UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Costs and benefits of plant defence suppression by Tetranychus evansi spider mites

Knegt, A.J.

Publication date
2019

Document Version
Final published version

License
Other

Link to publication

Citation for published version (APA):
Knegt, A. J. (2019). *Costs and benefits of plant defence suppression by Tetranychus evansi spider mites*. [Thesis, fully internal, Universiteit van Amsterdam].

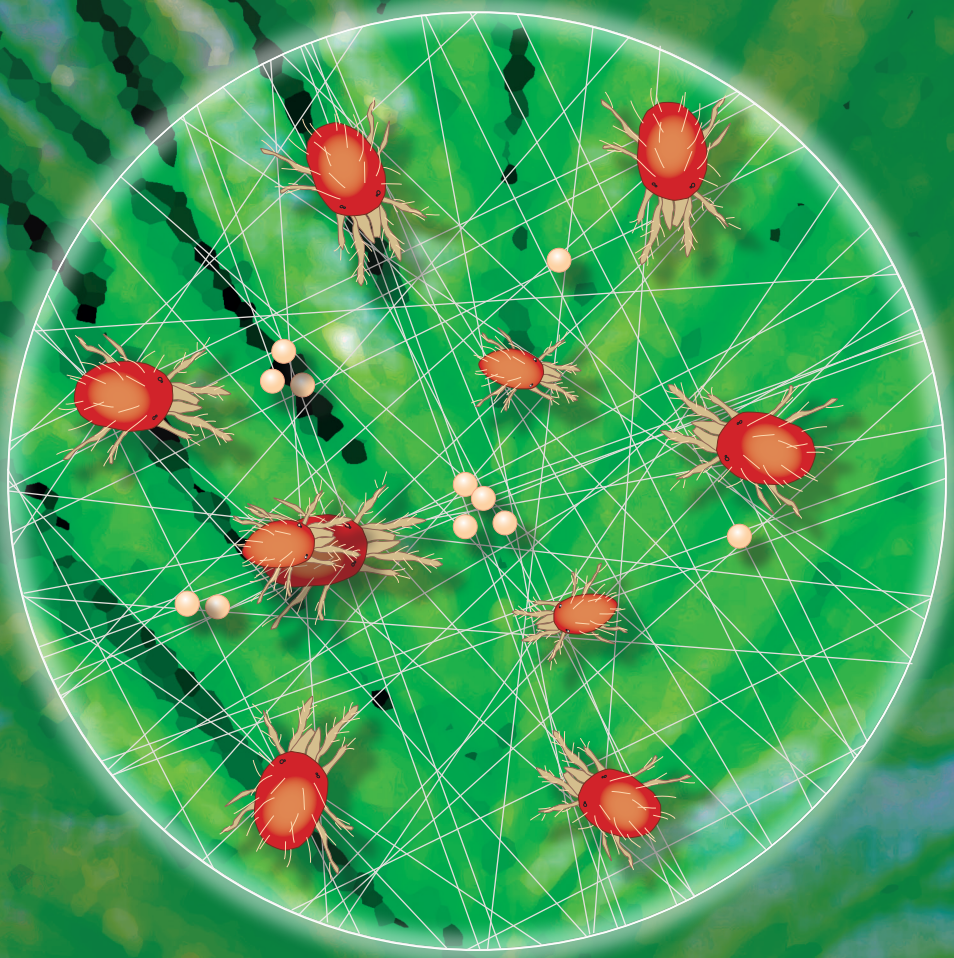
General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:09 Mar 2023

Costs and benefits of plant defence suppression by *Tetranychus evansi* spider mites



Bram Knegt

Costs and benefits of plant defence suppression by *Tetranychus evansi* spider mites

A.J. Knegt, 2019. Costs and benefits of plant defence suppression by *Tetranychus evansi* spider mites

PhD thesis, University of Amsterdam, The Netherlands

ISBN: 978 94 91407 72 7

Cover design: Jan van Arkel
Thesis lay-out: Jan Bruin (www.bred.nl)

Costs and benefits of plant defence suppression by *Tetranychus evansi* spider mites

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. K.I.J. Maex
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel

op woensdag 26 juni 2019, te 12:00 uur

door
Abram Josse Knegt

geboren te Amsterdam

Promotiecommissie

Promotor

Prof. dr. E.T. Kiers	Vrije Universiteit Amsterdam

Copromotor

Dr. C.J.M. Egas	Universiteit van Amsterdam

Overige leden

Prof. dr. P.H. van Tienderen	Universiteit van Amsterdam
Prof. dr. M.A. Haring	Universiteit van Amsterdam
Prof. dr. ir. T.B.S. Van Leeuwen	Universiteit Gent, België
Prof. dr. J.C. Bede	McGill University, Canada
Dr. I.M. Smallegange	Universiteit van Amsterdam

Faculteit der Natuurwetenschappen, Wiskunde en Informatica

Contents

6 Author affiliations

7 1
General introduction

23 2
Meta-analysis reveals intraspecific variation for plant-mediated interactions among herbivores

55 3
Tetranychus evansi spider mite populations suppress tomato defences to varying degrees

81 4
Detection of genetic incompatibilities in non-model systems using simple genetic markers: hybrid breakdown in the haplodiploid spider mite *Tetranychus evansi*

117 5
Spider mites maintain defence suppression after 60 generations of experimental evolution on tomato lines that lack inducible jasmonic acid defences

149 6
General discussion

171 Summary
174 Author contributions
175 Samenvatting
179 Acknowledgements

Author affiliations

Juan M. Alba
Livia M.S. Ataide
Rachid Chafi
Cleide R. Dias
Martijn Egas
Arne Janssen
Merijn R. Kant
Bram Knegt
Felipe Lemos
Tomas T. Meijer
Nigel Pearson
Tomos Potter
Yukie Sato
Bernardus C.J. Schimmel
Heike Staudacher
Department of Evolutionary and Population Biology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, Netherlands

Tomaz A. Barbosa
Ana M.G. Bernardo
Cleide R. Dias
Marcus V.A. Duarte
Felipe Lemos
Jussara Mencalha
Angelo Pallini
Pauliana A. da Silva
Neilier R. da Silva Júnior
Manuel Solís-Vargas
Department of Entomology, section Acarology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

Diogo P. Godinho
cE3c – Centre for Ecology, Evolution and Environmental Changes, University of Lisbon, Portugal

Dan Li
Academic Affairs Office, Shijiazhuang Tiedao University, 050043, Shijiazhuang, China

Elisa F. de Oliveira
Department of Entomology, Federal University of Lavras, Lavras, Minas Gerais, Brazil

Maria G.A. Oliveira
Manuel Solís-Vargas
Instituto de Biotecnologia Aplicada à Agropecuária, BIOAGRO, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

Maria G.A. Oliveira
Fabrício R. Ribeiro
Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

E. Toby Kiers
Department of Ecological Science, Vrije Universiteit, 1081 HV Amsterdam, Netherlands

Yukie Sato
Sugadaira Montane Research Center, University of Tsukuba, Sugadaira Kogen 1278-294, Ueda, Nagano 386-2204, Japan

Robert C. Schuurink
Department of Plant Physiology, Swammerdam Institute for Life Sciences, University of Amsterdam, Netherlands

1

General introduction

Main aim of the thesis

A wolf lurks in the bushes. She spotted several of her prey, and now she manoeuvres herself into a position for a sudden ambush. How shall she proceed? Shall she attempt to approach the deer from a distance, surprising them with her presence, but permitting them several seconds to respond? They could run away quickly, or dangerously injure her with their kicks. Shall she sneak even closer, so she can reach the deer in one leap, but risking to be noticed beforehand? She could also call on her pack members, so they can approach the deer from multiple directions. This will increase their chance of success, but she will then have to share the bounty with the rest of the pack. Or shall she scare the deer away, chasing them persistently, until one of them succumbs to exhaustion? She can endure longer distance running than her prey, but the long chase will wear her out substantially. Soft cracks sound as she progresses slowly. The deer raise their heads, wary of a potential threat. They have not spotted her yet. She should decide now. Which strategy will she choose?

Offensive behaviour is ubiquitous in nature. It is ubiquitous not only in predators, like wolves, but also in herbivores that attack plants. Although the offensive strategies of herbivores may be very different from those of wolves, the strategies are equally diverse and effective. Some herbivores, for example, detoxify plant defensive compounds, and this allows them to feed on otherwise unpalatable hosts (Smith 1955, Heckel 2014). Other herbivores cut the veins of their host plants to prevent being exposed to their defensive latex (Dussourd & Eisner 1987, Dussourd 2017), or they sequester defensive chemicals from their hosts, and use them as protection against predators (Duffey 1980, Heckel 2014). Why have some herbivores evolved the ability to detoxify host chemicals, whereas others specialise on cutting veins or sequestering host chemicals (Ali & Agrawal 2012, Blaazer et al. 2018)? Under which circumstances is it beneficial to employ a particular offensive strategy, and not another? Should the wolf charge now, or first chase her prey to exhaust them?

To gain insight into the evolution of offensive strategies, it is important to understand their costs and benefits in relation to the environment in which they are employed. In this thesis, I investigate costs and benefits of a recently described herbivore offensive

strategy: plant defence suppression. I will introduce this subject with an evolutionary perspective, and discuss what is known about the costs and benefits of defence suppression from plant-herbivore research. I will also address defence suppression in parasite-host systems, because its costs and benefits, as well as its mechanisms, may resemble those of plant defence suppression by herbivores (Guiget et al. 2016). Subsequently, I will introduce my study system, the tomato red spider mite *Tetranychus evansi*, and present an outline of my thesis.

