey-to-predator size ratios. *Heredity* 126:684-  
457 694.
- 458 Hochberg, M.E. (1998). Establishing genetic correlations involving parasite virulence. *Evolution*  
459 52:1865–1868.
- 460 Kerr, B., Neuhauser, C., Bohannan, B.J.M. & Dean, A.M. (2006). Local migration promotes  
461 competitive restraint in a host-pathogen ‘tragedy of the commons’. *Nature* 442:75–78.
- 462 Keymer, A. (1972). Density-dependent mechanisms in the regulation of intestinal helminth  
463 populations. *Parasitology* 84:573–587.
- 464 Lambrechts, L., Fellous, S., & Koella, J. C. (2006). Coevolutionary interactions between host and  
465 parasite genotypes. *Trends Parasitol.* 22:12-16.
- 466 Leggett, H.C., Benmayor, R., Hodgson, D.J. & Buckling, A. (2013). Experimental evolution of  
467 adaptive phenotypic plasticity in a parasite. *Curr. Biol.* 23:139–142.
- 468 Lion, S. & Boots, M. (2010). Are parasites “prudent” in space? *Ecol. Lett.* 13:1245–1255.
- 469 Mackinnon, M. & Read, A. (1999). Genetic relationships between parasite virulence and  
470 transmission in the rodent malaria *Plasmodium chabaudi*. *Evolution* 53:689-703.
- 471 Magalhães, S., Cailleau, A., Blanchet, E. & Olivieri, I. (2014). Do mites evolving in alternating host  
472 plants adapt to host switch? *J. Evol. Biol.* 27:1956–1964.
- 473 Mira, A.F., Marques, L.M., Magalhães, S. & Rodrigues, L.R. (2022). A method to measure the  
474 damage caused by cell-sucking herbivores. In *Environmental Responses in Plants: Methods and*  
475 *protocols, Methods in Molecular Biology* (ed. by Duque, P. & Szakonyi, D.). Humana, New York,  
476 pp. 299–312.
- 477 Noël, E., Lefèvre, S., Varoqui, M. & Duncan, A.B. (2022). The scale of competition impacts  
478 parasite virulence evolution. *Evol. Ecol.* <https://doi.org/10.1007/s10682-022-10199-0>
- 479 Plummer, M., Best, N., Cowles, K. & Vines, K. (2006). CODA: convergence diagnosis and output  
480 analysis for MCMC. *R news* 1:7–11.
- 481 Pollitt, L.C., Churcher, T.S., Dawes, E.J., Khan, S.M., Sajid, M., Basáñez, M.G., *et al.* (2013).  
482 Costs of crowding for the transmission of malaria parasites. *Evol. Appl.* 6:617–629.
- 483 Reece, S.E., Drew, D.R. & Gardner, A. (2008). Sex ratio adjustment and kin discrimination in  
484 malaria parasites. *Nature* 453:609–615.
- 485 Roissart, A. De, Wang, S. & Bonte, D. (2015). Spatial and spatiotemporal variation in  
486 metapopulation structure affects population dynamics in a passively dispersing arthropod. *J.*  
487 *Animal Ecol.* 84:1565–1574.
- 488 Roode, J.C. de, Gold, L.R. & Altizer, S. (2007). Virulence determinants in a natural butterfly-  
489 parasite system. *Parasitology* 134:657–668.
- 490 Roode, J.C. de, Yates, A.J. & Altizer, S. (2008). Virulence-transmission trade-offs and population  
491 divergence in virulence in a naturally occurring butterfly parasite. *Proc. Natl. Acad. Sci. U.S.A.*  
492 105:7489–7494.
- 493 Rotem, K.A. & Agrawal, A.A. (2003). Density dependent population growth of the two-spotted  
494 spider mite, *Tetranychus urticae*, on the host plant *Leonurus cardiaca*. *Oikos* 103:559–565.



