The scale of competition impacts parasite virulence evolution

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Title: The scale of competition impacts parasite virulence evolution

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Abstract

The structure of parasite populations can have important consequences for virulence evolution. Both the scale of competition, whether it is more intense in the within versus between host environment, and the mode of transmission, whether parasites transmit alone or in groups between hosts, influence population structure and can select for changes in virulence.

In a selection experiment, we evolved metapopulations of the spider mite parasite *Tetranychus urticae,* under local (fixed sampling per host patch) or global (sampling determined by host patch productivity) competition, with either co- (with a female parasite from the same host patch) or random (with parasites from different host patches) transmission. After 33 generations of evolution, the fecundity and virulence of each replicate population was measured.

Females from the 'local-random' regime were more virulent with higher fecundity than females from the 'global-random' regime. There was no difference in virulence or fecundity between females from the 'global-random' or 'global co-transmission' selection regimes.

These results show that the scale of competition, and not the type of transmission, impacted virulence evolution for *T. urticae*. Local competition may maintain higher levels of genetic diversity at the level of the metapopulation. As such, intense local, within-host, competition selected for more productive individuals better able to exploit their environment and be the one to transmit to a new host.

Introduction

Parasite existence in spatially sub-divided populations means that they are exposed to competition at different scales (Mideo et al., 2008, Vale, 2013). These contrasting scales might be within versus between host competition (Mideo et al., 2008) which can have very different consequences for life-history trait evolution (Vale, 2013). For instance, in the within-host environment selection favours traits that maximise growth, whereas among-hosts, traits favouring transmission to new hosts should be optimised (Coombs et al., 2007, Mideo et al., 2008). This is illustrated by experiments in which between-host transmission is relaxed (with artificial transmission) leading to high growth and virulence and sometimes the loss of structures required for transmission (Ebert, 1998). At the same time, the way parasites transmit between hosts can influence the composition of the parasite community through time, notably with whom competition will occur (Alizon, 2013). The relative importance of competition at different scales and the identity of competitors (e.g. kin or non-kin) can have lasting consequences for parasite evolution (Chao et al., 2000).

Parallels can be drawn between parasite life-cycles and metapopulation dynamics in free-living organisms regarding how the scale of competition should impact trait evolution (Grenfell & Harwood, 1997, Vale, 2013). In both, evolution across such spatially structured environments can depend upon whether competition is local (i.e. at the level of the host/patch) or global (i.e. at the level of the metapopulation of hosts/patches) (Olivieri et al., 1995, Buckling & Brockhurst, 2008). If contrasting within versus among host competition this can be approximated to whether each host contributes equal transmission stages to the next generation (local competition), or whether the number is proportional to parasite productivity (global competition) (Vale, 2013, Grenfell & Harwood, 1997). Note, that this type of population regulation is equivalent to models of soft or hard selection (Vale, 2013). Local and global competition are often considered as extremes, whereas reality may be somewhere in between (Debarre & Gandon, 2011, Ravigne et al., 2009), with parasites simultaneously subject to both. It is therefore critical to understand how selection at different scales adds up to impact parasite life-history and virulence.

Theoretical models have investigated how local versus global competition between co-infecting parasite genotypes should impact genetic diversity and consequences for virulence (Vale, 2013, Chao et al., 2000, Frank, 1996). In the first instance, global competition between coinfecting parasites may select for reduced genetic diversity via a selective sweep (Frank, 1996, Chao et al., 2000). In contrast, local competition (soft selection) should hinder a selective sweep, maintaining genetic diversity in the parasite population, meaning within-host competition may persist (Chao et al., 2000). What this means for virulence depends on how parasites interact, but if they compete for shared host resources global competition may select for more prudent parasites and local competition more virulent parasites that overexploit their host (Vale, 2013, Chao et al., 2000, Frank, 1996, Buckling & Brockhurst, 2008).

