

ORIGINAL ARTICLE

Genotype-specific interactions between parasitic arthropods

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Despite the ubiquity of coinfection, we know little of the effects of intra-specific genetic variability on coinfection by distinct parasite species. Here we test the hypothesis that parasite multiplication depends on the combination of parasite genotypes that coinfect the host (that is $\text{Genotype}_{\text{parasite}} \times \text{Genotype}_{\text{parasite}}$ interaction). To that aim, we infected tomato leaves with the ectoparasitic mites *Tetranychus urticae* and *Tetranychus evansi*. We tested all possible combinations between four *T. urticae* and two *T. evansi* populations sampled on different hosts or localities. There was no universal (that is genotype-independent) effect of coinfection on mite multiplication; in many cases the two species had no effect on each other. However, several combinations of *T. evansi* and *T. urticae* populations led to elevated *T. evansi* numbers. Similarly, *T. urticae* reproduction largely depended on the interaction between *T. urticae* and *T. evansi* populations. This evidence for genotype-by-genotype interaction between coinfecting parasites indicates that the effect of coinfection on parasite epidemiology and evolution may vary in space according to the genetic composition of local parasite populations; it further suggests the possibility of coevolution between parasites species that share the same hosts.

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INTRODUCTION

Understanding parasite epidemiology and evolution is a challenging task due to the large number of factors involved. One reason for this complexity is that parasites often share their hosts with other species with which they can interact (Pedersen and Fenton, 2007; Rigaud *et al.*, 2010). Coinfection by several parasites of distinct species can have both positive or negative effects on parasite fitness (Pedersen and Fenton, 2007). For example, HIV infection, and its associated immuno-suppression, facilitates infection by other infectious diseases such as tuberculosis (Narain *et al.*, 1992). Alternatively, infection by *Schistosoma* parasites has been suggested to protect hosts from malarial infection (Briand *et al.*, 2005). The environment can also modulate the effect that two parasites have on each other. For instance, the outcome of the competition between mosquito parasites can depend on the amount of food available to the host and infectious doses (Fellous and Koella, 2009). Unfortunately, logistic and conceptual limitations often restrict empirical approaches to simple designs unable to reflect the diversity of relationships that may occur between parasites. Indeed, most studies on coinfection by different parasite species neglect their intra-specific genetic variability (Pedersen and Fenton, 2007; Rigaud *et al.*, 2010). This source of variability is however very important as parasite populations are often genetically heterogeneous in space (for example Boubou *et al.*, 2011; Criscione and Blouin, 2004; Prugnolle *et al.*, 2005) leading to different epidemiologies in different contexts (for example Fellous *et al.*, 2012) and because genetic variability is essential to evolution and coevolution.

In some cases, parasite genetic factors have been shown to determine whether a parasite suffers from, benefits or is unaffected by the presence of a second parasite. Indeed, parasite fitness can depend on the genetics of the focal parasite (Hodgson *et al.*, 2004;

Seppälä *et al.*, 2009), as well as that of the parasite it shares its host with (Bell *et al.*, 2006). However, the combined, simultaneous effect of both parasite genetics—that is $\text{Genotype}_{\text{parasite}} \times \text{Genotype}_{\text{parasite}}$ interactions (further referred to as Gp.Gp interactions)—on their success has received scant attention. We know however that Gp.Gp interactions can be influential as experiments with two fish parasites of the *Diplostomum* genus found that whether *D. pseudospathaceum* succeeds, or fails, at infecting depends on an interaction between its genotype and that of its competitor (Seppälä *et al.*, 2012). In the present study, we focus on parasite multiplication once taxa have encountered on the same host. These approaches are complementary for two reasons. First, parasite fitness depends on infection success (Seppälä *et al.*, 2012) but also on multiplication within (and therefore transmission from) the host, all these stages being important. Second, theoretical models of parasite dynamics that consider parasite–parasite relationships usually distinguish between superinfection (that is when only one of several competing parasites survives in the host) from coinfection (that is when several parasites coexist in the host) (Adler and Mosquera, 2002; Alizon *et al.*, 2013). Among other things, coinfection would produce more virulent infection than superinfection; superinfection is predicted to favour greater diversity in parasite population than coinfection (Alizon, 2013).