- 495 Smitley, D.R. & Kennedy, G.G. (1985). Photo-oriented aerial-dispersal behavior of *Tetranychus*  
496 *urticae* (Acari: Tetranychidae) enhances escape from the leaf surface. *Ann. Entomol. Soc. Am.*  
497 78:609–614.
- 498 Thrall, P.H. & Burdon, J.J. (2003). Evolution of virulence in a plant host-pathogen metapopulation.  
499 *Science* 299:1735–1737.
- 500 Wickham, H. & Winston, C. (2016). *Ggplot2: Elegant graphics for data analysis*. 2nd edn.  
501 Springer International Publishing, Cham.
- 502 Wild, G., Gardner, A. & West, S.A. (2009). Adaptation and the evolution of parasite virulence in a  
503 connected world. *Nature* 459:983–986.
- 504 Wimp, G.M., Young, W.P., Woolbright, S.A., Martinsen, G.D., Keim, P. & Whitham, T.G. (2004).  
505 Conserving plant genetic diversity for dependent animal communities. *Ecol. Lett.* 7:776–780.
- 506 Zélé, F., Santos, I., Olivieri, I., Weill, M., Duron, O. & Magalhães, S. (2018a). Endosymbiont  
507 diversity and prevalence in herbivorous spider mite populations in South-Western Europe. *FEMS*  
508 *Microbiol. Ecol.* 94:fiy015.
- 509 Zélé, F., Santos, J.L., Godinho, D.P. & Magalhães, S. (2018b). Wolbachia both aids and hampers  
510 the performance of spider mites on different host plants. *FEMS Microbiol. Ecol.* 94:fiy187.
- 511

512 **Table 1. Genetic and environmental correlations between traits measured.** Genetic ( $r_g$ ) and environmental ( $r_e$ ) correlations - extracted from the genetic and  
513 residual error structure of the models, respectively, – were measured between **a)** the production of transmitting stages (adult daughters) and transmission, and  
514 between virulence and **b)** parasite replication, **c)** the production of transmitting stages (adult daughters) and **d)** juvenile mortality (i.e. proportion of eggs that did not  
515 develop into adults). All traits were measured per host (as the sum of the contribution of all females initially placed on the leaf). The deviance information criterion  
516 (DIC) for models with and without density included are shown. Models with the lowest DIC or the simplest model (when there was no difference in DIC values), are  
517 identified using an \*. Highest posterior density intervals (HPDI) and correlations coefficients ( $r_g$  and  $r_e$ ) are shown for the models including all data, and separately  
518 for each of the different densities. Intervals that do not include zero are shown in bold. Experiment: 1) continuous: continuous transmission; 2) end: transmission at  
519 the end of the infection period.

520

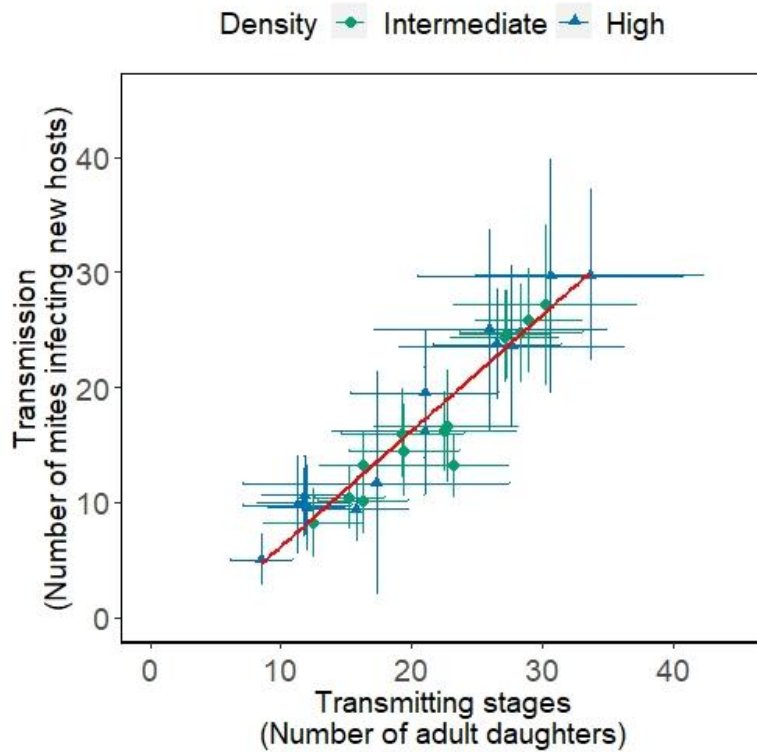
Section	Traits	Experiment	DIC		Across Densities		Density 5		Density 10		Density 20	
			No density	Density	genetic correlation	environmental correlation	genetic correlation	environmental correlation	genetic correlation	environmental correlation	genetic correlation	environmental correlation
a	Transmitting stages vs transmission	continuous	3632*	3632	HPDI: <b>0.94, 0.99</b> ; rg= 0.99	HPDI: <b>0.99, 0.99</b> ; re= 0.99	na	na	HPDI: <b>0.48, 0.99</b> ; rg= 0.99	HPDI: <b>0.99, 0.99</b> ; re= 0.99	HPDI: <b>0.81, 0.99</b> ; rg = 0.99	HPDI: <b>0.79, 0.99</b> ; re= 0.99

