

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

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10

Abstract

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

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

Introduction:

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

One important environmental factor that may affect the outcome of competition in coinfections scenarios is the relative densities of each competitor in the within-host environment (5, 7, 17-19). Higher-Increasing densities of a competitor may increasingly reduce a focal parasite's growth (7, 18). Alternatively, the impact of a competitor may depend on focal parasite densities (5, 17, 19). This may occur to the extent that For example, interspecific competition no longer may not only affects traits at higher-lower intraspecific densities (17). Interactions among parasites may also impact transmission-related traits, independently of growth and virulence, such as triggering dispersal from hosts infected with competitors or impacting whether a new host becomes infected. Indeed, certain parasites avoid or choose a host, or a host tissue, depending on its infection status (20, 21). This means that multiple parasites in the environment have the potential to impact parasite life-history ecology and evolution at different scales, not restricted to the within-host environment.

A key aspect that may affect how parasite interactions in coinfections modify parasite traits is whether they are genetically correlated. Indeed, if that is the case, then any genetic change in one trait driven by the presence of competitors will affect the genetic value of other traits, which has major consequences for the evolutionary trajectories of populations. In contrast, if the correlation is purely

environmental, then no direct evolutionary consequences are expected, but the ecological impact of the parasite, such as parasite severity or epidemic onset, may be modified.

This study ~~uses-used~~ inbred lines of the spider mite *Tetranychus urticae* to investigate how ~~the presence of~~ an interspecific competitor, the closely related species *T. evansi*, impacts virulence, the number of adult daughters produced and transmission to a new host patch as well as the potential correlations, genetic or environmental, among these traits. In *T. urticae*, there is genetic variation for dispersal distance (22, 23), which here we refer to, ~~as~~ and is the same as transmission, and for host use (24), which may be correlated with virulence. Moreover, dispersal is a plastic trait, with individuals having higher dispersal at elevated intraspecific densities (22) and in the presence of kin (25). ~~Previous work has investigated how dispersal is linked to other life-history traits in *T. urticae* (23, 26, 27).~~ Moreover, ~~S~~selection for higher dispersal has been shown to be associated with higher diapause incidence and lower fecundity (23) and dispersing individuals had smaller eggs (26) and fewer offspring surviving to adulthood when laying eggs at higher densities (27). Further, in a recent companion study to this one conducted with the same inbred lines, we found a positive genetic correlation between virulence and the number of adult daughters produced when transmission was possible during the infectious period (28). Hence, there are both genetic and environmental relationships between transmission and other life-history traits. Finally,

~~On tomato plants,~~ The outcome of competition between *T. evansi* ~~generally outcompetes and~~ *T. urticae*. ~~However, outcomes~~ can change ~~not only~~ due to variation across populations (29) but also depending on the sequence of arrival, as *T. evansi* excludes *T. urticae* except when the latter arrives first and occupies *T. evansi*'s preferred niche (30). In sum, competitive interactions between these mite species are strong and strongly impacted by both genetic and environmental factors. Therefore, the system composed of these two spider mite species is ideal to address how the presence of a competitor affects virulence, parasite growth (number of adult daughters) and transmission, as well as the potential (genetic) interactions among these traits.

Materials and Methods

Biological system

Spider mites are macroparasites of plants, including many economically important crops, with their complete life cycle occurring on their host plant (31). Both *T. urticae* and *T. evansi* females lay eggs on leaves, which take ~4 days to hatch. The juvenile stage comprises 1 nymph stage and 2 deuteronymph stages, with adults emerging after approximately 14 days in our laboratory (25°C, 16:8 L: D cycle). All

stages feed by injecting their stylet into parenchyma cells and sucking out the cytoplasm, which leaves chlorotic damage on the leaf surface, our measure of virulence (32). *T. urticae* is a generalist species, feeding on more than 1000 different plant species (33), whereas *T. evansi* is a specialist species, mostly feeding on *Solanaceae* plants (34). In natural systems, co-occurrence of different spider mite species in the same geographical area is common, leading to co-infection of the same host plant (35).