In turn, random transmission whereby parasites transmit independently from a host in contrast to co-transmission, when parasites transmit in groups, will also influence population structure and life-history trait evolution. The co-transmission of parasites, either vertically from mother to offspring or in groups by a vector or collection of transmission stages, can maintain interactions between the same parasite lineages for multiple generations (Vautrin & Vavre, 2009, Alizon, 2013). Co-transmission is synonymous with budding dispersal in free-living organisms (Gardner et al., 2009, Faria et al., 2020) which can increase relatedness over time, but also align the interests of the group (independently of relatedness), selecting for cooperation and reduced virulence (Alizon, 2013, Frank, 1996) , (but see (Kamiya et al., 2018)). Indeed, the scale of competition and interactions with kin are often coupled (Wild et al., 2009, Lion & Boots, 2010) and levels of relatedness between parasites can change outcomes for virulence evolution (Chao et al., 2000, Frank, 1996, Buckling & Brockhurst, 2008).

Despite this, empirical investigations of how transmission type and competition impact virulence evolution have mostly remained separated. Selection experiments have shown that virulence can evolve in response to direct within-host competition (Ford et al., 2016, Garbutt et al., 2011) or cooperation (Raymond et al., 2012). Importantly, these studies do not contrast how virulence evolves when competition is more intense in the within versus between host environment. At the same time, other studies have shown that lower levels of parasite virulence can evolve following persistent vertical transmission over multiple generations (Magalon et al., 2010, Messenger et al., 1999). A few studies have explored the role of spatial structure for virulence evolution (Boots & Mealor, 2007, Kerr et al., 2006) including for parasites with vertical and horizontal transmission (Berngruber et al., 2013). Yet no study has manipulated the scale of competition and transmission type to investigate how the interplay between both impacts virulence evolution.

In this experiment we used a fully crossed design to investigate the consequences of global and local competition, with random and co-transmission, for parasite virulence evolution in meta-populations of the spider mite *Tetranychus urticae*. *T. urticae* compete for shared host resources, feeding on plant material causing substantial damage (Kant et al., 2004) (Figure S1). Their population structure means they are exposed to both local and global competition at different life-stages; offspring remain close to their natal patch and mate locally, after which females disperse (Mitchell, 1973). *T. urticae* also have different dispersal types which might be more (or less) representative of either random or co-transmission; aerial dispersal, when they leave their host plant in groups carried in the wind by threads of silk, can be considered a type co-transmission (Li & Margolies, 1994). Alternatively, they can also walk to find new host plants (Azandeme-Hounmalon et al., 2014), which may be more in line with random transmission.

We investigate the interplay between the scale of competition and transmission type for virulence evolution. We predict that local resource competition may select for higher levels of virulence if it maintains genetic diversity for longer than global competition (Chao et al., 2000). Co-transmission is predicted to select for cooperation (Kummerli et al., 2009, Alizon, 2013), which means that this may select for more prudent mites to avoid the tragedy of the commons (Frank, 1996). Indeed, there is intense density dependent competition in *T. urticae* with many juveniles dying before they become adult (Godinho et al., 2021).

Methods

Biological model

T. urticae Koch (Acari: Tetranychidae), is a generalist herbivore with a host range of over 1100 plant species (Migeon & Dorkeld, 2019, Helle & Sabelis, 1985). *T. urticae* are haplodiploid. Females lay eggs on the leaf surface which hatch approximately 4 days later. Juveniles go through a larval stage and two nymph stages before becoming adult. Upon emergence as adults, mating occurs and females disperse to new patches of host (new leaves on the same or different plant) and the life-cycle starts again. One generation, from egg to adult takes ~14 days in our laboratory at 25˚C. *T. urticae* feed by injecting their stylet into plant cells and sucking out the cytoplasm contents. They can empty up to 22 cells min^{-1} (Jeppson, in (Alba et al., 2015)), leaving white chlorotic lesions on the leaf surface (Kant et al., 2004). Both juvenile and adult stages feed in the same way; all *T. urticae* life-stages are spent on the host plant.

The population used in this experiment was made by mixing 10 different field populations maintained in Lisbon since 2013 (see (Zele et al., 2018)). They were transferred to Montpellier in November 2015, mixed together and maintained on bean plants (variety Pongo) in a large plastic box. Prior to the experiment, to equalise maternal effects, 10 groups of 40 females were collected and each placed on a large patch of bean leaves (10 - 15 leaves on water saturated cotton wool in a plastic box 255mm x 183mm x 77mm) with a paint brush, to lay eggs. The offspring of these females were used to seed the selection experiment. Prior to, and during, the experiment *T. urticae* were always maintained at 25˚C, with a 16: 8 light: dark cycle.