b	Virulence vs parasite replication	continuous	4956*	5007	HPDI: <b>0.91, 0.94</b> ; rg= 0.93	HPDI: <b>0.49, 0.65</b> ; re= 0.57	na	na	HPDI: -0.99, 0.80; rg= 0.04	HPDI: <b>0.30, 0.54</b> ; re= 0.40	HPDI: <b>0.96, 0.99</b> ; rg= 0.97	HPDI: <b>0.24, 0.58</b> ; re= 0.42
		end	6109*	6182	HPDI: <b>0.79, 0.99</b> ; rg= 0.99	HPDI: <b>0.51, 0.65</b> ; re= 0.59	HPDI: -0.96, 0.99; rg= 0.09	HPDI: <b>0.07, 0.40</b> ; re= 0.25	HPDI: -0.97, 0.99; rg= 0.09	HPDI: <b>0.28, 0.57</b> ; re= 0.43	HPDI: <b>0.92, 0.99</b> ; rg= 0.99	HPDI: <b>0.30, 0.61</b> ; re= 0.51

c	Virulence vs transmitting stages	continuous	4244	4240*	HPDI: <b>0.83, 0.99</b> ; rg= 0.99	HPDI: -0.24, 0.03; re=-0.09	na	na	HPDI: <b>0.51, 0.99</b> ; rg= 0.99	HPDI: <b>0.23, 0.64</b> ; re= 0.39	HPDI: <b>0.44, 0.99</b> ; rg= 0.99	HPDI: <b>0.15, 0.24</b> ; re= 0.16
		end	5564*	5585	HPDI: -0.84, 0.99; rg= 0.09	HPDI: <b>-0.29, -0.08</b> ; re= -0.15	HPDI: -0.99, 0.99; rg= 0.09	HPDI: <b>0.13, 0.45</b> ; re= 0.28	HPDI: -0.99, 0.97; rg=-0.01	HPDI: -0.10, 0.25; Re= 0.02	HPDI: <b>-0.99, -0.13</b> ; rg= -0.99	HPDI: <b>-0.45 -0.06</b> ; re= -0.27

d	Virulence vs juvenile mortality	continuous	1882	1869*	HPDI: -0.99, 0.05; rg= -0.81	HPDI: <b>0.03, 0.29</b> ; re= 0.17	na	na	HPDI: -0.97, 0.28; rg= -0.72	HPDI: -0.07, 0.35; re= 0.22	HPDI: -0.94, 0.58; rg= -0.60	HPDI: -0.06, 0.31; re= 0.14
		end	2698	2649*	HPDI: -0.43, 0.99; rg= 0.09	HPDI: <b>0.25, 0.47</b> ; re= 0.38	HPDI: -0.97, 0.99; rg= 0.08	HPDI: <b>0.01, 0.34</b> ; re= 0.2	HPDI: -0.85, 0.98; rg= 0.08	HPDI: <b>0.15, 0.47</b> ; re= 0.28	HPDI: <b>0.71, 0.99</b> ; rg= 0.98	HPDI: <b>0.27, 0.60</b> ; re= 0.44