Plant defence and evolution of herbivore offense

Plants evolved various mechanisms to defend themselves against herbivores. Lipids in plant cuticles, for example, reduce herbivore population growth (Way & Murdie 1965, Eigenbrode & Espelie 1995), and leaf hairs can secrete chemical compounds that are toxic to herbivores (Thurston & Webster 1962, Glas et al. 2012). Some defences, however, are costly to maintain (Cipollini et al. 2014), and plants therefore express them only in response to a threat of herbivory. An example of such an inducible defence response (Chester 1933, Karban & Baldwin 1997) is the production of proteinase inhibitors and polyphenol oxidases, i.e., compounds that directly reduce herbivore performance (Green & Ryan 1979, Felton et al. 1989, Zhu-Salzman et al. 2008). Additionally, plants can also increase their indirect defences, such as production of volatiles that recruit natural enemies of the herbivores (Baldwin & Schultz 1983, Heil 2014).

In response to well-defended host plants, herbivores have evolved a great diversity of mechanisms to overcome these defences. Herbivore offensive traits, as defined by Karban & Agrawal (2002), are traits that increase the herbivore host use efficiency and performance. Examples of such traits are detoxification of plant defensive compounds, suppression of plant defence mechanisms, but also rolling the leaves of host plants to reduce leaf toughness (Berenbaum 1978, Sagers 1992, Kadioglu et al. 2012), or diluting defensive compounds by attacking plants in large numbers (Wood 1973, Raffa 2001). As in plant defence, herbivore offensive adaptations are often expressed only, or with increased intensity, when the herbivore is exposed to a particular challenge (Després et al. 2007). Monarch butterfly larvae, for example, cut the latex veins of their milkweed hosts to prevent exposure to the sticky latex that can glue them to the leaf surface (Zalucki & Brower 1992). However, they only engage into this behaviour when they sense compounds present in latex exudates (Helmus & Dussourd 2005).

The variation and plasticity in herbivore offenses suggests that different environments require different offensive solutions. Therefore, to understand the evolution of herbivore offensive strategies, it is necessary to have insight into their costs and benefits in relation to the environment. Why do some herbivores, for example, evolve to detoxify a wide range of host chemicals, whereas others specialise on inducing nutritious galls a single host plant species? The debate over the costs and benefits of wide versus spe-

cialized host ranges has intrigued biologists for decades (Futuyma & Moreno 1988, Forister et al. 2012). Herbivores with a wide host range benefit from abundant food availability, but have to deal with various plant defence mechanisms. Their diet may consist of plants with a variety of defensive secondary metabolites (Schultz 1988, Mithöfer & Boland 2012), and to prevent the toxic or digestive inhibitory effects of these compounds, herbivores metabolize them with detoxifying enzymes (Smith 1955, Heckel 2014). The production of such detoxification enzymes, however, can be costly, thereby limiting herbivore performance on well-defended hosts (Creswell et al. 1992, Agrawal et al. 2002, Castañeda et al. 2010).

Alternatively, herbivores can also specialise on one or a limited number of host plant species, in which they manipulate their hosts to produce high-quality food. However, specialized diets entail costs of restricted food availability (Castagneyrol et al. 2014), as well as of searching for suitable host plants (Bell 1990, Kennedy & Gray 1993). Galling herbivores, for example, employ salivary enzymes to induce growth of nutritious tissues in their host plants that serve as food for the herbivore and simultaneously provide shelter against their natural enemies (Price et al. 1987, Stone & Schönrogge 2003). Such preingestive manipulation of hosts increases the predictability and palatability of the ingested tissues, but can only be effective in a limited number of plant species due to physiological differences among plants (Inbar et al. 2004, Price 2005).

Costs and benefits of defence suppression

Only less than two decades ago, a previously undescribed herbivore offensive strategy was reported: by manipulating a plant's physiological processes, herbivores were found to prevent expression of inducible plant defences, and thereby increase their own performance (Musser et al. 2002, Kant et al. 2015, Stahl et al. 2018). The first empirical evidence was presented by Musser et al. (2002, 2005), who demonstrated that saliva of the caterpillar *Helicoverpa zea* contains glucose oxidase, an enzyme that suppresses the inducible defence response of tobacco and tomato plants. Subsequently, defence suppression was found to be employed by various herbivores, including thrips, whiteflies, aphids, leafhoppers, beetles, mealybugs and mites (Kant et al. 2015). Often, defence-suppressing herbivores inject salivary effectors into their host plants to interfere with plant defence signalling, but some herbivores employ symbiotic viruses or phytoplasma to do the job for them (Yang et al. 2008, Stahl et al. 2018).

Under what circumstances is it beneficial to suppress the defences of host plants, compared to, for example, detoxifying their defensive compounds? What are the costs and benefits of defence suppression in relation to the environment? No hypotheses can be derived from predator-prey research, because no examples of offensive strategies analogous to defence suppression are known (but see Losey & Denno [1998] for an example of positive, trait-mediated predator-predator interactions). However, theoreti-

cal and experimental work of defence suppression by parasites, where it is often referred to as immunosuppression or immune evasion, has long been recognized as an important offensive strategy of parasites (Ehrlich 1909). Therefore, research into immune evasion has progressed further than in plant-herbivore research (Guiget et al. 2016), and I will shortly review host defence suppression by parasites before discussing experimental evidence from plant-herbivore systems.

Costs and benefits of immune evasion by parasites

All major parasitic groups employ immune evasion: viruses (Vossen et al. 2002), bacteria (Hornef et al. 2002), fungi (Marcos et al. 2016), protists (Sacks & Sher 2002), helminths (Maizels & McSorley 2016) and parasitoids (Schmidt et al. 2001). They evade or suppress the immune system of their hosts through a variety of mechanisms, which can be categorized as avoidance of recognition by the host, interference with host internal signalling, and disabling of host immune effectors (Schmid-Hempel 2005, 2008). *Salmonella typhimurium* bacteria, for example, vary the structure of lipopolysaccharides in their cell envelopes to prevent elicitation of an immune response by their hosts (Guo et al. 1997). Pox viruses, as another example, produce decoy receptors that bind host signalling proteins that induce apoptosis (Smith et al. 1990). Because apoptosis prevents viral replication in infected cells, suppression of apoptosis through these decoy receptors enhances pox virus virulence (Reading et al. 2002).

The most important benefit of immune evasion for parasites is to delay clearance, i.e., to prevent extermination of the parasite within the host (Frank & Schmid-Hempel 2008). Prolonged infection increases the likelihood of transmission, and therefore parasite fitness (Anderson & May 1979, Alizon et al. 2009). At the same time, however, efficient immune evasion is tailored to specific immune systems, and therefore likely entails the cost of a restricted host range (Schmid-Hempel 2008). Furthermore, immune evasion can be established by one or a few parasite individuals, but may benefit other parasites in the same host. In this perspective, immune evasion can be seen as a public good to which some parasites contribute but not all (Rankin et al. 2007). Within-host competition with 'cheaters' thus poses a considerable cost to pathogens that pay costs to collectively suppress their host's immune responses (Rundell et al. 2016).

Additionally, within-host competition increases optimal parasite virulence, as parasite competitive ability is tightly associated with damage to its host (Mideo 2009). A major cost of virulence, however, is death of the host, and thereby reduced chances for successful transmission (Anderson & May 1979, Alizon et al. 2009). Because immune evasion produces favourable hosts also for other parasites, it increases the likelihood of coinfestation (Cornet & Sorci 2010, Price et al. 2012) as well as competitor population size (Graham 2008). Therefore, immune evasion may provide further impetus for the evolution of increased parasite virulence (Kamiya et al. 2018). At the same time, howev-

er, when virulence increases too much, the costs of earlier host death may render investment into immune evasion unprofitable (Kamiya et al. 2018). Consequently, costs and benefits of immune evasion strongly depend on the presence of competing parasites.

Costs and benefits of defence suppression by herbivores

In line with the parasite-host literature, experimental plant-herbivore studies indicate that plant defence suppression by herbivores increases herbivore performance (reviewed by Kant et al. 2015). Additionally, defence suppression also reduces the recruitment of natural enemies through plant volatiles (Zhang et al. 2009, 2013). Costs appear to derive mostly from altered ecological interactions. Defence suppressing *Aculops lycopersici* russet mites, for example, increased the performance of competing spider mites on their shared tomato hosts, with detrimental effects on russet mite population growth (Glas et al. 2014). Similarly, *Pieris brassicae* butterfly egg extracts suppressed defence of their *Arabidopsis thaliana* hosts, which increased the performance of competing *Spodoptera littoralis* larvae (Bruessow et al. 2010), and *Brevicoryne brassicae* aphids suppressed inducible defences in their cabbage hosts, thereby increasing growth and development of competing *P. brassicae* butterfly larvae (Soler et al. 2012). Defence suppression can also enhance competitor performance indirectly: suppression of inducible defences by *Bemisia tabaci* whiteflies reduced attraction of natural enemies of *Tetranychus urticae* spider mites that coinfested their bean hosts (Zhang et al. 2009). More generally, fitness consequences of plant-mediated interactions among arthropod herbivores have been demonstrated in various systems (Denno & Kaplan 2007).

Although it has been established that herbivores suppress plant defence through the action of salivary enzymes or through vectoring viruses and phytoplasma (Stahl et al. 2018), it is unknown whether the production of these enzymes or maintenance of symbioses imposes metabolic costs. Metabolic costs can limit herbivores in their use of a particular offensive strategy, e.g., by prohibiting herbivores to detoxify many different host defensive compounds at the same time (Beccera 1997, Ali & Agrawal 2012). Metabolic costs of herbivore defence suppression may similarly restrict the range of hosts in which they can suppress defences, depending on the specificity of their defence-suppressing effectors.