To investigate the effect of Gp.Gp interactions between parasites we studied the coinfection between two phytophagous, ectoparasitic mites, *Tetranychus evansi* and *Tetranychus urticae*. Plant immune response to these mites include proteinase inhibitors that are regulated by jasmonic acid and salicylic acid pathways. *T. evansi* can manipulate host immune system and down-regulate the production of proteinase inhibitors (Sarmento *et al.*, 2011a). By contrast, *T. urticae* has not been shown to inhibit plant defences, but some genotypes are less prone to

activating them than others (Kant *et al.*, 2008). These two species have also been shown to influence each other's growth rate in captivity (Sarmiento *et al.*, 2011b) and distribution in the wild (Ferragut *et al.*, 2013). We experimentally established competition between the two species placing several adult mites of each species on the same tomato leaf, a host plant where natural coinfection occurs in the field (Alain Migeon, personal communication; Simon Fellous, personal observation). We recorded offspring production and competition avoidance behaviour over one cycle of reproduction. Mite genotypes were manipulated by using four *T. urticae* populations collected on different host plant species: tomato (*Solanum lycopersicum*), citrus tree (*Citrus aurantium*) and rose bay (*Nerium oleander*) and two *T. evansi* populations representing the two major phylotypes encountered in Europe (Boubou *et al.*, 2011) both sampled on tomato (*Solanum lycopersicum*). Choice of assay population was not trivial as coevolution and local adaptation can deplete genetic diversity and conceal potent Gp.Gp interactions (Heath and Nuismer, 2014). We hence chose to contrast allopatric populations of mites that were sampled on different host species and localities and we knew were genetically distinct. The life-histories of the four *T. urticae* populations we employed were investigated in a previous study (Figure 1 in Fellous *et al.*, 2014), revealing reproducible phenotypic—and therefore genetically based—differences among them.

MATERIALS & METHODS

Biological system

The two-spotted spider mite, *T. urticae*, is a cosmopolitan and highly polyphagous mite found on more than a thousand plant species (Migeon and Dorkeld, 2006–2014). This mite feeds by piercing leaf parenchyma cells with its stylet and sucking out cell content (Tomczyk and Kropczynska, 1985). The species has an arrhenotokous reproductive system: diploid females produce haploid males with unfertilised eggs (Helle and Bolland, 1967). A life cycle can be completed in as little as 10 days on suitable hosts and at optimal temperature (c. 23 °C). After mated-females colonise new host plants, mites undergo several reproductive cycles until reaching carrying capacity or plant death (Helle and Sabelis, 1985).

The biology of *T. evansi* is in many ways similar to that of *T. urticae*. However it has a narrower host range as it is mostly found on solanaceous plants. It has a recent history of invasion: two major phylotypes are present in Europe (Boubou *et al.*, 2011). The first phylotype invaded Africa and later on Europe, whereas the second one seemingly arrived directly from South America.

All mites were maintained before the experiment in our standard rearing conditions. *T. urticae* were fed detached leaves of bean, *Phaseolus vulgaris*, while *T. evansi* received *Solanum nigrum* leaves. These leaves were placed on water-soaked cotton wool in plastic boxes in a controlled-temperature chamber at 23 °C with 16 h of light per day.

Experimental populations

Our aim being to study the effects of intra-specific genetic variability on mite development, we selected experimental populations that we were certain would exhibit genetic differences based on the available literature and our experience. We thus assayed populations that originated from distinct regions and host species. Recent theory shows that restricting analyses to sympatric populations increases the risk of false negatives, that is of not finding G × G patterns even though they influence evolution (Heath and Nuismer, 2014). This derives from the strong depleting effects that adaptation and coevolution can have on standing genetic variation. The most common genotypes (that is those that would be most likely to be sampled) may thus not reflect existing genetic diversity. In other words, statistical interactions between genotypes from the same locality may largely underestimate the spread of functional genotype-by-genotype interactions at play in the field. However, G × G patterns can only lead to coevolution if assayed genotypes do encounter. In other words, for functional interactions between genotypes to be more than laboratory artifacts

and influence natural processes, chosen genotypes must be representative of natural genetic diversity (see details in Heath and Nuismer, 2014).