The selection experiment

The scale of competition (global or local) and transmission (co- or random) were manipulated in a fully factorial design with 4 selection regimes 1. 'local random', 2. 'local co-transmission', 3. 'global random' and 4. 'global co-transmission' (each replicated 3 times). Each replicate population comprised 96 females distributed in pairs across 48, 4 cm^2 bean leaf patches, each considered an individual host, placed on water saturated cotton wool in a plastic box. Females were left to lay eggs, and 14 days later 96 adult female offspring re-distributed across another

48 bean leaf patches. The only difference between selection regimes each generation was the

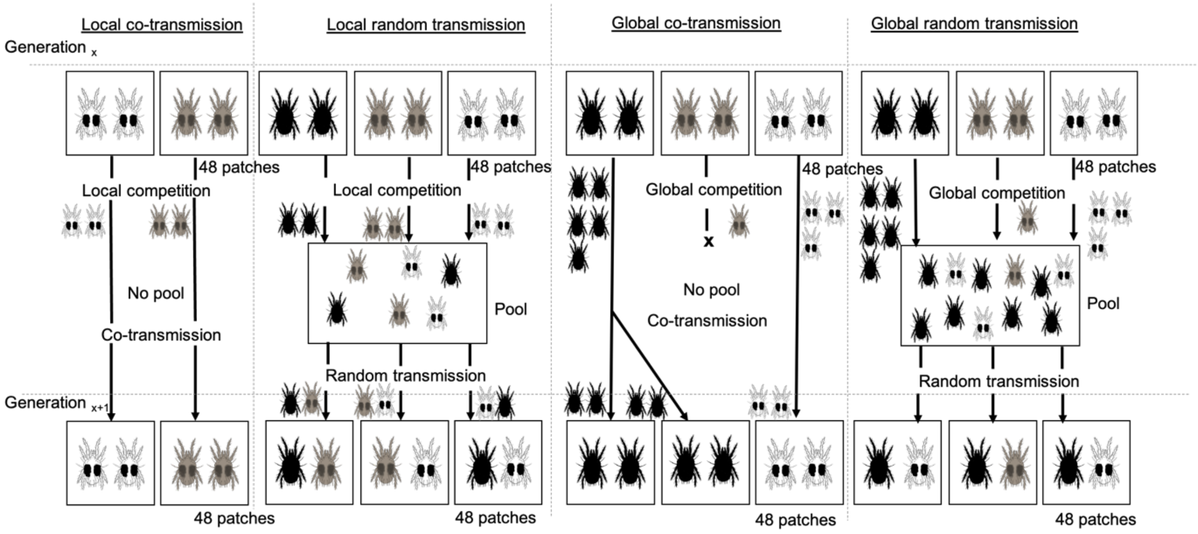

Figure 1: outlines the scale of competition and transmission in the different selection regimes between generations. Within each selection regime mites of the same colour shared the same host in Generation$_{x}$. In the 'local co-transmission' regime two daughters were transferred from the same host (white square) together to a new host in Generation$_{x+1}$. In the 'local random transmission' treatment two daughters from each of the 48 hosts were placed on a large leaf where they were mixed, before being placed in randomly chosen pairs on a new host in Generation$_{x+1}$. In the 'global co-transmission' regime the number of daughters on each host was counted before being transferred in pairs to new hosts in Generation$_{x+1}$, the number of which being a function of relative host fecundity. In the 'global random transmission' regime all daughters from each host were placed together on a large leaf and mixed, and then randomly paired females were then placed on a new leaf patch. Note, all replicates of the 'local co-transmission' treatment were lost by generation 14 of the experiment.

mixing or not of daughters among host patches and the contribution of each to the next generation (Figure 1 and (Rodrigues et al., 2021)). All replicates of the 'local co-transmission' selection regime were lost at generation 14.