Additionally, in parasite-host systems metabolic costs of defence suppression make parasites susceptible to exploitation by 'cheaters' that do not invest in defence suppression but nevertheless reap its benefits (Rundell et al. 2016). Defence-suppressed hosts are actually more prone to coinfestation than non-suppressed hosts, and also allow larger densities of secondary pathogens (Graham 2008, Cornet & Sorci 2010, Price et al. 2012). Because metabolic costs of defence suppression by herbivores have hitherto not been investigated, it is not known if defence suppressing herbivores face costly intraspecific competition with non-suppressors. It is also not known if plant defence suppres-

sion increases the likelihood of herbivore coinfestation. However, variation in defence suppression among herbivore genotypes indicates that such costs may exist (Kant et al. 2008, Alba et al. 2015).

Study system

In this thesis, I investigate costs and benefits of defence suppression by herbivorous *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae) spider mites (FIGURE 1.1). The tomato red spider mite *T. evansi* originated in South America (Boubou et al. 2011, 2012), where it mainly infests host plants from the Solanaceae family, such as tomato, potato, and eggplant (Navajas et al. 2013, Migeon & Dorkeld 2018). In its native range, *T. evansi* is not considered a pest of agricultural importance (Furtado et al. 2007), and populations are most likely controlled by the predatory mite *Phytoseiulus longipes* and the entomopathogenic fungus *Neozygites floridiana* (Ribeiro et al. 2009, da Silva et al. 2010). However, over the last century, *T. evansi* has expanded its range to African, European, Asian and North-American regions with a subtropical or Mediterranean climate (Migeon et al. 2009, Navajas et al. 2013, Migeon & Dorkeld 2018). In its invasive range, *T. evansi* infests a wider range of host plants, replaces local spider mite species, and causes more harm to agriculture (Saunyama and Knapp 2003, Ferragut et al. 2013, Murungi et al. 2014, Azandémè-Hounmalon et al. 2015). These observations, together with the

FIGURE 1.1. A *Tetranychus evansi* female among several eggs and web on a leaf surface. Photo by Jan van Arkel.

Box 1

Biology of the tomato red spider mite *Tetranychus evansi*

Spider mites feed on their hosts by injecting their stylets into the plant's parenchyma and mesophyll cells, and sucking out the cell contents (Bensoussan et al. 2016). This generates chlorotic spots on infested plants, typical of spider mite injury, and plants infested with *T. evansi* likely suffer reduced fitness (Liu et al. 2017). *Tetranychus evansi* can reach rapid population growth due to its short generation time of ~10 days under benign conditions (Bonato 1999, Gotoh et al. 2010), and natural populations usually contain a few to thousands of individuals (Rosa et al. 2005). As all members of its family, *T. evansi* spins silken webs with which it covers its feeding site to avoid competitors and predators (de Moraes & Lima 1983, Sarmento et al. 2011b). Spider mites have a haplodiploid mode of reproduction, where haploid males develop from unfertilized eggs, and diploid females from fertilized eggs (Schrader 1923, Helle & Sabelis 1985). Consequently, a single virgin female can colonise a new host plant, mate with her sons, and establish a new local population. Together with a short generation time, this mode of reproduction allows for fast local population growth.

absence of natural enemies in its invasive range (Ferragut et al. 2013, Navajas et al. 2013) and a large potential distribution in newly colonised areas (Migeon et al. 2009), have made the European and Mediterranean Plant Protection Organization to consider *T. evansi* an invasive pest (EPPO 2004).

Interactions between *T. evansi* and their host plants have predominantly been studied in cultivated tomato, *Solanum lycopersicum*. In general, upon infestation with spider mites, tomato plants accumulate jasmonic acid (JA) and salicylic acid (SA), two plant hormones involved in the majority of defensive responses against herbivores (Walling 2000, Campos et al. 2014). Through a signaling cascade, these hormones induce the production of defence-associated compounds such as proteinase inhibitors, polyphenol oxidases and pathogenesis-related proteins (Li et al. 2002a, Kant et al. 2004, Martel et al. 2015). *Tetranychus evansi* is likely susceptible to this inducible, direct defence response, as its oviposition rates correlate negatively with tomato JA levels (Ataide et al. 2016), and are compromised on plants previously infested with defence-inducing spider mites (Sarmento et al. 2011a, b, de Oliveira et al. 2016, Godinho et al. 2016). In addition, the spider mite-induced accumulation of JA and SA leads to the production of a blend of volatiles (Ament et al. 2001, Kant et al. 2004), which can be used by natural enemies to

locate prey (Dicke et al. 1990, Kant et al. 2004). This indirect plant defence response is potentially effective against *T. evansi*, as infested tomato plants were more attractive to the predatory mite *P. longipes* than uninfested plants (Sarmento et al. 2011a).

Tetranychus evansi is able to suppress the inducible defence responses of tomato plants (Sarmento et al. 2011a). Through injecting salivary effector proteins into the plant while feeding (Jonckheere et al. 2016, Villarroel et al. 2016, Schimmel et al. 2017a), *T. evansi* delays or reduces expression of plant genes associated with plant defence, and suppresses the production of proteinase inhibitors (Sarmento et al. 2011a, Alba et al. 2015, de Oliveira et al. 2016, Godinho et al. 2016, Schimmel et al. 2017a, b). This suppression of tomato JA and SA-dependent plant defence by *T. evansi* occurs downstream of hormone accumulation (Alba et al. 2015), and is most effective at local feeding sites (Sarmento et al. 2011a, Schimmel et al. 2017a, b).

Costs and benefits of defence suppression by *Tetranychus evansi*

Evidence for benefits of defence suppression for *T. evansi* comes from a number of studies. Sarmento et al. (2011a, b) showed that *T. evansi* had higher fecundity on plants previously attacked by conspecifics than on unattacked plants. Similar results were found by Godinho et al. (2016), de Oliveira et al. (2016), and Schimmel et al. (2017b), although the benefit differed from ~1 egg per day (Godinho et al. 2016) to ~5 eggs per day (Sarmento et al. 2011a) among these studies. Another benefit of suppression of inducible plant defence could be reduced attraction of natural enemies through plant-produced volatiles. In the only study where this has been tested, Sarmento et al. (2011a) found that the specialist predator *P. longipes* preferred tomato plants infested with *T. evansi* over uninfested plants. This means that suppression of indirect tomato defence by *T. evansi* is not effective against this natural enemy, but the efficacy of defence suppression against recruitment of other natural enemies, such as entomopathogenic fungi (Elliot et al. 2000, Hountondji et al. 2005) or generalist predators remains to be tested.

Costs of defence suppression for *T. evansi* arise from altered ecological interactions. *Phytoseiulus longipes* predatory mites, for example, prefer eggs from *T. evansi* that fed from defence-suppressed tomato plants, compared to eggs from *T. evansi* that fed from artificially induced plants (Ataide et al. 2016). Potentially, *T. evansi* can sequester plant compounds with defensive properties against natural enemies, and transfer them to their eggs. Defence suppression would then reduce the amount of these defensive compounds in their diet, and hence reduce the defences in their eggs. Additionally, competing spider mite species can benefit from the suppressed defences of host plants shared with *T. evansi* (Sarmento et al. 2011a, b, Alba et al. 2015, Godinho et al. 2016, de Oliveira et al. 2016). Whether defence suppression by *T. evansi* increases the likelihood of coinfestation is unknown. However, when it does happen, it likely compromises *T. evansi* fit-

ness, as *T. evansi* are susceptible to the defences induced by other spider mite species (Sarmento et al. 2011a, b, Godinho et al. 2016, de Oliveira et al. 2016).

However, studies investigating competition between *T. evansi* and *T. urticae* have found mixed results: some confirmed (Sato et al. 2014), but other rejected (Sarmento et al. 2011b) a negative effect of defence-inducing *T. urticae* on *T. evansi* population growth. This may be explained by phenotypically plastic *T. evansi* behaviour in the presence of competitors. For example, *T. urticae* cues induce *T. evansi* to produce more and denser web, which hinders *T. urticae* feeding (Sarmento et al. 2011b). Likewise, *T. evansi* males interfere with *T. urticae* reproduction by preferentially mating *T. urticae* females over conspecific females, potentially reducing *T. urticae* population growth (Sato et al. 2014, 2016, Clemente et al. 2016, 2018). Moreover, when challenged with *T. urticae* on the same leaflet, *T. evansi* can actually suppress plant defence to even lower levels than before the introduction of *T. urticae*, and simultaneously increase its fecundity (Schimmel et al. 2017a). These effects appear to be restricted to the local *T. evansi* feeding sites only, and thus may not benefit competing *T. urticae* (Schimmel et al. 2017a, b). By mitigating ecological costs of defence suppression, such traits 'buffer' (Frank 2007) herbivores against negative selection on defence suppression (Blaazer et al. 2018). In this context, buffering traits may co-evolve with defence suppression, and the existence of such traits thus suggests a cost of competition for defence-suppressing herbivores like *T. evansi*.

Lastly, intraspecific competition could theoretically also impose costs on defence suppression in *T. evansi*. Analogous to the costs of immune evasion associated with the invasion of 'cheaters' in parasite-host systems (Rundell et al. 2016), non-suppressing *T. evansi* could potentially invade a population of defence suppressors. Although it has been found that *T. evansi* prefer plants infested with conspecifics over uninfested plants (Sarmento et al. 2011a), metabolic costs associated with defence suppression, which would be required for such intraspecific public good games (Rankin et al. 2007), have to date not been investigated.