Our choice of study populations followed the reasoning described in the previous paragraph. For *T. urticae* we used four populations from three different host plants (*Solanum lycopersicum*, *Nerium oleander* and *Citrus aurantium*) and three south-European localities. Published data and our own on-going population genetics work shows that, despite mite specialisation to the host plant (Fellous *et al.*, 2014), there is substantial gene flow between *T. urticae* populations encountered on these plants and localities (Magalhães *et al.*, 2007). For *T. evansi* we used each of the two European phylotypes (Boubou *et al.*, 2011), which geographic distributions converge in southern Europe.

T. urticae populations were found c. 6 months earlier by a small number of individuals collected in the field and experienced strong bottlenecks during their first generations in the lab (Fellous *et al.*, 2014). Two populations derived from individuals collected on tomato, *S. lycopersicum*: Population A was sampled in southern France and population B in Crete. Another population derived from individuals collected on *N. oleander*, and the last one from individuals collected on *C. aurantium*, both in Spain. These are further referred to as *T. urticae* populations C and D, respectively. Genetic (or heritable epigenetic) differences between these populations are supported by their significantly different performance on tomato and other plants (Fellous *et al.*, 2014). We used two *T. evansi* populations, both collected on tomato, from each of the two major phylotypes encountered in Europe (Boubou *et al.*, 2011). The *T. evansi* populations, further referred to as one and two, correspond to phylotypes one and two, respectively, as described in (Boubou *et al.*, 2011). Population one was sampled at Beau-soleil (south-east of France) and population two near Sainte-Marie-la-Mer (south-west of France).

Experimental design

All combinations of *T. urticae* and *T. evansi* populations were tested with a full-factorial design (for coinfection treatments). We also included single infections of each species as controls treatments. There were thus eight coinfection treatments and six single infection treatments.

Replicates were organised in five blocks, each containing every treatment, and were run one week apart. Mites used for each block were produced independently by having c. 50 females of each population lay eggs on a tomato leaf during 24 h.

An experimental unit (that is, one replicate) was made of two tomato leaflets (*S. lycopersicum*, cultivar Heinz) placed on a bed of cotton wool soaked with water. For simplicity, we refer to these leaflets as leaves through the manuscript. Leaves partly overlapped (c. 2% of surface) to allow mite movement between them. Three adult, 3–7 days old females and one adult male per species (that is, the parental generation) were placed on the inoculated leaf at the beginning of the experiment (Figure 1). Coinfection treatments thus received twice more mites than single infection treatments, so as to study the effect of parasite co-occurrence without changing the initial density of the focal species. Adults were left on the leaves for five days, when we removed them after recording their survival and position (on the inoculated or non-inoculated leaves; Figure 1). This brief oviposition period synchronised the development of mite offspring and therefore avoided generation overlaps. It was an essential point in our protocol as the morphological differences between *T. urticae* and *T. evansi* become conspicuous at the adult stage only. Counts of adult offspring, and their position, occurred 18 days post-inoculation, after one reproductive cycle (Figure 1).

We studied dispersal by depositing, at the beginning of experiment, the individuals on a single tomato leaf (that is inoculated) in contact with a second leaf (that is non-inoculated) on the same cottonwool bed. Dispersal was estimated, after 5 and 18 days, as the proportion of individuals that were present on the non-inoculated leaf.

The experiment was conducted in a controlled-temperature chamber at 23 °C with 16 h of light per day.