Spider-mite populations

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

Prior to each experiment, cohorts of 40 mated female spider mites from each inbred line were isolated on a bean patch (2-3 leaves placed together). These females were allowed to lay eggs for 48h. Fourteen days later, the mated daughters of these females, of approximately the same age, were used in the experiments. The same procedure was used to create cohorts of *T. evansi*. All spider-mite populations, inbred lines and cohorts used in these experiments were maintained on bean leaves (*Phaseolus vulgaris*; variety Pongo) placed on water saturated cotton wool, in small plastic boxes (255 mm length x 183 mm width x 77 mm height), at 25°C with a 16:8 L: D cycle, at 60% relative humidity. Not all inbred lines are represented in each experiment due to too few individuals available at the start of the experiment (N = between 12-14 to 164 lines).

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

Females of each *T. urticae* inbred line were randomly assigned to one 'intraspecific density' treatment (5, 10 and 20 females), with or without 'interspecific competition' (10 *T. evansi* females) (Figure 1a). In all treatments, females were placed on a 2 x 2 cm bean leaf patch placed on wet cotton wool in plastic boxes. There were 3 to 13 replicates for each inbred line per treatment combination (intraspecific density x interspecific competition) distributed across 3 blocks. Variation in the number of replicates per line arose due to differences in the number of adult females produced among inbred lines in the synchronised cohorts. All females were allowed to feed and lay eggs on their leaf patches for 4 days. After this period, females were killed, the number of eggs was counted and a photograph of each patch was taken using a Canon EOS 70D camera. The damage inflicted by these adult female

spider mites on each host patch, used as a measure of virulence, was determined using ImageJ and Ilastik 1.3, as described in (28). Succinctly, the background from each photo was removed in ImageJ, ~~subsequently using then we used~~ Ilastik ~~we top~~ distinguished damaged area from healthy leaf and ~~then finally in ImageJ~~ the damaged area was calculated via the colour contrast between damaged and undamaged leaf tissue ~~in ImageJ~~. Because some leaf veins were incorrectly assigned as damage by Ilastik, uninfested bean leaf patches were left in the experimental boxes for the same period of time and photographed; these control patches were used to establish an average baseline level of falsely assigned damage, which was subtracted from each measurement to estimate the actual damage (hereafter: 'damage'). After a period of 14 days, the female offspring surviving on each patch were counted. Only females were counted because the males of both species are not easily distinguishable, females are the main dispersers in these species and the number of females produced correlates with transmission (28). The data on damage inflicted and production of adult females, for the "intraspecific density" treatments in the absence of *T. evansi* are published elsewhere (28).

Experiment 2. Impact of interspecific competition on transmission

We measured differences in dispersal traits (transmission) for the different *T. urticae* inbred lines assigned to the same 'intraspecific density' and 'interspecific competition' treatments as in Experiment 1. Adult *T. urticae* females were placed in groups of 5, 10 or 20 on a 2cm² bean leaf patch on wet cotton wool alone or with 10 *T. evansi* females. This first host patch was connected, in a row, to 2 other bean ~~leaf host~~ patches via 3 x 1 cm Parafilm bridges from day 1 of the experiment (Figure 1b). This experimental setup was replicated across several boxes. Females were allowed to feed and disperse across ~~the~~ patches, and the number of mites on each patch ~~was~~ counted on days 1, 2, 3, 6, and 9 after the beginning of the experiment. There were ~~3-1~~ to ~~13-9~~ replicates for each inbred line per treatment combination (intraspecific density x interspecific competition) distributed across 2 blocks. Variation in the number of replicates is again due to the number of offspring emerging as adult females among lines.

Statistical analysis

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

Impact of interspecific competitors on within-host traits

In Experiment 1 General Linear Mixed Models (GLMM) were used to investigate how intraspecific density, interspecific competition and their interaction affected virulence and the number of female offspring becoming adult. These analyses included intraspecific density as a covariate and interspecific competition as a fixed factor. Next, we used a GLMM to test whether the relationship between virulence and the production of adult daughters (transmitting stages) changed with interspecific competition and if this effect varied across intraspecific densities. In this model, the number of adult daughters remained the response variable, with the linear, quadratic and saturating terms for virulence, intraspecific density, interspecific competition and their interactions, up to and including 3-way interactions, included as explanatory variables. Full models were simplified by removing non-significant terms in a stepwise fashion. Inbred line and block were included in these models as random factors.