Thesis outline

To test hypotheses about the costs and benefits of defence suppression it is necessary to compare populations with different defence suppression phenotypes. Therefore, much of my work has concentrated on searching for heritable variation in defence suppression in *T. evansi*. This search was guided by my expectations of evolutionary costs and benefits of defence suppression outlined above.

How much do *T. evansi* spider mites benefit through increased fecundity from suppressing the defence response of their tomato hosts? And how much can other spider mite species benefit from suppressed defences of a host shared with *T. evansi*? These questions were answered by a number of studies (Sarmento et al. 2011a, b, Godinho et al. 2016, de Oliveira et al. 2016, Schimmel et al. 2017a, b), but the absolute and relative

size of the benefit varied substantially among these studies. Therefore, in CHAPTER 2, I quantitatively review past evidence of plant-mediated interactions among *T. evansi* and *T. urticae* on tomato plants, including unpublished studies. Because multiple studies were performed with the same lab populations, this meta-analysis allowed us to test if defence suppression changes over generations.

Since identity and density of host plants, competitors, and natural enemies differ across environments, I expected different *T. evansi* populations to suppress plant defence to different degrees. For example, the absence of natural enemies from invasive *T. evansi* populations may decrease need for *T. evansi* to suppress recruitment of natural enemies by their host plants. In CHAPTER 3 I quantify variation in defence suppression among eleven *T. evansi* populations from various locations around the world.

Tetranychus evansi populations are differentiated into two lineages, characterized by genetic differences at nuclear and mitochondrial loci (Gotoh et al. 2009). These lineages co-occur in nature (Boubou et al. 2012), and interlineage crosses suffer from post-zygotic reproductive incompatibility (Gotoh et al. 2009). A small proportion of hybrids is nevertheless viable, and natural F1 and F2 hybrids have been reported in the field that harbour increased genetic variation compared to their parental lineages due to recombination (Boubou et al. 2012). In CHAPTER 4, I perform reciprocal interlineage crosses, and investigate if viable hybrid offspring indeed have recombined genotypes. Furthermore, by using a new two-locus genetic statistic, I investigate whether Bateson-Dobzhansky-Muller incompatibilities underlie post-zygotic incompatibility between *T. evansi* lineages, and also whether heterosis contributes to viability of hybrid offspring.

If recombination among the two *T. evansi* lineages also affects the genes involved in defence suppression, then *T. evansi* populations with different levels of defence suppression can potentially be selected from a genetically diverse population of interlineage hybrids. Considering this possibility, in CHAPTER 5, I established a population of interlineage hybrids, and exposed them to host plants manipulated for their expression of JA-dependent inducible plant defences. Using an experimental evolution approach over approximately 60 generations, I test the hypothesis that if defence suppression entails metabolic costs, it will be selected against or erode due to antagonistic pleiotropy (Cooper & Lenski 2000) or genetic drift (Halligan & Keightley 2009), in cases where defence suppression is not necessary or possible.

Last, in CHAPTER 6, I discuss the results obtained in this thesis, and their implications for our understanding of the evolution of plant defence suppression by herbivores. Furthermore, I make several suggestions for further research on herbivore defence suppression, as well as for how to meaningfully categorize herbivore offensive strategies.

References

Agrawal AA, Vala F, Sabelis MW (2002). Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? *The American Naturalist*, **159**, 553-565.

Alba JM, Schimmel BCJ, Glas JJ, Ataide LMS, Pappas ML, Villarroel CA, Schuurink RC, Sabelis MW, Kant MR (2015). Spider mites suppress tomato defences downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist*, **205,** 828-840.

Ali JG, Agrawal AA (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, **17**, 293-302.

Alizon S, Hurford A, Mideo N, van Baalen M (2009). Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology*, **22**, 245-259.

Ament K, Kant MR, Sabelis MW, Haring MA, Schuurink RC (2004). Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiology*, **135**, 2025-2037.

Anderson RM, May RM (1979). Population biology of infectious diseases: part I. *Nature*, **280**, 361-367.

Ataide LMS, Pappas ML, Schimmel BCJ, Lopez-Orenes A, Alba JM, Duarte MVA, Pallini A, Schuurink RC, Kant MR (2016). Induced plant-defenses suppress herbivore reproduction but also constrain predation of their offspring. *Plant Science*, **252,** 300-310.

Azandémè-Hounmalon GY, Affognon HD, Assogba Komlan F, Tamò M, Fiaboe KKM, Kreiter S, Martin T (2015). Farmers' control practices against the invasive red spider mite, *Tetranychus evansi* Baker and Pritchard in Benin. *Crop Protection*, **76**, 53.58.

Baldwin IT, Schultz JC (1983). Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science*, **221**, 277-279.

Beccera JX (1997). Insects on plants: macroevolutionary chemical trends in host use. *Science*, **276**, 253-256.

Bell WJ (1990). Searching behavior patterns in insects. *Annual Review of Entomology*, **35**, 447-467.

Bensoussan, N., Santamaria, M.E., Zhurov, V., Diaz, I., Grbić, M. & Grbić, V. (2016) Plant-herbivore interaction: dissection of the cellular pattern of *Tetranychus urticae* feeding on the host plant. *Frontiers in Plant Science*, **7**, 1105.

Berenbaum M (1978). Toxicity of a furanocoumarin to armyworms: a case of biosynthetic escape from insect herbivores. *Science*, **201**, 532-534.

Bernays EA (1991). Evolution of insect morphology in relation to plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **333**, 257-264.

Bernays EA, Janzen DH (1988). Saturniid and sphingid caterpillars: two ways to eat leaves. *Ecology*, **69**, 1153-1160.

Blaazer CJH, Villacis-Perez EA, Chafi R, Van Leeuwen T, Kant MR, Schimmel BCJ (2018). Why do herbivorous spider mites suppress plant defenses? *Frontiers in Plant Science*, **9**, 1-16.

Blanchet FG, Roslin T, Kimura MT, Huotari T, Kaartinen R, Gripenberg S, Tack AJM (2018). Related herbivore species show similar temporal dynamics. *Journal of Animal Ecology*, **87**, 801-812.

Bonato, O. (1999) The effect of temperature on life history parameters of *Tetranychus evansi* (Acari: Tetranychidae). *Experimental and Applied Acarology*, **23**, 11-19.

Boubou A, Migeon A, Roderick GK, Auger P, Cornuet J-M, Magalhães S, Navajas M (2012). Test of colonisation scenarios reveals complex invasion history of the red tomato spider mite *Tetranychus evansi*. *PLoS One*, **7**, e35601.

Boubou A, Migeon A, Roderick GK, Navajas M (2011). Recent emergence and worldwide spread of the red tomato spider mite, *Tetranychus evansi*: genetic variation and multiple cryptic invasions. *Biological Invasions*, **13**, 81-92.

Broadway RM (1997). Dietary regulation of serine proteinases that are resistant to serine proteinase inhibitors. *Journal of Insect Physiology*, **43**, 855-874.

Bruessow F, Gouhier-Darimont C, Buchala A, Metraux J-P, Reymond P (2010). Insect eggs suppress plant defence against chewing herbivores. *The Plant Journal*, **62**, 876-885.

Campos ML, Kang J-H, Howe GA (2014). Jasmonate-triggered plant immunity. *Journal of Chemical Ecology*, **40**, 657-675.

Castagneyrol B, Jactel H, Vacher C, Brockerhoff E, Koricheva J (2014). Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *Journal of Applied Ecology*, **51**, 134-141.

Castañeda LE, Figueroa CC, Nespolo RF (2010). Do insect pests perform better on highly defended plants? Costs and benefits of induced detoxification defences in the aphid *Sitobion avenae*. *Journal of Evolutionary Biology*, **23**, 2474-2483.

Chester KS (1933). The problem of acquired physiological immunity in plants. *The Quarterly Review of Biology*, **8**, 275-324.

Cipollini D, Walters D, Voelckel C (2014). Costs of resistance in plants: from theory to evidence. *Annual Plant Reviews*, **47**, 263-308.

Clemente SH, Rodrigues LR, Ponce R, Varela SAM, Magalhães S (2016). Incomplete species recognition entails few costs in spider mites, despite first-male precedence. *Behavioral Ecology and Sociobiology*, **70**, 1161-1170.

Clemente SH, Santos I, Ponce R, Rodrigues L, Varela SAM, Magalhães S (2018). Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly. *Behavioral Ecology*, **29**, 321-327.

Cooper VS, Lenski RE (2000). The population genetics of ecological specialization in evolving *Escherichia coli* populations. *Nature*, **407**, 736-739.

Cornet S, Sorci G (2010). Parasite virulence when the infection reduces the host immune response. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1929-1935.

Creswell JE, Merritt SZ, Martin MM (1992). The effect of dietary nicotine on the allocation of assimilated food to energy metabolism and growth in fourth-instar larvae of the southern armyworm, *Spodoptera eridania* (Lepidoptera: Noctuidae). *Oecologia*, **89**, 449-453.

da Silva FR, de Moraes GJ, Gondim^{jr} MGC, Knapp M, Rouam SL, Paes JLA, de Oliveira GM (2010). Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screen-house tomatoes. *Neotropical Entomology*, **39**, 991-995.

Denno RF, Kaplan I (2007). Plant-mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. In: *Ecological communities: plant mediation in indirect interaction webs* (eds Ohgushi T, Craig TP, Price PW). Cambridge University Press, London.