Statistical analyses

We used generalised linear models (GLM) to assess the effects of *T. urticae* and *T. evansi* genotypes on mite multiplication. To analyse the numbers of live

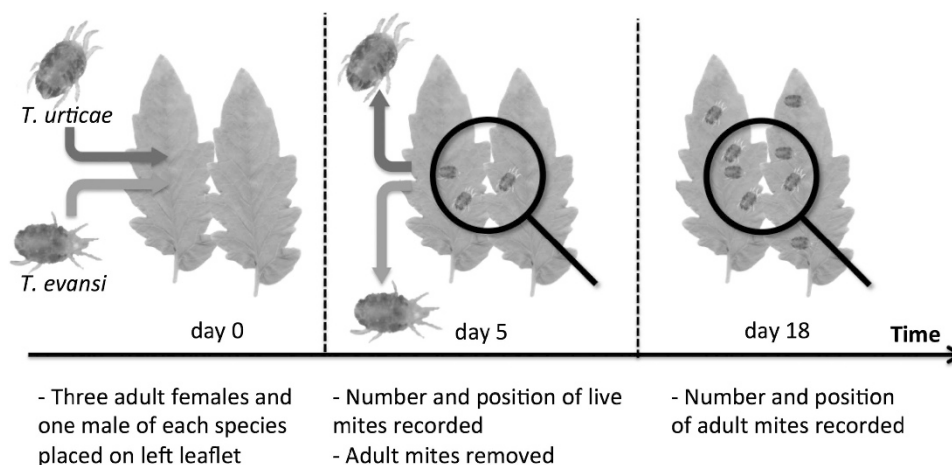


Figure 1 Experimental set-up. Adult mites were placed on tomato leaves on a bed of wet cotton wool. Each experimental unit contained two tomato leaflets, which were in contact hence permitting mite movement among them.

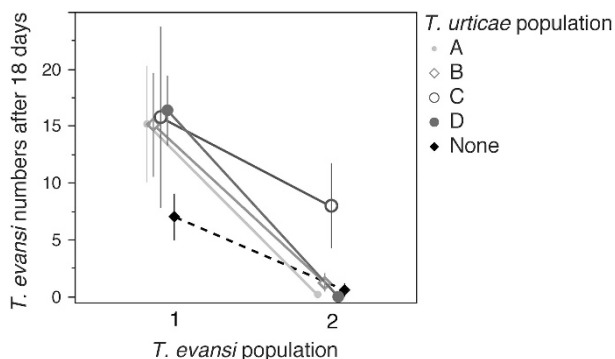


Figure 2 Number of *T. evansi* offspring 18 days after inoculation of tomato leaves. Because young *T. evansi* and *T. urticae* are difficult to discriminate we only counted adult individuals. Symbols indicate means and error-bars indicate s.e.

individuals 18 days post-inoculation (that is, the response variable) we employed models with Poisson distributions and log links. We first tested whether mite numbers were affected by the presence of the other mite species independent of its genotype (that is, general effects of coinfection). Initial models contained two factors describing the population of the focal mite species, a factor describing the absence or presence of the second mite species and their interaction. Later, we investigated interactions between mite genotypes with models containing the two factors describing their genotypes and their interaction. Mite genotypes were treated as fixed factors because they did not represent random selections of mite populations but were selected for their *a-priori* differences. Block was included in early models but not in the final ones as it was never significant. We used least-squares contrasts with likelihood-ratios tests for *post-hoc* tests. Analyses were carried out with the statistical package JMP 10.0.2.

Note that since we manually infected hosts with adult parasites, and therefore by-passed the first step of natural infections, we focused on the quantitative multiplication of mites not taking into account behaviours associated host plant choice.

For brevity, analyses of survival at the parental population (that is, after 5 days) and mite movement to the non-inoculated leaf after 18 days are reported in Supplementary Materials (Supplementary Tables S1 and S3, Supplementary Figures S1 and S2).

RESULTS

Parasite survival and reproduction

After 18 days, that is after approximately one reproductive cycle, the presence of *T. urticae* (independent of its population) had a marginally non-significant effect on *T. evansi* numbers ($X^2 = 3.78$, $df = 1$, $P = 0.052$). *T. urticae* presence indeed tended to facilitate the development of *T. evansi* (Figure 2). There was however no general effect (that is population-independent) of *T. evansi* presence on *T. urticae* numbers ($X^2 = 0.0001$, $df = 1$, $P = 0.99$).