Impact of interspecific competitors on transmission

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

Genetic variance for within-host traits and transmission

Broad-sense heritability, $H^2 = \frac{Var(G)}{Var(G)+Var(E)}$ (37), for each trait in each experiment was determined by extracting the proportion of total variance in models explained by inbred line (among inbred line

variance) by re-running models for within-host and transmission related life-history traits including all terms as random (competition, line, density, block and patch nested within block for dispersal score and/or density) to obtain all variance components. Note that these models did not include interaction ~~s-between~~ terms. Traits were divided by the total number of adult females placed on a patch (e.g. traits per capita) since ~~traits-they~~ are passed from parents to offspring at the level of an individual, not in groups of individuals. The significance of each model was assessed by comparing the Akaike's Information Criterion (AIC) of a models including inbred line with a model excluding it. A significant H^2 indicates that trait variance is significantly explained by differences in the additive or dominance genetic variance across individuals and/or by differences in maternal effects.

Correlations between within-host traits and transmission

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

First, we reported the Pearson's correlation coefficient across mean trait values for each of the inbred lines. Next, we extracted the standard errors for each correlation coefficient and associated p-values from a PROC MIXED COVTEST model as described in (38) using SAS Studio. As measures were taken in different experimental set-ups we bootstrapped (with replacement) the mean value for each inbred line at each density and interspecific competition treatment 20 times and randomly paired the different values.

~~In order to~~To randomly couple, multiple times ~~the~~, measures in the different experiments, ~~we bootstrapped (with replacement) the mean value for each inbred line at each density and interspecific competition treatment 20 times. The actual mean values for each inbred line for pairs of traits were coupled and the 20 bootstrapped means randomly coupled. Mean~~Trait values were standardised across all lines for each density and competition treatment, ~~t~~, such that ~~e~~ each variable had a mean of zero and standard deviation of one. This ~~means~~was so that values ~~of~~for pairs of traits were of a similar scale as required for the PROC MIXED COVTEST in SAS Studio. ~~Only inbred lines with 3 or more replicates were included for a given density and interspecific competition treatment combination.~~

~~Genetic correlations were done using a PROC MIXED COVTEST model as described in (38) using SAS Studio. Briefly, each paired value was given an identity and the columns for each pair of traits~~

stacked giving 2 columns, one showing the 2 traits measured (labelled as trait) and the other the values (the response variable). Trait was included in the model as an explanatory variable. There were 2 random terms in the model. The first random term included an interaction between 'trait' and inbred line, thus assessing the variance among inbred lines variance for each trait. The error structure for this term was specified as 'unr', which tells the model to assess the genetic correlation across the 2 traits. A The second random term was included in the model with the trait pair 'ID' nested within inbred line. The significance of the genetic correlation from each model was given using a log-likelihood ratio test by comparing the log likelihood of the aforementioned model with a model where the error structure was defined as 'un(1)', thus constraining the covariance matrix to zero.

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

Results

Impact of interspecific competitors on within-host traits

Interspecific competitors had no effect on the virulence of *T. urticae* at any intraspecific density (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 and S1; Table S2). When virulence was included as a covariate, the presence of interspecific competitors did not change the relationship between virulence and the production of adult daughters, i.e. transmitting stages (interspecific competition*virulence; $F_{1, 635} = 2.17$, $p = 0.1416$, interspecific competition*virulence²; $F_{1, 637} = 0.03$, $p = 0.8532$, Figure 2; Table S1).

A significant interaction between intraspecific density and virulence ($F_{1, 643} = 16.07$, $p < 0.0001$) showed that the shape of the relationship between virulence and the production of adult daughters, i.e. transmitting stages, changed at ~~the~~ different densities (positive at low densities, no relationship at intermediate densities, and negative at high densities). This was corroborated with a significant quadratic terms for virulence in a second model investigating factors affecting the number of adult daughters (virulence; $F_{1, 648} = 12.45$, $p = 0.0004$, and virulence²; $F_{1, 643} = 9.36$, $p = 0.0023$; Figure 2; Table S2). However, as these results were not influenced by interspecific competition they are not discussed further here, as they are presented elsewhere (28).