Després L, David J-P, Gallet C (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology and Evolution*, **22**, 298-307.

de Moraes GJ, Lima HC (1983). Biology of *Euseius concordis* (Chant) (Acarina: Phytoseiidae) a predator of the tomato russet mite. *Acarologia*, **24**, 251-255.

de Oliveira EF, Pallini A, Janssen A (2016). Herbivores with similar feeding modes interact through the induction of different plant responses. *Oecologia*, **180,** 1-10.

Dicke M, van Beek TA, Posthumus MA, Dom NB, van Bokhoven H, de Groot A (1990). Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. *Journal of Chemical Ecology*, **16**, 381-396.

Duffey SS (1980). Sequestration of plant natural products by insects. *Annual Review of Entomology*, **25**, 447-477.

Dussourd DE (2017). Behavioral sabotage of plant defenses by insect folivores. *Annual Review of Entomology*, **62**, 15-34.

Dussourd DE, Eisner T (1987). Vein-cutting behaviour: insect counterploy to the latex defense of plants. *Science*, **237**, 898-901.

Ehrlich P (1909). Ueber Partialfunktionen der Zelle. *Münchener Medizinische Wochenschrift*, **56**, 217-222.

Eigenbrode SD, Espelie KE (1995). Effects of plant epicuticular lipids on insect herbivores. *Annual Review of Entomology*, **40**, 171-194.

Elliot SL, Sabelis MW, Janssen A, van der Geest LPS, Beerling EAM, Fransen J (2000). Can plants use entomopathogens as bodyguards? *Ecology Letters*, **3**, 228-235.

EPPO (2004). Introduction of *Tetranychus evansi* in some Mediterranean countries: addition to the EPPO alert list. European and Mediterranean Plant Protection Organization. Reporting Service no. 05 – 2004. Num. article: 2004/080. https://gd.eppo.int/reporting/article-1601

Felton GW, Donato K, Del Vecchio RJ, Duffey SS (1989). Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. *Journal of Chemical Ecology*, **15**, 2667-2694.

Ferragut, F., Garzón-Luque, E. & Pekas, A. (2013) The invasive spider mite *Tetranychus evansi* (Acari: Tetranychidae) alters community composition and host-plant use of native relatives. *Experimental and Applied Acarology*, **60**, 321-341.

Forister ML, Dyer LA, Singer MS, Stireman^{III} JO, Lill JT (2012). Revisiting the evolution of ecological specialization, with emphasis on insect–plant interactions. *Ecology*, **93**, 981-991.

Frank SA (2007). Maladaptation and the paradox of robustness in evolution. *PLoS One*, **2**, e1021.

Frank SA, Schmid-Hempel P (2008). Mechanisms of pathogenesis and the evolution of parasite virulence. *Journal of Evolutionary Biology*, **21**, 396-404.

Furtado IP, de Moraes GJ, Kreiter S, Tixier M-S, Knapp M (2007). Potential of a Brazilian population of the predatory mite *Phytoseiulus longipes* as a biological control agent of *Tetranychus evansi* (Acari: Phytoseiidae, Tetranychidae). *Biological Control*, **42**, 139-147.

Futuyma DJ, Moreno G (1988). The evolution of ecological specialization. *Annual Review of Ecology, Evolution and Systematics*, **19**, 207-233.

Glas JJ, Alba JM, Simoni S, Villarroel CA, Stoops M, Schimmel BCJ, Schuurink RC, Sabelis MW, Kant MR (2014). Defense suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. *BMC Biology*, **12,** 98.

Glas JJ, Schimmel BCJ, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012). Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. *International Journal of Molecular Sciences*, **13**, 17077-17103.

Godinho DP, Janssen A, Dias T, Cruz C, Magalhães S (2016). Down-regulation of plant defence in a resident spider mite species and its effect upon con- and heterospecifics. *Oecologia*, **180,** 161-167.

Gotoh T, Araki R, Boubou A, Migeon A, Ferragut F, Navajas M (2009). Evidence of co-specificity between *Tetranychus evansi* and *Tetranychus takafujii* (Acari: Prostigmata, Tetranychidae): comments on taxonomic and agricultural aspects. *International Journal of Acarology*, **35**, 485-501.

Gotoh T, Sugimoto N, Pallini A, Knapp M, Hernandez-Suarez E, Ferragut F, Ho C, Migeon A, Navajas M, Nachman G (2010). Reproductive performance of seven strains of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae) at five temperatures. *Experimental and Applied Acarology*, **52,** 239-259.

Graham AL (2008). Ecological rules governing helminth microparasite coinfection. *Proceedings of the National Academy of Sciences USA*, **105**, 566-570.

Green TR, Ryan C (1979) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science*, **175**, 776-777.

Guiget A, Dubreuil G, Harris MO, Appel HM, Schultz JC, Pereira MH, Giron D (2016). Shared weapons of blood- and plant-feeding insects: surprising commonalities for manipulating hosts. *Journal of Insect Physiology*, **84**, 4-21.

Guo L, Lim KB, Gunn JS, Bainbridge B, Darveau RP, Hackett M, Miller SI (1997). Regulation of lipid A modifications by *Salmonella typhimurium* virulence genes phoP-phoQ. *Science*, **276**, 250-253.

Halligan DL, Keightley PD (2009). Spontaneous mutation accumulation studies in evolutionary genetics. *Annual Review of Ecology, Evolution and Systematics*, **40**, 151-172.

Heckel DG (2014). Insect detoxification and sequestration strategies. *Annual Plant Reviews*, **47**, 77-114.

Heil M (2014). Herbivore-induced plant volatiles: targets, perception and unanswered questions. *New Phytologist*, **204**, 297-306.

Helle W, Sabelis MW (1985). *Spider Mites. Their biology, natural enemies and control*. Elsevier Science Publishers, Amsterdam, Netherlands.

Helmus MR, Dussourd DE (2005). Glues or poisons: which triggers vein cutting by monarch caterpillars? *Chemoecology*, **49**, 45-49.

Hornef MW, Wick MJ, Rhen M, Normark S (2002). Bacterial strategies for overcoming host innate and adaptive immune responses. *Nature Immunology*, **3**, 1033-1040.

Hountondji FCC, Sabelis MW, Hanna R, Janssen A (2005). Herbivore-induced plant volatiles trigger sporulation in entomopathogenic fungi: the case of *Neozygites tanajoae* infecting the cassava green mite. *Journal of Chemical Ecology*, **31**, 1003-1021.

Inbar M, Wink M, Wool D (2004). The evolution of host plant manipulation by insects: molecular and ecological evidence from gall-forming aphids on *Pistacia*. *Molecular Phylogenetics and Evolution*, **32**, 504-511.

Jonckheere W, Dermauw W, Zhurov V, Wybouw N, Van den Bulcke J, Villarroel CA, Greenhalgh R, Grbić M, Schuurink RC, Tirry L, Baggerman G, Clark RM, Kant MR, Vanholme B, Menschaert G, Van Leeuwen T (2016). The salivary protein repertoire of the polyphagous spider mite *Tetranychus urticae*: a quest for effectors. *Molecular and Cellular Proteomics*, **15,** 3594-3613.

Kadioglu A, Terzi R, Saruhan N, Saglam A (2012). Current advances in the investigation of leaf rolling caused by biotic and abiotic stress factors. *Plant Science*, **182**, 42-48.

Kamiya T, Mideo N, Alizon S (2018). Coevolution of virulence and immunosuppression in multiple infections. *Journal of Evolutionary Biology*, **31**, 995-1005.

Kant MR, Ament K, Sabelis MW, Haring MA, Schuurink RC (2004). Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiology*, **135,** 483-495.

Kant MR, Jonckheere W, Knegt B, Lemos F, Liu J, Schimmel BCJ, Villarroel CA, Ataide LMS, Dermauw W, Glas JJ, Egas M, Janssen A, Van Leeuwen T, Schuurink RC, Sabelis MW, Alba JM (2015). Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany*, **115**, 1015-1051.

Kant MR, Sabelis MW, Haring MA, Schuurink RC (2008). Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proceedings of the Royal Society B*, **275,** 443-452.

Karban R, Agrawal AA (2002). Herbivore offense. *Annual Review of Ecology and Systematics*, **33**, 641-664.

Karban R, Baldwin IT (1997). *Induced Responses to Herbivory*. Chicago University Press, Chicago, USA.

Kennedy N, Gray RD (1993). Can ecological theory predict the distribution of foraging animals? A critical analysis of experiments on the ideal free distribution. *Oikos*, **68**, 158-166.

Krieger RI, Feeny PP, Wilkinson CF (1971). Detoxication enzymes in the guts of caterpillars: an evolutionary answer to plant defenses? *Science*, **172**, 579-581.

Li X, Schuler MA, Berenbaum MR (2002b). Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature*, **419**, 712–715.

Li C, Williams MM, Loh Y, Lee GI, Howe GA (2002a). Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. *Plant Physiology*, **130,** 494-503.

Liu J, Legarrea S, Kant MR (2017). Tomato reproductive success is equally affected by herbivores that induce or that suppress defenses. *Frontiers in Plant Science*, **8**, 1-12.

Losey JE, Denno RF (1998). Positive predator-predator interactions: enhanced predation rates and synergistic suppression of aphid populations. *Ecology*, **79**, 2143-2152.

Maizels RM, McSorley HJ (2016). Regulation of the host immune system by helminth parasites. *Journal of Allergy and Clinical Immunology*, **138**, 666-675.