Instead, effects of the two mite species on each other turned out to be population specific, as revealed by the significant interactions between *T. urticae* and *T. evansi* populations (Table 1, Figures 2 and 3). While *T. evansi* population one benefited from the presence of all *T. urticae* populations (contrast *With T. urticae* population A, B, C and D vs *Without T. urticae*: $X^2 = 4.45$, $df = 1$, $P = 0.035$) (Figure 2), *T. evansi* population two only benefited from the presence of *T. urticae* population C (contrast *With T. urticae* population C vs *Without T. urticae*: $X^2 = 37.8$, $df = 1$, $P = 0.009$) (Figure 2). The interaction between *T. urticae* and *T. evansi* populations remained marginally non-significant ($P = 0.055$) when only using the *T. urticae* populations sampled on tomato (Supplementary Table S2).

Similarly, the identity of the *T. evansi* population had a significant effect on *T. urticae* numbers (see significant interaction between *T. urticae* and *T. evansi* populations in Table 1, Figure 3). For example, in the case of *T. urticae* population B, *T. evansi* population two led to greater mite numbers than with *T. evansi* population one (contrast *With T. evansi* population two vs *With T. evansi* population one: $X^2 = 6.27$, $df = 1$, $P = 0.012$). Interestingly, these same two *T. evansi* populations tended to have opposite effects on *T. urticae* population D, as these were more numerous in the presence of *T. evansi* population one than with population two (contrast *With T. evansi* population one vs *With T. evansi* population two: $X^2 = 3.5$, $df = 1$, $P = 0.061$). The interaction between *T. urticae* and *T. evansi* populations was not significant anymore when only using the *T. urticae* populations sampled on tomato (Supplementary Table S2). This is not surprising as *T. urticae* population C, which was sampled on *Nerium oleander*, was excluded.

Table 1 Analyses of mite numbers 18 days after leaf inoculation

Species	Factors	d.f.	χ^2	P-value
<i>T. urticae</i>	<i>T. urticae</i> population	3	22.3	<0.0001
	<i>T. evansi</i> population	1	2.33	0.127
	<i>T. urticae</i> * <i>T. evansi</i> populations	3	12.0	0.007
<i>T. evansi</i>	<i>T. urticae</i> population	3	11.4	0.01
	<i>T. evansi</i> population.	1	44.9	<0.0001
	<i>T. urticae</i> * <i>T. evansi</i> populations.	3	11.5	0.009

Generalised linear modelling with Poisson distribution and a log-link.

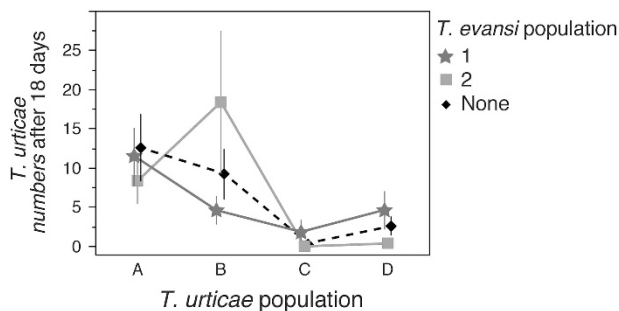


Figure 3 Number of *T. urticae* offspring after 18 days after inoculation of tomato leaves. Because young *T. evansi* and *T. urticae* are difficult to discriminate we only counted adult individuals. Symbols indicate means and error-bars indicate s.e.

Within host movements and competition avoidance

In our experimental set-up, parental mites were deposited on one of two adjacent leaves (that is the inoculated leaf). There were very little movements between the inoculated and non-inoculated leaves during the 5 days before parents were removed, hence preventing us from testing the hypothesis that competitor avoidance was controlled by interactions between *T. evansi* and *T. urticae* genotypes. However, we noticed that the only three instances where *T. evansi* females were found on the non-inoculated leaf were in the presence of *T. urticae* population C (Supplementary Figure S3). A Fisher’s exact test revealed that *T. urticae* population C did increase *T. evansi* movement from the inoculated to the non-inoculated leaf ($P=0.008$). There was nonetheless no effect of *T. urticae* population identity on *T. urticae* movements after 5 days: analyses of mite positions after 18 days provided no support for interactions between mite populations and are reported as Supplementary materials.