Impact of interspecific competitors on transmission

The dispersal score was affected by both interspecific competition ($F_{1, 44759} = 20.8268$, $p < 0.0001$) and intraspecific density ($F_{1, 4229-1177} = 15.8616.69$, $p < 0.0001$), with a significant interaction between these two factors ($F_{1, 44554} = 8.767.68$, $p = 0.00320058$; Figure 3, Table S3). Models investigating the effect of interspecific competition separately at each density showed that there was only a significant effect of competition in the low density treatment (Table S4). This meant that ~~This interaction showed that, at low and intermediate intraspecific densities,~~ *T. urticae* females were more likely to leave ~~the patch earlier in the presence of T. evansi~~ the first host patch in the presence of interaspecific competitors only at low densities (Table S3S4). The interaction between *T. urticae* density and the quadratic term for time was also significant ($F_{1, 1872-1844} = 2223.0257$, $p < 0.0001$), with values of the dispersal score saturating through time for patches in the intermediate and high density treatments (Figure 3, Table S3). Note, the dispersal score captured time to arrive on₁ and maximum numbers on patches 2 and 3 (Figure ~~S3-S2~~ and Table ~~S4S5~~). We observed an effect of intraspecific density on all these underlying traits and of interspecific competition on the time to arrive to patch 2 and 3, the latter dependeding on the density of intraspecific competitors, as there was a significant interaction between the two factors (Table S45).

Genetic variance for within-host traits and transmission

~~In-For~~ the within-host ~~environment~~ traits, we found ~~low but~~ significant broad-sense heritability for the number of adult daughters ($H^2-H^2 = 0.057$) and virulence ($H^2 = 0.060$). For measures of transmission, inbred line explained a significant portion of the variance (H^2) for time to reach host patches 2 ($H^2 = 0.10062$) and 3 ($H^2 = 0.03036$) and the dispersal score ($H^2 = 0.0394$), but not the maximum number of individuals on host patch 2 or 3 (Table S1).

Correlations between within host traits and transmission

We ~~aimed to explore~~ d the genetic relationships between traits related with transmission between hosts (i.e., day arriving on and maximum number on each patch) and traits measured in the within host-environment (i.e., virulence and number of adult daughters) ~~in 6 different treatment combinations of intraspecific density and interspecific competition.. Note, we only looked at correlations between~~ for traits that showed significant among line variation (virulence, number of

adult daughters, day arriving on patches 2 and 3; Table S1), giving a total of 24 correlations. Of these, 6 models did not converge. Of the remaining 18 models ~~investigating genetic correlations between traits~~, only 1 was ~~marginally~~ significant (Table 1). ~~The significant correlation shows that there was, showing~~ a negative correlation between virulence and the time to arrive on host patch 2 at high *T. urticae* densities in the presence of interspecific competition (Table 1; Figure S3). ~~This shows~~ Collectively these results show that virulence and the number of adult daughters measured in the within-host environment are mostly independent of traits measuring transmission between hosts.

Discussion

In this study, we found that interspecific competition did not modify virulence, the production of adult daughters (i.e., transmitting stages) or the relationship between these traits. However, the presence of interspecific competitors ~~led to earlier~~ increased transmission of *T. urticae* to new host patches at low ~~and intermediate~~ intraspecific densities, which may be a mechanism to escape interspecific competition (at higher intraspecific densities this effect may be masked by more intense intraspecific competition). ~~For the most part, differences in transmission between hosts were mostly~~ genetically unrelated to measures of virulence or the number of adult daughters produced in the within-host environment. ~~The absence of genetic correlations between among the traits measured, despite them being heritable, means that~~ Therefore, selection ~~will~~ is expected to act on ~~them each~~ trait independently. ~~Hence, that is,~~ selection for virulence or the number of adult daughters is mostly unlinked to that on traits that foster early transmission. This means that selection for faster spread across host patches is not necessarily associated with higher virulence.