Marcos CM, de Oliveira HC, de Melo WCMA, da Silva JF, Assato PA, Scorzoni L, Rossi SA, de Paula e Silva ACA, Mendes-Giannini MJS, Fusco-Almeida AM (2016). Anti-immune strategies of pathogenic fungi. *Frontiers in Cellular and Infection Microbiology*, **6**, 1-22.

Martel C, Zhurov V, Navarro M, Martinez M, Cazaux M, Auger P, Migeon A, Santamaria ME, Wybouw N, Diaz I, Van Leeuwen T, Navajas M, Grbić M, Grbić V (2015). Tomato whole genome transcriptional response to *Tetranychus urticae* identifies divergence of spider mite-induced responses between Tomato and Arabidopsis. *Molecular Plant-Microbe Interactions*, **28**, 343-361.

Metz JAJ, Gyllenberg M (2001). How should we define fitness in structured metapopulation models? Including an application to the calculation of evolutionarily stable dispersal strategies. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 499-508.

Mideo N (2009). Parasite adaptations to within-host competition. *Trends in Parasitology*, **25**, 261-268.

Migeon A, Dorkeld F (2018). Spider Mites Web: a comprehensive database for the Tetranychidae. http://www.montpellier.inra.fr/CBGP/spmweb.

Migeon A, Ferragut F, Escudero-Colomar LA, Fiaboe K, Knapp M, de Moraes GJ, Ueckermann E, Navajas M (2009). Modelling the potential distribution of the invasive tomato red spider mite, *Tetranychus evansi* (Acari: Tetranychidae). *Experimental and Applied Acarology*, **48**, 199-212.

Mithöfer A, Boland W (2008). Recognition of herbivory-associated molecular patterns. *Plant Physiology*, **146**, 825-831.

Murungi LK, Salifu D, Masinde P, Wesonga J, Nyende A, Knapp M (2014). Effects of the invasive tomato red spider mite (Acari: Tetranychidae) on growth and leaf yield of African nightshades. *Crop Protection*, **59**, 57-62.

Musser RO, Cipollini DF, Hum-Musser SM, Williams SA, Brown JK, Felton GW (2005). Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. *Archives of Insect Biochemistry and Physiology*, **58**, 128-137.

Musser RO, Hum-Musser SM, Eichenseer H, Pfeiffer M, Ervin G, Murphy JB, Felton GW (2002). Caterpillar saliva beats plant defences. *Nature*, **416**, 599-600.

Navajas M, de Moraes GJ, Auger P, Migeon A (2013). Review of the invasion of *Tetranychus evansi*: biology, colonization pathways, potential expansion and prospects for biological control. *Experimental and Applied Acarology*, **59,** 43-65.

Price PA, Jin J, Goldman WE (2012). Pulmonary infection by *Yersinia pestis* rapidly establishes a permissive environment for microbial proliferation. *Proceedings of the National Academy of Sciences USA*, **109**, 3083-3088.

Price PW (2005). Adaptive radiation of gall-inducing insects. *Basic and Applied Ecology*, **6**, 413-421.

Price PW, Fernandes GW, Waring GL (1987). Adaptive nature of insect galls. *Environmental Entomology*, **16**, 15-24.

Rankin DJ, Bargum K, Kokko H (2007). The tragedy of the commons in evolutionary biology. *Trends in Ecology and Evolution*, **22**, 643-651.

Reading PC, Khanna A, Smith GL (2002). Vaccinia virus CrmE encodes a soluble and cell surface tumor necrosis factor receptor that contributes to virus virulence. *Virology*, **292**, 285-298.

Ribeiro AEL, Gondim MGC jr, Calderan E, Delalibera I jr (2009) Host range of *Neozygites floridana* isolates (Zygomycetes: Entomophthorales) to spider mites. *Journal of Invertebrate Pathology*, **102**, 196-202.

Rosa AA, Gondim MGC jr, Fiaboe KKM, de Moraes GJ, Knapp M (2005). Predatory mites associated with *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae) on native solanaceous plants of coastal Pernambuco State, Brazil. *Neotropical Entomology*, **34**, 689-692.

Rundell EA, McKeithen-Mead SA, Kazmierczak BI (2016). Rampant cheating by pathogens? *PLoS Pathogens*, **12**, e1005792.

Sachs D, Sher A. (2002). Evasion of innate immunity by parasitic protozoa. *Nature Immunology*, **3**, 1041-1047.

Sagers CL (1992). Manipulation of host plant quality: herbivores keep leaves in the dark. *Functional Ecology*, **6**, 741-743.

Sanchez A, Gore J (2013). Feedback between population and evolutionary dynamics determines the fate of social microbial populations. *PLoS Biology*, **11**, e1001547.

Sarmento, R.A., Lemos, F., Bleeker, P.M., Schuurink, R.C., Pallini, A., Oliveira, M.G.A., Lima, E.R., Kant, M.R., Sabelis, M.W. & Janssen, A. (2011a) A herbivore that manipulates plant defence. *Ecology Letters*, **14**, 229-236.

Sarmento, R.A., Lemos, F., Dias, C.R., Kikuchi, W.T., Rodrigues, J.C.P., Pallini, A., Sabelis, M.W. & Janssen, A. (2011b) A herbivorous mite down-regulates plant defence and produces web to exclude competitors. *PLoS One*, **6**, e23757.

Sato, Y., Alba, J.M. & Sabelis, M.W. (2014) Testing for reproductive interference in the population dynamics of two congeneric species of herbivorous mites. *Heredity*, **113**, 495-502.

Sato, Y., Staudacher, H. & Sabelis, M.W. (2016) Why do males choose heterospecific females in the red spider mite? *Experimental and Applied Acarology*, **68**, 21-31.

Saunyama IGM, Knapp M (2003). Effect of pruning and trellising of tomatoes on red spider mite incidence and crop yield in Zimbabwe. *African Crop Science Journal*, **11**, 269-277.

Schimmel BCJ, Ataide LMS, Chafi R, Villarroel CA, Schuurink RC, Kant MR (2017a). Overcompensation of defense suppression by herbivores in response to competition. *New Phytologist*, **214,** 1688-1701.

Schimmel BCJ, Ataide LMS, Kant MR (2017b). Spatiotemporal heterogeneity of tomato induced defense responses affects spider mite performance and behavior. *Plant Signaling & Behavior*, **12**, e1370526.

Schmid-Hempel P (2005). Natural insect host-parasite systems show immune priming and specificity: puzzles to be solved. *BioEssays*, **27**, 1026-1034.

Schmid-Hempel P (2008). Parasite immune evasion: a momentous molecular war. *Trends in Ecology and Evolution*, **23**, 318-326.

Schmidt O, Theopold U, Strand M (2001). Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. *BioEssays*, **23**, 344-351.

Schrader F (1923). Haploidie bei einer Spinnmilbe. *Archiv für Mikroskopische Anatomie*, **97**, 610-622.

Schultz JC (1988). Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology*, **69**, 896-897.

Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jerzy R, Dower SK, Cosman D, Goodwin RG (1990). A receptor for tumor necrosis-factor defines an unusual family of cellular and viral proteins. *Science*, **248**, 1019-1023.

Smith J (1955). Detoxification mechanisms in insects. *Biological Reviews*, **30**, 455-475.

Soler R, Badenes-Pérez FR, Broekgaarden C, Zheng S-J, David A, Boland W, Dicke M (2012). Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. *Functional Ecology*, **26**, 156-166.

Stahl E, Hilfiker O, Reymond P (2018). Plant–arthropod interactions: who is the winner? *The Plant Journal*, **93**, 703-728.

Stone GN, Schönrogge K (2003). The adaptive significance of insect gall morphology. *Trends in Ecology and Evolution*, **18**, 512-522.

Thurston R, Webster JA (1962). Toxicity of *Nicotiana gossei* domin to *Myzus persicae* (Sulzer). *Entomologia Experimentalis et Applicata*, **5**, 233-238.

Villarroel CA, Jonckheere W, Alba JM, Glas JJ, Dermauw W, Haring MA, Van Leeuwen T, Schuurink RC, Kant MR (2016). Salivary proteins of spider mites suppress defences in *Nicotiana benthamiana* and promote mite reproduction. *The Plant Journal*, **86,** 119-131.

Vossen MTM, Westerhout EM, Söderberg-Nauclér C, Wiertz EJHJ (2002). Viral immune evasion: a masterpiece of evolution. *Immunogenetics*, **54**, 527-542.

Walling L (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, **19**, 195-216.

Way MJ, Murdie G (1965). An example of varietal variations in resistance of Brussels sprouts. *Annals of Applied Biology*, **56**, 326-328.

Yang J-Y, Iwasaki M, Machida C, Machida Y, Zhou X, Chua N-H (2008). βCl, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. *Genes and Development*, **22**, 2564-2577.

Zalucki MP, Brower LP (1992). Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: Danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). *Chemoecology*, **3**, 81-93.

Zhang P-J, Broekgaarden C, Zheng S-J, Snoeren TAL, van Loon JJA, Gols R, Dicke M (2013). Jasmonate and ethylene signaling mediate whitefly-induced interference with indirect plant defense in *Arabidopsis thaliana*. *New Phytologist*, **197**, 1291–1299.

Zhang P-J, Zheng S-J, van Loon JJA, Boland W, David A, Mumm R, Dicke M (2009). Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *Proceedings of the National Academy of Sciences USA*, **106**, 21202-21207.

Zhu-Salzman K, Luthe DS, Felton GW (2008). Arthropod-inducible proteins: broad spectrum defenses against multiple herbivores. *Plant Physiology*, **146**, 852-858.