DISCUSSION

This experiment revealed that when two parasites of different species coinfect the same host their multiplication can be determined by interactions between their genotypes (that is Gp.Gp interaction). More precisely, we found that, during the first reproductive cycle of an infection (that is 18 days), numbers of ecto-parasitic mites *T. urticae* and *T. evansi* either increased or remained unaffected by the presence of the other species, and that this depended on the combination of mite populations in presence.

Epidemiological and evolutionary implications

Genotype-by-genotype interactions are important for ecological dynamics as they foster different demographics in localities populated

by different genotypes. Since parasite populations are often genetically structured (that is, all genotypes are not encountered everywhere) (for example Boubou *et al.*, 2011; Criscione and Blouin, 2004; Prugnolle *et al.*, 2005), Gp.Gp interactions suggest that the effect each parasite has on the others should differ among locations. In some cases, one species may tend to exclude the other while favouring its spread in other locations—as when *T. evansi* population two encountered *T. urticae* population C in our experiment.

For coevolution between two organism to occur, a number of conditions must be filled. The first one is that their fitness depends on functional genotype-by-genotype interactions (Health and Nuismer, 2014; Thompson, 2005). These refer to situations where the phenotype of an organism is determined by the interaction between its genome and that of the other organism it may coevolve with. Functional genotype-by-genotype interactions can be characterised at the molecular level, but also evidenced by statistical genotype-by-genotype interactions, as we do here (Heath and Nuismer, 2014). It is also important that, for each species, focal genotypes may not be restricted to isolated localities, a condition that would be fulfilled by our choice of mite populations (see Methods). Our results therefore suggest possible coevolution between parasites that share the same host. This type of coevolution would be analogous to better-known host-parasite coevolution—which is based on $\text{Genotype}_{\text{host}} \times \text{Genotype}_{\text{parasite}}$ interactions (Agrawal and Lively, 2002), and coevolution between mutualists of different trophic levels (for example Health *et al.*, 2012; Thompson, 2005). In addition, coevolution between parasite species would necessitate that the two species co-occur frequently on the same hosts so as to substantially affect the selective pressures they are under. As such, it may be faster during epidemic phases or when hosts exhibit low resistance to infection, hence creating so-called coevolutionary hot-spots (Thompson, 2005).

The composition of the host population also exerts strong selective pressures: parasites, such as *T. urticae*, readily adapt to specific host species or genotypes (Carletto *et al.*, 2009; Fry, 1990; Legros and Koella, 2010; Magalhães *et al.*, 2007). The *T. urticae* populations we used in our experiment were sampled on three different host plant species. Therefore, the different phenotypes of our *T. urticae* populations that were expressed in situation of coinfection may result from their specialisation to different hosts. It would explain why *T. urticae* population C stood out as eliciting both dispersal movements of *T. evansi* (Supplementary Figure S3) and significantly increasing *T. evansi* multiplication (Figure 2). When parasites’ genotype-specific effects are due to adaptation to different hosts, local composition of host community would control coinfection outcomes through parasite specialisation and local-adaptation.

The effect of coinfection on the evolution of parasite virulence has been extensively studied theoretically (Mideo *et al.*, 2008; Alizon, 2013). In most models predicted evolutionary trajectory relies on the effects coinfecting parasites have on each other. However, no model to our knowledge takes Gp.Gp interactions into account. Indeed, they all assume that coinfection is either facilitative, neutral or detrimental; not that it varies according to the players involved. The integration of such variability would be an important challenge associated with reconciling theory on virulence evolution with empirical data (Alizon *et al.*, 2013).

Underlying processes

The mechanism behind the interactions between *T. evansi* and *T. urticae* are unknown, identifying them would go beyond the aim of the present work. Since the mites mostly exhibited facilitative effects, classical means of competition—such as resource limitations

and spiteful interactions—do not apply. Similarly, it was reported that *T. evansi* can alter *T. urticae* population growth by depositing silk-web on the leaf surface (Sarmiento *et al.*, 2011b). But web production was negligible in our experiment. We estimated mite performance by counting the number of offspring produced on plants exposed for a short to adult mites. This would accurately reflect reproductive output rate (that is number of offspring per unit of time). But other components of fitness such as life-time reproductive success (which depends on lifespan) and offspring condition were not taken into account.