521

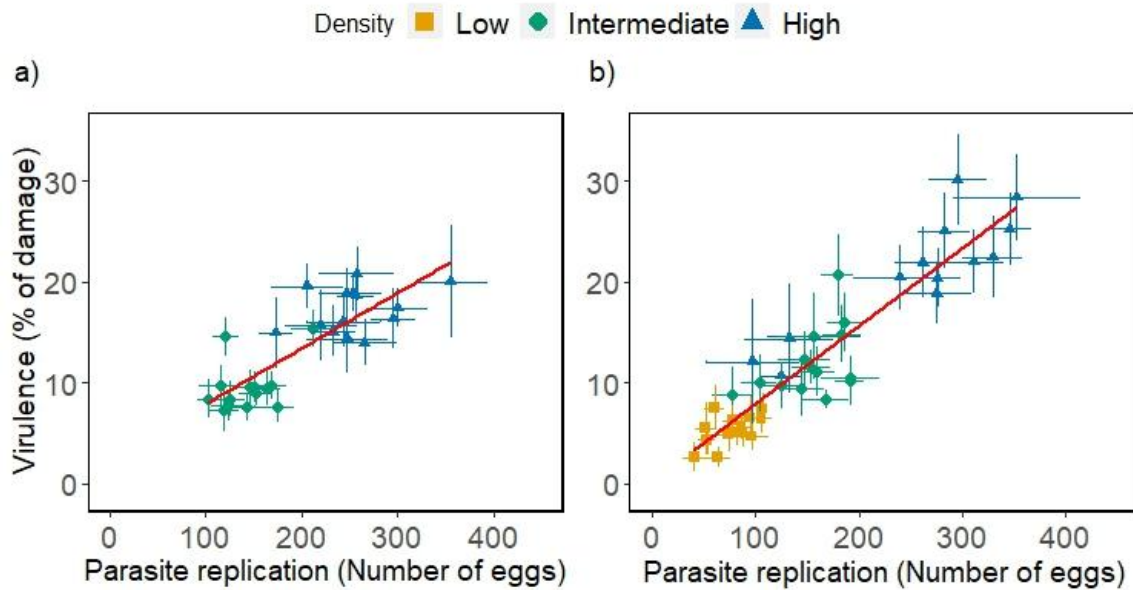


522

523 **Figure 1. Correlation between transmitting stages and transmission.** The number of  
 524 daughters produced (transmitting stages) and transmission (the number of mites infecting new  
 525 hosts) for inbred lines of *T. urticae* infesting hosts at different starting densities (10 females =  
 526 green circles; 20 females = blue triangles) with the possibility of continuous transmission; dots are  
 527 the mean for each inbred line  $\pm$  standard error; the regression (fitted with geom\_smooth function)  
 528 is represented in red.