2

Meta-analysis reveals intraspecific variation for plant-mediated interactions among herbivores

Bram Knegt, Juan M. Alba, Livia M.S. Ataide, Tomaz A. Barbosa, Ana M.G. Bernardo, Rachid Chafi, Cleide R. Dias, Marcus V.A. Duarte, Diogo P. Godinho, Arne Janssen, Merijn R. Kant, Felipe Lemos, Dan Li, Jussara Mencalha, Elisa F. de Oliveira; Maria G.A. Oliveira, Angelo Pallini, Fabrício R. Ribeiro, Bernardus C.J. Schimmel, Pauliana A. da Silva, Neilier R. da Silva Júnior, Manuel Solís-Vargas, E. Toby Kiers & Martijn Egas

Abstract

Theory predicts that plant-mediated interactions among herbivores drive diffuse coevolution of plants and herbivores. A coevolutionary response in herbivores requires intraspecific variation for induction or suppression of plant responses that affect other community members. However, research on the extent of such variation in plant-herbivore systems is lacking. We investigated variation in plant-mediated interactions between two species of herbivorous spider mites on tomato plants, through a meta-analysis of 134 published and unpublished datasets. The two spider mite species differ profoundly in the way they interact with their tomato host: whereas generalist *Tetranychus urticae* induces plant defence responses that hamper its performance, specialist *Tetranychus evansi* suppresses these, thereby maintaining a high performance. Considering that both species can occur on the same host, we asked (i) to what extent modulation of plant defence responses by spider mites affect spider mite performance, (ii) how variable these effects are, and (iii) what factors affect this variation. On average, feeding by *T. evansi* enhanced the performance of conspecific and heterospecific mites, whereas feeding by *T. urticae* decreased it. These plant-mediated interactions varied largely among studies and 18-29% of the variation was explained by the length of time that populations had been cultured in lab environments. Longer lab culturing produced weaker interactions. We found no significant effect of several characteristics of the experimental procedure, such as the proportion of leaves that were infested or the duration of fecundity assays, nor whether performance of *T. evansi* or *T. urticae* was measured. Our results demonstrate that plant-mediated interactions affect spider mite fitness, and that *T. urticae* and *T. evansi* harbour intraspecific variation for these interactions. This variation enables selection for phenotypic changes over generations of lab culturing, and suggests that spider mites have the potential to adapt to plant-mediated ecological interactions.

Introduction

Plants are often attacked by multiple herbivore species. Each herbivore species causes plants to respond, characterized by changes in plant morphology and physiology (Chester 1933, Karban & Baldwin 1997). For example, some plants develop more trichomes after herbivore infestation (Agrawal 1998), whereas others adjust their nutrient content (Cook et al. 1978) or produce anti-nutritive compounds (Green & Ryan 1972). These changes play a large role in the interactions between plants and herbivores (Kant et al. 2015), but are also relevant for other community members, such as predators and parasitoids of herbivores (Faeth 1986), or other herbivores that feed on the same plant. Root feeding by scarabaeid beetle larvae, for example, can induce a change in aboveground nitrogen content in their hosts that benefits phloem-feeding aphids (Gange & Brown 1989). Such indirect, plant-mediated interactions among herbivores (Benz 1974, Denno & Kaplan 2007) play a role in competition among herbivores (Kaplan & Denno 2007), and set in motion a cascade of effects with important consequences for community dynamics (Ohgushi 2005, Kessler & Halitschke 2007, Poelman et al. 2008, Stam et al. 2014).

Herbivore-induced plant responses and associated changes in community dynamics also play an important role in evolutionary processes, in particular in diffuse (co)evolution and eco-evolutionary dynamics. In diffuse coevolution, reciprocal selection between two species is shaped by the presence of a third (Janzen 1980, Fox 1981, Strauss et al. 2005). Plant-mediated interactions among herbivores may drive diffuse coevolution, because herbivores can affect reciprocal selection between plants and other herbivores by inducing a change in plant traits (Inouye & Stinchcombe 2001, Strauss & Irwin 2004). Indeed, Wise & Rausher (2013) demonstrated diffuse evolution of plant resistance to herbivory, such that the presence of multiple herbivores constrained the evolution of increased plant resistance against specific species. Additionally, recent reviews suggest that induced plant responses make plant-herbivore communities suitable study systems for eco-evolutionary dynamics (Utsumi 2011, Ohgushi 2016). Eco-evolutionary dynamics describe the evolutionary response of a community member to ecological interactions and its concurrent feedback on community properties (Schoener 2011). Plant-mediated herbivore interactions can impose selection on induced trait change in plants, because they can affect plant fitness (Agrawal 1998) when the induced trait change in plants is heritable (Zangerl & Berenbaum 1990). In turn, evolutionary change in induced plant response will affect plant-mediated interactions among herbivores, because their strength depends on the degree of phenotypic plasticity in the plant (Ohgushi 2005). Plant-mediated herbivore interactions thus form an eco-evolutionary feedback loop with plant phenotypic plasticity at its core (Ohgushi 2016).

Although the above considerations indicate that plant-mediated herbivore interactions may shape the evolution of plant phenotypic plasticity, few studies have investigated evolutionary responses to indirect, plant-mediated interactions in herbivores. An evo-

lutionary response in herbivores requires heritable intraspecific variation for their induction of plant defence or for their susceptibility to it. Such variation could arise through differential selection among herbivore populations in different environments such as different host plants (Gould 1979, Siepielski et al. 2013), through mutation, or through fluctuating selection within populations (Kalisz 1986, Siepielski et al. 2009). For example, the evolution of feeding preference in leaf beetles varied depending on the degree to which a community of other herbivores induced leaf regrowth in willows (Utsumi et al. 2013). Subsequently, the leaf beetle genotypes with different feeding preference induced different responses in their willow hosts which affected community composition (Utsumi 2015). More generally, numerous studies have demonstrated genetic variation for ecologically relevant traits in herbivores (reviewed by Via 1990, Gloss et al. 2016), and the relevance of plant-mediated interactions for herbivore fitness is supported by a substantial amount of evidence from various systems (reviewed by Denno & Kaplan 2007, Kant et al. 2015). Yet, there is limited experimental evidence for intraspecific variation in induction of plant responses and its effects on other herbivores. Notably, intraspecific variation in pairwise plant-herbivore interactions cannot be extrapolated to tripartite plant-mediated herbivore interactions, because this variation may or may not be relevant, depending on how other herbivores respond to differences in plant induction, on what response these other herbivores induce in a plant themselves, and on interactive effects on plant defence (Strauss et al. 2005).

Given the importance of intraspecific variation in plant-mediated herbivore interactions for diffuse coevolution and eco-evolutionary dynamics, and the paucity of experimental evidence, the purpose of the present study was to quantify such variation in one well-studied plant-herbivore system. Using meta-analysis, we investigated plant-mediated interactions between two spider mite species, *T. urticae* and *T. evansi*, on tomato plants (*Solanum lycopersicum*). Meta-analysis has been used to compare plant-mediated herbivore interactions across systems (Denno et al. 1995, Nykänen & Koricheva 2004, Leimu & Koricheva 2006, Kaplan & Denno 2007, Johnson et al. 2012), but here we synthesize studies investigating interactions between the same three species only. We chose plant-mediated interactions between these spider mites on tomato as our study system for three reasons. First, the two spider mite species differ profoundly in the way they interact with their tomato host: whereas *T. urticae* induces plant defence responses that hamper its performance (Li et al. 2002, 2004; Kant et al. 2008), *T. evansi* suppresses these, thereby maintaining a high performance (Sarmento et al. 2011a, Alba et al. 2015). This allowed us to assess if intraspecific variation differs among species with negative or positive plant-mediated effects on herbivore performance. Second, experimental evidence suggests that intraspecific variation for plant-mediated interactions is present in these species, because Kant et al. (2008) and Alba et al. (2015) found that different genotypes of *T. urticae* and *T. evansi* induced different responses in tomato plants, with conse-

quences for the performance of other *T. urticae* mites. Additionally, Kant et al. (2008) showed that *T. urticae* strains differ in their susceptibility to herbivore-induced tomato responses. Third, a substantial number of studies investigating plant-mediated interactions among spider mites on tomato is available, enabling meta-analysis as a tool for assessments of intraspecific variation.

We performed three meta-analyses, comparing spider mite performance assessed through oviposition rates (Sabelis 1991) on (i) *T. evansi*-infested tomato plants vs. uninfested plants, (ii) *T. urticae*-infested plants vs. uninfested plants, and (iii) *T. evansi*-infested plants vs. *T. urticae*-infested plants. Specifically, we ask to what extent spider mite-induced plant responses affect performance of both species, how variable these effects are, and what factors contribute to this variation. Our aim is to provide a detailed assessment of variation in plant-mediated interactions among herbivores, and assess the role of intraspecific variation in these interactions.

Materials and methods

Study system

Spider mites (Acari: Tetranychidae) are small (<0.5 mm) herbivorous arthropods that pierce plant parenchyma cells and feed on their contents (Bensoussan et al. 2016). The two-spotted spider mite *T. urticae* is a generalist herbivore reported on >1100 plant species (Migeon & Dorkeld 2017), and has a cosmopolitan distribution. In contrast, the tomato red spider mite *T. evansi* is considered a specialist on solanaceous plants such as tomato, originates from South America, and has recently become invasive in many areas with a Mediterranean climate (Navajas et al. 2013). In invaded areas, *T. evansi* has displaced native tetranychid mites, including *T. urticae* (Ferragut et al. 2013, Azandémè-Hounmalon et al. 2015).