Briefly, the scale of competition was manipulated by controlling the number of adult females from each host contributing to the next generation; under local competition each host contributed equal numbers (2-4 females in 'local random', adjusted to accommodate mortality), whereas under global competition it was a function of host patch productivity. We only recorded patch productivity each generation in the ‘global co-transmission’ treatment. The numbers of offspring across patches were variable within each replicate (replicate 1: mean 10.32, variance 15.46; replicate 2: mean 10.37, variance 19.25; replicate 3: mean 10.72, variance 34.03). On average 18.2 ± 0.67 SE (38%) host patches contributed no offspring to the next generation, 17.7 ± 0.80 SE (37 %) contributed 2 offspring (1 patch) to the next generation, 9.0 ± 0.35 SE (18.7%) contributed 4 offspring (2 patches) to the next generation and 3.9 ± 0.23 SE (8%) host patches contributed 6 or more offspring (3 or more patches) to the next generation (see Figure S2). This indicates that the selection regime in the ‘global’ competition treatments lead to reductions in genetic diversity faster than in the ‘local’ competition regimes.

Transmission between generations was imposed by females either being placed in pairs with another female from the same maternal host (co-transmission), or randomly paired with a female from another host (random transmission). In the random transmission treatments, females were paired from distant locations on the mixing patch to increase the probability of being transferred with a mite from another patch (Figure 1). The selection experiment continued for 33 generations. At this point, adult female offspring from the 48 hosts within each selection regime were mixed, and 96 adult females haphazardly chosen and placed on a large leaf patch (~10 leaves) and allowed to lay eggs. This was repeated for a second generation to equalise maternal effects across selection regimes (Generation 33 + 2).

Measuring virulence & fecundity

Adult females from each selection regime were placed on 4 cm^2 bean leaf patches alone, or in groups of 5, 10 or 20, with 3 replicates per selection regime at each density. These different densities were chosen to have a range within which differences in virulence might be observed. In particular, the single female treatment to see if intrinsic levels of virulence differed following selection under the different regimes, 2 females per patch to replicate experimental conditions and the higher densities in case expression of virulence was contingent on more intense competition for resources. Note, in the selection regimes competition was not for resources per se on the 4cm^2 patch, but among females to have their daughters represented in the population the following generation (between females sharing a patch when competition was local, and among females on different patches when competition was global).

Females laid eggs for 4 days, at which point they were removed, eggs on each patch counted using a binocular microscope and virulence measured. The virulence measured was the feeding damaged caused by the adult females placed on the leaves for 4 days. This was done by photographing each leaf with a Canon EOS 70D camera with a 15 – 55mm lens (Figure S1). Images were then treated in FijiImageJ. First the background surrounding each leaf patch was removed and the area of each leaf calculated based on a cm rule placed beside each patch in each photograph. A grid was then placed over each leaf image, each cell measuring 0.05 mm^2. The number of cells containing damage was then counted and used as a measure of virulence.

In this experiment we use fecundity as a measure of *T. urticae* fitness. In a separate experiment, we show a positive correlation between the number of eggs and the number of adult female offspring, the dispersing stage in this species, at densities of 10 females on a leaf patch. At higher densities (20 females per patch), when intense competition between offspring developing on the leaf patch occurs, no relationship between fecundity and number of adult daughters was observed. It would therefore seem at lower densities (10 females and under) fecundity is a good proxy for fitness.

Statistical analysis

General linear mixed models were used to investigate how selection regime impacted total log fecundity and total log virulence per host at each of the different densities. In separate models, ‘local-random’ with ‘global-random’, and ‘global-random’ with ‘global co-transmission’ were compared. As all replicates of the ‘local co-transmission’ regime were lost by generation 14 of the experiment, these were the models that made sense in terms of treatment comparisons. In each model, selection regime and density were considered fixed factors, and population nested within selection regime and box as random factors. Models were simplified by removing non-significant terms in a stepwise manner. We also did a Spearman’s rank correlation between per capita virulence and per capita fecundity. The initial analyses were done on total virulence as this is the harm the host experiences. In contrast, the correlation between traits was done on per capita values, because this is what selection will act upon and to control for density. These analyses were implemented in JMP 14.