Impact of interspecific competitors on within-host traits

We found no significant impact of *T. evansi* on virulence or the production of adult daughters in *T. urticae*. This may stem from the fact that *T. evansi* is a poor competitor on bean plants. These results may have been very different had this experiment been done on a host plant to which *T. evansi* is better adapted. ~~Indeed~~ For instance, *T. evansi* is generally found to be the superior competitor on tomato plants, often excluding *T. urticae* ((19, 39) but see (29, 30)). As interspecific competition did not modify these traits, it also did not lead to changes in their interaction. Our results contrast with other studies that show that the impact of interspecific competition on parasite growth and virulence can change in response to the relative densities of each parasite in coinfection (5, 7, 18). ~~Indeed~~ Instead, as previously found in the absence of competitors (28), we here find a positive

relationship between virulence and transmission at low densities, no relationship at intermediate densities and a negative relationship at high densities. ~~[As shown in this previous publication (28), this result is because of intense within-host intraspecific competition among juvenile *T. urticae* developing on the host patch. At higher densities, despite many more eggs being laid, fewer offspring become adults (28). Our results contrast with other studies that show that the impact of interspecific competition on parasite growth and virulence can change in response to the relative densities of each parasite in coinfection (5, 7, 18).~~

Impact of interspecific competitors on transmission

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

The finding that interspecific competition causes *T. urticae* to move to a new host faster means that coinfection may be an important driver of epidemic spread. Coinfection can cause individuals to become superspreaders, when an infected host is responsible for a disproportionate number of transmission events (40). Here, we only measured the number of spider mites moving from one host patch to another, which is not the same as the number of new hosts infected. Nevertheless, it gives an idea of the number of transmission stages leaving an infected host, which is a measure of infection potential, similar to parasite shedding (10, 41-43). These different effects of parasite intraspecific densities and coinfection could be used to predict parasite spread in natural populations and to manage or control epidemics, for instance by identifying (and isolating or treating) the most infectious individuals (44). ~~Additionally, moving to uninfected hosts faster may foster coexistence with *T. evansi* in the host population, given that the order of arrival strongly affects the outcome of competition between these two species (30).~~

Whereas some studies have shown that the intensity of interspecific parasite competition modulates the effect of intraspecific competition within the host (5, 7, 17, 19, 30), the effect on transmission is

less clear. From the dispersal literature, it is clear that intra- and interspecific competition can interact to shape the movement of organisms at different scales (45). However, parasite studies are rare (e.g. (10) for an example with different parasite strains) and do not measure other traits (e.g., virulence), which are key to evaluate the impact of interspecific competition on disease epidemics. Thus, ~~in the between-host environment~~ it is ~~less as yet un~~clear how the relative impact of inter and intraspecific competition among parasites ~~plays out~~affects transmission. ~~The fact that the effect of~~This is especially true because we here found that interspecific competition ~~modifying~~affects transmission ~~changes with differently depending on~~ intraspecific density ~~may be very relevant for these macro-parasites~~. New infections are often seeded by low densities of mites (a few adult females), but during the course of infection densities become very high until the host is completely overexploited (46). Whether coinfections with *T. evansi* will foster the spread of *T. urticae* through host populations may ~~well thus~~ depend on how often coinfections involve ~~high versus low intraspecific densities~~early or late stages of infection. One possible scenario is that *T. evansi* promoting *T. urticae* transmission will result in the latter arriving first to a host plant, which will give a headstart to *T. urticae* and, as a consequence facilitate coexistence due to priority effects ~~-(30)(34)~~. In turn, this headstart may result in *T. urticae* reaching higher densities before the arrival of *T. evansi*, which may reduce the impact of *T. evansi* on transmission. ~~This would then create a negative feedback on the~~diminish the effect of interspecific competitors on transmission, thus becoming a self-limiting mechanismcreating a negative feedback loop, such that this effect would only be detected transiently. —

Correlations between within-host traits and transmission

~~Our results indicate~~We showed that within-host traits are mostly genetically independent of measures of transmission between hosts. ~~There~~Indeed, there was no genetic relationship among traits in ~~23/24~~17/18 possible tests across treatment combinations, despite these traits being genetically determined ~~(i.e., there was a significant amount of variation explained by the genetic backgrounds of the inbred lines)~~. These results contrast with previous ~~a study~~ly published findings in which we found a genetic correlation between adult daughters (i.e transmitting stages) and transmission (28). However, ~~previously in that case,~~ transmission was measured from hosts where virulence was inflicted, and, thus, highly dependent on within-host processes that lead to the production of transmitting stages. Here, by obtaining independent measures for virulence and transmission, we did not find that these traits are genetically linked and ~~by removing the connection mediated by within-host processes,~~ we also do not observe an effect of intra and interspecific competition on this relationship. Only ~~one~~1 genetic correlation ~~between traits measured in the within-host environment and transmission~~ werewas found to be significant, that between virulence and the time to arrive on host patch 2.