Genotype-by-genotype interactions are best known between hosts and parasites (Lambrechts *et al.*, 2006). They can have significant effects on epidemiology (Fellous *et al.*, 2012) and underly host-parasite coevolution (Betts *et al.*, 2014). Genotype_{host} × Genotype_{parasite} interactions often result from genotype-specific effects of parasites on host immunity (Lambrechts *et al.*, 2013). A similar mechanism is therefore likely for Gp.Gp interactions. Some *T. urticae* populations activate plant immunity, while others manage to not activate it (Alba *et al.*, 2015; Kant *et al.*, 2008). It was also reported that *T. evansi* can inhibit plant defences (Sarmiento *et al.*, 2011a). We conducted our experiment on detached leaves rather than whole plants, some immune components may thus have been altered (Schmelz *et al.*, 2001). But published data shows that local (that is non-systemic) defences are potent against *T. evansi* and *T. urticae* (Sarmiento *et al.*, 2011a). Therefore, differences among mite populations and species in their ability to activate and resist plant defences likely contribute to population-specific interactions between these parasites (Paterson, 2013). In fact, this may even be a general mechanism for Gp.Gp interactions as indirect interactions between parasites are often mediated by the host immune system (Frank, 2002). However, the role of host immunity for Gp.Gp interactions was recently tested with *Diplostomum* parasites of fish—which infection success rely on Gp.Gp interactions (Seppälä *et al.*, 2012)—finding no evidence for genotype-specific priming of the host immune system (Rellstab *et al.*, 2013).

Competitive interactions between *T. evansi* and *T. urticae* would have led to a reduction in *T. urticae* abundance and effective host range in southern Europe after *T. evansi* invasion (Ferragut *et al.*, 2013). On tomato, competition was investigated by Sarmiento and collaborators (Sarmiento *et al.*, 2011b). In their experiment *T. evansi* reduced *T. urticae* numbers within one generation. This discrepancy with our results may derive from genetic differences among the mite populations assayed as these authors only tested one population per species. Similarly, the host plant cultivar used by Sarmiento (Santa Clara I-5300, CA, USA) is different from ours. Cultivars of the same plant species often vary broadly in terms of defences against parasites and pests (Krips *et al.*, 1998; Xuéreb and Thiéry, 2006). The putative importance of plant immunity (and mite counter-defences) for Gp.Gp interactions suggests that host genetic identity may have been an important factor. Prior experience may have played a role too: we produced the parental generation of *T. urticae* on bean rather than tomato as in Sarmiento *et al.* (2011b). Mite ability to exploit certain hosts is indeed known to be influenced by the host species that previous generations infected (Magalhães *et al.*, 2011). Finally, Sarmiento *et al.* (2011b) carried-out their experiment on whole tomato plants, while we used detached leaves, as is common for *T. urticae* phenotyping (for example Agrawal *et al.*, 2002; Magalhães *et al.*, 2011). All these elements illustrate how using lab data to make quantitative prediction on parasite dynamics may be deceptive. It is clear Gp.Gp interactions occur, but their importance to field dynamics remains to be elucidated.

CONCLUSIONS

Our experiment revealed that the multiplication of two coinfecting plant ectoparasites relied on Genotype_{parasite} × Genotype_{parasite} interactions. Indeed, the combination of parasite populations present determined mite reproduction and whether coinfection was beneficial or neutral. It follows that the local genetic composition of parasite populations may determine the reciprocal influence two coinfecting parasites have on each other and cascade on their epidemiologies. Ultimately this might lead to coevolution between parasites sharing the same host. Besides, the genetic identity of co-occurring parasites being determined by local selective pressures (for example nature of available hosts) coinfection outcome may vary with local host community composition.

DATA ARCHIVING

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.fg271>.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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