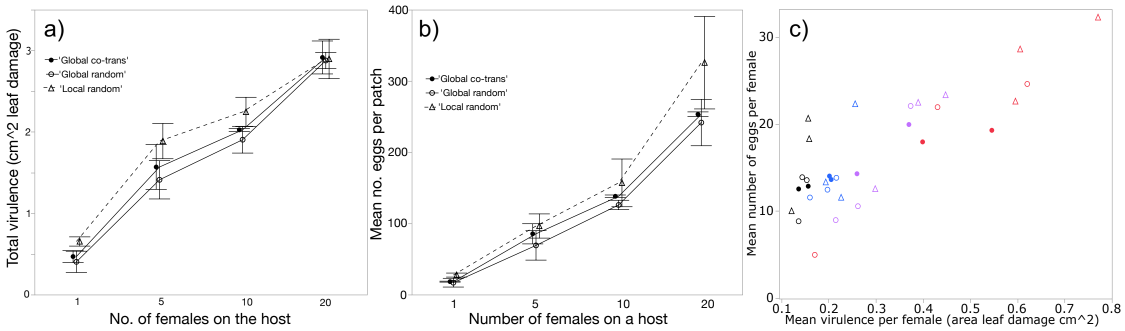

Figure 2: shows mean virulence measured as total area of leaf damage (a) and total fecundity (b) on each host patch when 1, 5, 10 or 20 females were sharing a host in the 'local random transmission' (open triangles, dashed line), 'global random transmission' (open circles, solid line) and 'global co-transmission' (closed circles, solid lines) selection regimes (± standard error). Relationship between mean virulence per female and mean fecundity per female across all female densities, per replicate, in the in the 'local random transmission' (open triangles), 'global random transmission' (open circles) and 'global co-transmission' (closed circles) regimes. The different density treatments are denoted by different colours; 1 female per host = red, 5 females per host = purple, 10 females per host = blue and 20 females per host = black (c).

Results

Virulence and fecundity

Females from the 'local-random' regime were slightly more virulent ($F_{1,5} = 6.89$, $p = 0.0513$; Figure 2a) and had higher fecundity ($F_{1,4} = 8.72$, $p = 0.0449$; Figure 2b) than females from the 'global random' regime (Table S1). There was no difference in total virulence ($F_{1,3} = 3.06$, $p = 0.5652$; Figure 2a) or total fecundity ($F_{1,3} = 3.50$, $p = 0.1409$; Figure 2b) when comparing females from the 'global random' and 'global co-transmission' selection regimes (Table S1).

There was a positive correlation between mean per capita virulence and mean per capita fecundity ($r = 0.71$, $n = 33$, $p < 0.0001$; means per population at each density; Figure 2c).

Discussion

Our results illustrate the relative importance of global versus local competition for parasite virulence evolution. *T. urticae* from the ‘local random’ regime were more virulent, with higher fecundity, than *T. urticae* from the ‘global random’ regime. In contrast, there was no difference in virulence or fecundity between females from the ‘global co-transmission’ and ‘global random’ selection regimes.

Why ‘local random’ more virulent than ‘global random’?

Our finding that mites from the ‘local random’ regime are more virulent, with higher fecundity than mites from the ‘global random’ regime, is consistent with the prediction that intense local competition can select for mites better able to exploit their local environment in the presence of competitors (Chao et al., 2000). This may be because local competition maintains genetic diversity over longer time-frames than global competition, thus increasing the intensity of within-host competition (Chao et al., 2000). This would mean that responses to selection continued in the ‘local random’ regime after a potential selective sweep in the ‘global random’ regime prevented any further responses to selection (Frank, 1996, Chao et al., 2000).

At the same time under global competition, a selective sweep is predicted to select for lines with the highest productivity (Chao et al., 2000). We found the opposite result to be true with females from the ‘local random’ regime having the highest levels of fecundity. Consistent with this finding, a separate experiment with these selection lines found females from the ‘local random’ regime to also have the highest number of offspring becoming adult, followed by females from the ‘global random’ regime, with females from the 'global co-transmission’ regime having the lowest numbers of offspring becoming adult (see Figures S4 and S5, (Rodrigues et al., 2021); note in this experiment co-transmission is referred to as 'budding dispersal'). We think one reason for this may be due to individuals in the global competition regimes suffering from higher levels of inbreeding depression. Indeed, despite being haplodiploid, spider mites have been found to suffer from inbreeding (Tien et al., 2015).