Moreover, the sign of this correlation hinged on ~~and again these were dependent on the intensity of~~
~~intraspecific competition and the presence of interspecific competition~~: it was negative in the
~~presence of *T. evansi*, but with no correlation positive in its~~ between these traits in the ~~. This was a~~
~~negative correlation between virulence and time to arrive on host patch~~ correlation was at high
~~intraspecific densities, in the presence~~ absence of competition. ~~of *T. evansi*, was a negative correlation~~
~~between virulence and time to arrive on host patch 2. As this was dependent on the presence of~~
~~interspecific competition, t~~ This means that ~~these~~ more virulent lines are responding to the presence
of *T. evansi* as a trigger to leave the first host patch sooner.

How such direct measures of transmission ~~in the between host environment~~, independent of within-
host processes (e. g. virulence), actually scale up and affect the spread of *T. urticae* across a population
of potential hosts is not straightforward and may well depend on the presence and relative densities
of interspecific competitors ~~on such potential hosts~~ (20). Moreover, different life-history strategies
could co-exist in a parasite population, some maximising fitness ~~in the within host environment~~ and
others maximising the spread across ~~the hosts population~~. If genetic variation for within-host traits
and transmission are uncoupled, then contrasting selection pressures in each environment may
maintain variation for both across scales.

Conclusion

Our results show that interspecific competition may increase the rate of parasite spread across hosts
and that this trait is genetically independent of traits measured in the within-host environment, i.e.,
virulence and ~~the~~ production of adult daughters (transmitting stages). ~~This may mean that Therefore,~~
parasites selected for higher virulence locally are not those necessarily favoured in travelling wave
epidemics, or those that spread far to seed infections in new host populations. ~~Here, we distinguish~~
~~traits pertaining from the within host environment that may affect transmission indirectly, from~~
~~between host traits that are more direct measures of transmission.~~ In the future it would be
interesting to explore how ~~these the~~ traits ~~measured in this study are actually~~ related to ~~traits those~~
~~favouring affecting~~ the infection of a greater quantity of hosts or ~~parasite spread over~~ longer ~~distant~~
~~distances~~ spread.

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Figure legends

Figure 1: Experimental set up in a) Experiment 1, in which adult female *T. urticae* (black and white spider mites) were placed in groups of 5, 10 or 20 on a 2cm² bean leaf patch with or without 10 *T. evansi* (red spider mites), b) Experiment 2, in which adult female *T. urticae* were placed in groups of 5, 10 or 20 on a 3 x 2cm² bean leaf patch ~~es-system~~ with or without 10 *T. evansi*. ~~The picture-figure here~~ only depicts the low density treatment.

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

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

557 **Table 1:** Summary of genetic correlations between transmission-related traits ~~measured in the between and within-host environment~~ at each of the different
558 intraspecific densities, in the presence ~~and/or~~ absence of interspecific competition. The Pearson's correlation across mean trait values for the different inbred
559 lines is presented for each pair of traits \pm the standard error calculated from the PROC MIXED COVTEST on the bootstrapped data. The χ^2 and log likelihood
560 test comparing models with and without the genetic correlation are also shown. All values of $p < 0.05$ were corrected using Bonferroni corrections (counting
561 6 tests per pair of traits). Significant correlations are shown in bold.

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		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	Virulence	$r_g = -0.10 \pm 0.28$ 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 $\chi^2 = 0.3$, $p = 0.5839$	$r_g = -0.19 \pm 0.275$ SE $\chi^2 = 0.04$, $p = 0.5271$	$r_g = 0.49 \pm 0.24$ SE $\chi^2 = 2.7$, $p = 0.1003$	$r_g = -0.67 \pm 0.14$ SE $\chi^2 = 8.1$, $p = 0.0264$
	No. adult daughters	$r_g = -0.22 \pm 0.27$ 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	Virulence	$r_g = -0.01 \pm 0.28$ 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
	No. adult daughters	$r_g = -0.07 \pm 0.03$ 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

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