529

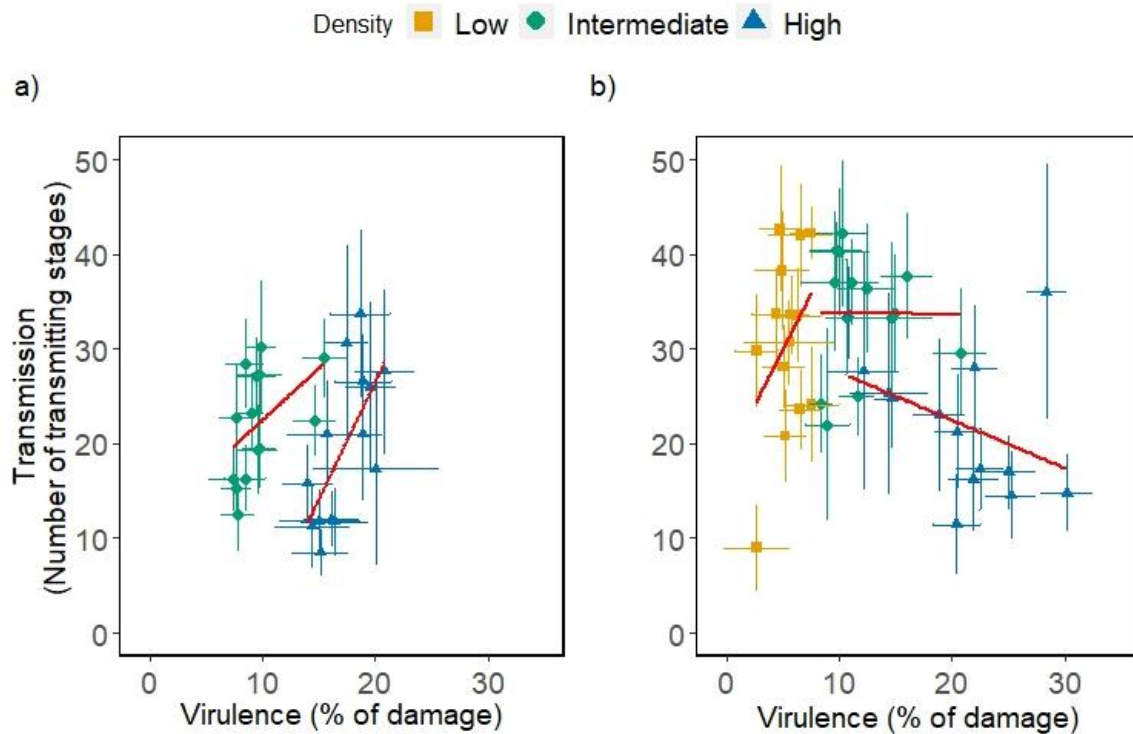
530



531

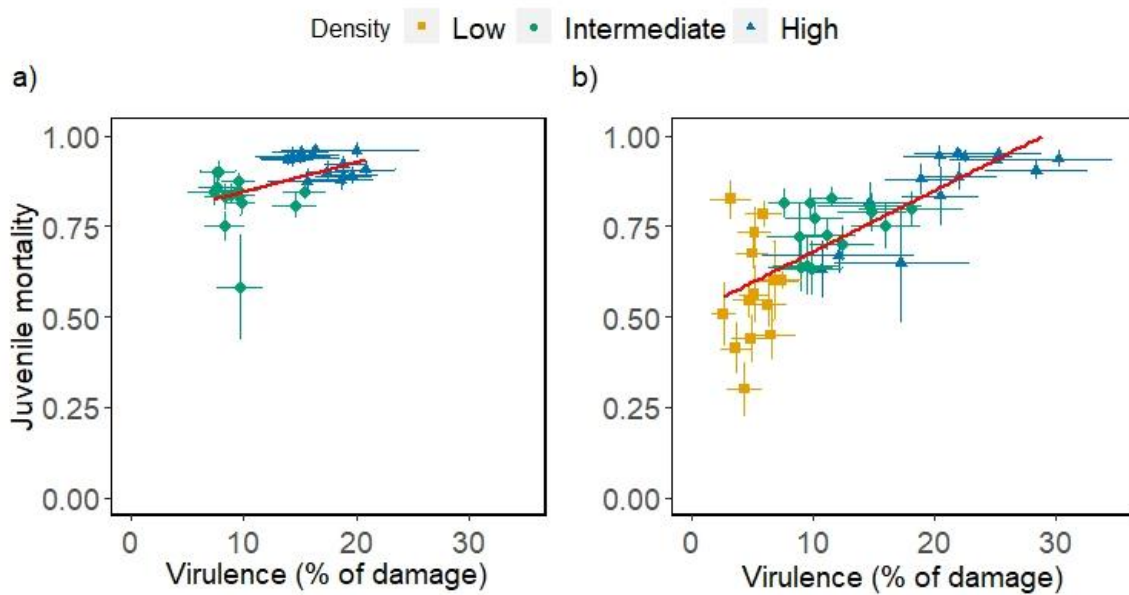
532 **Figure 2. Correlation between parasite replication and virulence in inbred lines of *T.***  
533 ***urticae* infecting a host patch.** The total damage inflicted on the host patch (i.e., virulence) and  
534 the total number of eggs laid (parasite replication) were measured in a set-up with a) continuous  
535 transmission during the infection period and b) transmission at the end of the infection period.  
536 Colours represent different densities (5 females = yellow squares; 10 females = green circles; 20  
537 females = blue triangles); dots are the mean for each inbred line  $\pm$  standard error; Because there  
538 is no effect of density on these correlations, regressions (in red) are represented across densities  
539 (fitted with the `geom_smooth` function).

540



541

542 **Figure 3. Correlation between virulence and transmission in inbred lines of *T. urticae***  
 543 **infecting a host patch.** The total damage inflicted on the host patch (i.e., virulence) and the total  
 544 number of transmitting stages (transmission) were measured in a set-up with a) continuous  
 545 transmission during the infection period and b) transmission at the end of the infection period.  
 546 Colours represent different densities (5 females = yellow squares; 10 females = green circles; 20  
 547 females = blue triangles); dots are the mean for each inbred line  $\pm$  standard error; Because there  
 548 are differences among densities, regressions (in red) are represented for each density (fitted with  
 549 the geom\_smooth function).



551 **Figure 4. Correlation between virulence and juvenile mortality in inbred lines of *T. urticae* infecting a host patch.**

552 The damage inflicted on the host patch (i.e., virulence) and juvenile mortality (i.e. proportion of eggs that did not develop  
 553 into adults) were measured in a set-up with a) continuous transmission during the infection period and b) transmission at  
 554 the end of the infection period. Densities are coded in yellow squares (5 females), green circles (10 females) and blue  
 555 triangles (20 females); dots are the mean for each inbred line  $\pm$  standard error; regressions (fitted with geom\_smooth  
 556 function) are shown in red.