Our experiment measured how the scale of competition in the within versus among host environment affected virulence evolution. Other studies have investigated how local versus global transmission at the among host level affects virulence evolution. Under this latter scenario, local transmission among hosts should select lower levels of virulence to reduce competition with kin (Wild et al., 2009, Lion & Boots, 2010). This hypothesis has been tested in a number of systems and has been shown to select for reduced infectivity (Boots & Mealor, 2007) or virulence (Kerr et al., 2006).

Why no difference between 'global random' and 'global co-transmission'?

Transmission of pairs of females from the same host patch each generation aligns the interests of parasites, therefore selecting for cooperation compared to random dispersal, and at the same time increases the probability of interactions with kin (Gardner et al., 2009, Faria et al., 2020, Alizon, 2013). Furthermore, when competition is global, co-transmission can increase cooperative or kin selected behaviours compared to under random dispersal (Kummerli et al., 2009). This may be expected to select for lower virulence levels as competition is less intense between individuals sharing a host, thus reducing the tragedy of the commons (Frank, 1996, Alizon, 2013). Indeed, increased interactions between kin under co-transmission in *T. urticae* selected for reduced male harm in this same experiment (in this paper co-transmission is referred to as budding dispersal (Rodrigues et al., 2021)), but not lower levels of virulence. This might be because virulence is a necessary by-product of within-host/patch growth, such that it is not possible to reduce virulence without also greatly reducing productivity. Other studies showing that budding dispersal increases cooperation rely on public goods that do not directly harm the resource or host (Kummerli et al., 2009). Another possibility is that changes in virulence were manifest in different, unmeasured ways. There is senescence of leaf tissue surrounding areas that *T. urticae* have fed that is detectable 7 days after feeding (Alba et al., 2015). As such, one way *T. urticae* could cooperate to reduce virulence would be to concentrate their feeding, as opposed to disparate feeding across the leaf, to reduce collateral leaf damage.

Local versus global competition for parasites in the real world

Many parasites will probably encounter both local and global competition at different stages of their life-cycles. In our selection experiment the local and global regimes were two extremes on a continuum and thus there was not the opportunity for both types of competition to co-occur within the same regime, which may be a more realistic selection scenario (Ravigne et al., 2009, Debarre & Gandon, 2011).

Adaptations to exploit or overcome competitors directly in the within-host environment are consistent with the occurrence of local competition, such as toxins to kill competitors (Chao et al., 2000). An extreme example of strict local competition is parasitoid wasps with one larva emerging per host (Wang et al., 2019, Gallet et al., 2018), but this is probably rare. Parasite population management strategies may also impose local or global competition with possible unforeseen consequences for virulence or transmission evolution (Vale, 2013).

The relationships between the scale of competition, and interactions with kin

In many systems the scale of competition and the frequency of interactions with kin are often coupled, for example, vertically transmitted symbionts will often experience local competition with kin (Frank, 1996, Vautrin & Vavre, 2009). However, changes in the scale of competition may very well arise via effects symbionts have on their hosts that determine their representation in the population the next generation. For instance, hosts infected with one parasite may outcompete hosts infected with another (Duncan et al., 2018, Wille et al., 2002, Vautrin & Vavre, 2009). This would be more representative of global competition. Thus, whether global or local competition prevails under vertical transmission may depend upon the effects parasites/symbionts have on their hosts.

Previous work has shown that selection under different population structures can have important consequences for parasite evolution (Griffin et al., 2004, Kummerli et al., 2009, Kerr et al., 2006, Berngruber et al., 2013, Boots & Mealor, 2007). Our study adds to this body of work indicating that the scale of competition can also impact virulence evolution. Contrary to expectation we did not find that increased interactions with kin reduced virulence evolution under ‘co-transmission’, possibly due to constraints with growth. It will be interesting to investigate whether parasites with other modes of virulence can select for reduced virulence under different population structures which could have important consequences for parasite management strategies.

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Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: All authors consent to publication.

Availability of data: All data will be placed in a public repository (Dryad or other) upon publication.

Authors contributions: ABD conceived of the idea for the experiment and wrote the manuscript. ABD and SL performed the experiment. EL, MV and ABD analysed the data.

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