Data collection and inclusion criteria

We searched for published literature using the queries '*Tetranychus evansi*' [AND] 'tomato' or '*Tetranychus urticae*' [AND] 'tomato' in the electronic database *Web of Science*, resulting in 273 papers (accessed May 26, 2017). We also collected unpublished datasets from colleagues. Datasets (published and unpublished) were only included if they met the following criteria:

(1) The experiment included a treatment where intact tomato plants (*S. lycopersicum*) of any variety were infested with defence-suppressing *T. evansi* spider mites, and a treatment where plants were infested with defence-inducing *T. urticae* spider mites.
(2) The experiment included measurements of *T. evansi* and/or *T. urticae* oviposition rates on these infested plants.
(3) The treatments and oviposition rate measurements were performed using the same leaflet(s), but were separated in space or time.

Applying these criteria resulted in the inclusion of seven published studies in this meta-analysis: Sarmento et al. (2011a, b), Alba et al. (2015), de Oliveira et al. (2016), Godinho et al. (2016) and Schimmel et al. (2017a, b), and unpublished studies from nine groups of authors. Most studies provided more than one dataset. The final collection consisted of 134 datasets (TABLE S2.1).

Experimental procedure of the included studies

The experimental procedure of most included studies adhered to the following design. One or several leaflets of tomato plants with 3-6 fully expanded leaves were infested with *T. urticae* or *T. evansi* for 1-7 days (hereafter 'treatments'). Subsequently, the infested leaflets were cleaned with a fine brush, removing all mites, eggs and web, and the damaged leaf area was subsequently reinfested with adult *T. evansi* or *T. urticae* females of standardized age (hereafter 'secondary infestation'), of which oviposition rate was measured over 2-5 days. Some studies followed a different design, in which treatments and oviposition rate measurements were conducted simultaneously by confining the treatment mites and the mites of which oviposition rate was measured to separate areas on the leaflet. This difference in procedure is accounted for in the analysis by including separation method as a covariate.

Effect size calculation

For all datasets, we obtained either the raw data on oviposition rates per female or a summary specifying the mean, standard deviation and sample size per treatment. When multiple females oviposited on the same plant, we treated the plant as the biological unit and averaged their oviposition rates into one value per plant. We used Hedges' g as the effect size metric (TABLE 2.1), which is a standardized mean difference between treatments corrected for small sample bias (Hedges 1981).

Many datasets contained dependency structures, complicating the computation of effect sizes. For example, some experiments were performed in blocks in time, and others compared several experimental treatments to the same control treatment. We accounted for such complex data structures following the recommendations of Borenstein et al. (2009, pp. 214-245), explained in SUPPLEMENTARY NOTE S1.

Covariates

To identify potential sources of variation in effect size, we listed the following covariates for each dataset:

[A] Research group characteristics:

(1) Research group: the research group in which the experiments were conducted. Categorical variable, levels: University of Amsterdam, Federal University of Viçosa, University of Lisbon.

TABLE 2.1. Effect size calculation.

Comparison	Abbreviation used in figures	Formula for effect size
T. evansi-infested plants vs. uninfested control plants	*T. evansi* – control	Effect size = mean oviposition rate on *T. evansi*-infested plants – mean oviposition rate on uninfested control plants
T. urticae-infested plants vs. uninfested control plants	*T. urticae* – control	Effect size = mean oviposition rate on *T. urticae*-infested plants – mean oviposition rate on uninfested control plants
T. evansi-infested plants vs. *T. urticae*-infested plants	*T. evansi* – *T. urticae*	Effect size = mean oviposition rate on *T. evansi*-infested plants – mean oviposition rate on *T. urticae*-infested plants

(2) First author: the first author of the publication, or, in the case of unpublished data, the experimenter that organized the data collection. Categorical variable, levels: RA Sarmento, EF de Oliveira, DP Godinho, JM Alba, CR Dias, D Li, LMS Ataíde, B Knegt, BCJ Schimmel, LMS Ataíde, M Solís-Vargas, F Lemos, FR Ribeiro.

[B] Characteristics of the experimental procedure:

(3) Environment: the type of environment in which the experiment was conducted. Categorical variable, levels: greenhouse, climate room.

(4) Proportion leaves infested: the number of treated leaves per plant divided by the total number of fully expanded leaves. Numerical variable, range: 0.22-1.

(5) Infestation level: the estimated infestation level by mites during treatments. This variable is calculated as the density of mites per leaflet multiplied by the duration of the treatment in days. Numerical variable, range: 6-480 mite days.

(6) Separation method: the method by which the mites that were used during treatments were separated from the mites of which the oviposition rate was measured. Categorical variable, levels: in time, in space, in time and space.

(7) Arena: in some experiments damaged leaf tissue was cut from the treated leaflets (e.g., leaf discs) and placed on wet cotton wool before the start of oviposition rate measurements, but in others the plant was kept intact. This variable specifies the status of the leaflet during the oviposition rate measurements. Categorical variable, levels: intact, excised.

(8) Number of females tested: the number of adult females that were placed together on the same leaflet for oviposition rate measurements. Numerical variable, range: 1-25 adult females.

(9) Oviposition duration: the number of days over which oviposition rates were measured. Numerical variable, range: 2-5 days.

[C] Characteristics of plants and mites:
(10) Plant variety: the variety of cultivated tomato used. Categorical variable, levels: Castlemart, Santa Clara, Aguamiel, Moneymaker, UC32.
(11) *Tetranychus evansi* population: the population of *T. evansi* that was used for treatments and/or oviposition rate measurements. Categorical variable, levels: 'Viçosa-1' (Sarmento et al. 2011a), 'Algarrobo-1' (Alba et al. 2015).
(12) *Tetranychus urticae* population: the population of *T. urticae* that was used for treatments and/or oviposition rate measurements. Categorical variable, levels: 'Brazil' (Sarmento et al. 2011a), 'Santpoort-2' (Alba et al. 2015), '*Ricinus communis*' (Godinho et al. 2016).
(13) *Tetranychus evansi* host variety: the variety of cultivated tomato on which *T. evansi* was reared. Categorical variable, levels: Castlemart, Santa Clara, Agua Miel, Moneymaker.
(14) *Tetranychus urticae* host species: the host plant species on which *T. urticae* was reared. Categorical variable, levels: *Solanum lycopersicum*, *Phaseolus vulgaris*.
(15) Time in culture (*T. evansi*): the duration that the *T. evansi* population had been cultured in the lab. We expressed this variable in estimated number of generations, assuming a generation time of 14 days at 25 °C on tomato (Bonato 1999). Numerical variable, range: 2-363 generations.
(16) Time in culture (*T. urticae*): the duration that the *T. urticae* population had been cultured in the lab. We expressed this variable in estimated number of generations, assuming a generation time of 14 days at 25 °C on bean (Watson 1964) and tomato (Egas & Sabelis 2001). Numerical variable, range: 54-389 generations.
(17) Species of secondary infestation: the species used for oviposition rate measurements. Categorical variable, levels: *T. evansi*, *T. urticae*.

The leaf area (cm^2) on which oviposition rate measurements were performed could not be included as a covariate, because this was not standardized in many datasets. The age of the leaflets on which the experiments were performed was also not included, because not enough datasets reported on which leaves the experiments were performed. The females used for oviposition rate measurements came from age-standardized cohorts, such that they were 2-10 days old. At this age both spider mite species have a constant oviposition rate (Watson 1964, Bonato 1999), and the age of females used for oviposition rate measurements was therefore not included.

Collinearity analysis

If values of one covariate correlate with values in another covariate, the variables are collinear and their influence on the effect size cannot be assessed independently. Collinearity also confounds statistical tests, which can be avoided by removing collinear variables from a statistical model. Removed variables are not meaningless, but the effect

of a remaining variable can be attributed to any variable in the set of variables that are collinear. We assessed collinearity among covariates using a procedure explained in SUPPLEMENTARY NOTE S2. Briefly, we calculated pairwise correlations among all variables, as well as variance inflation factors to investigate multicollinearity, and considered variables to be collinear when their pairwise correlation was larger than 0.5 or when they had a variance inflation factor larger than 2 (Zuur et al. 2010). When we found a pair of variables to be collinear, we removed the variable that explained variation in the dependent variable the least, i.e., with the lowest statistical power, from our model.

Model selection

After the collinearity analysis, we removed covariates with a non-significant effect from the final model with a backward model simplification procedure explained in SUPPLEMENTARY NOTE S3. We did not include interaction effects among covariates because the number of datasets was too small to properly investigate interactions.

Pooling of datasets to avoid pseudoreplication

Most studies provided more than one dataset. These datasets often differed by only one or a few of the covariates. For example, datasets 06-09 are all part of the publication by de Oliveira et al. (2016), and the only difference between them is the infestation level inflicted during treatments (TABLE S2.1). However, this covariate was removed from the statistical models due to collinearity (TABLE 2.2), rendering datasets 06-09 identical for all remaining covariates. Because these datasets were collected at the same time and by the same experimenters, retaining each dataset as independent would incur pseudoreplication. After removing a covariate from the statistical model due to collinearity or non-significance, we therefore pooled datasets that became identical by all remaining covariates. We only pooled datasets from the same study.

The initial, complete collection of datasets with their effect size, variance, sample size and covariates is given in TABLE S2.1. For each meta-analysis, the final set of covariates as well as the variables that were excluded, is given in TABLE 2.2. The final effect sizes, variances, sample sizes and covariates, after datasets were pooled, are given in TABLES S2.2-S2.4.

Meta-analysis

Meta-analysis can be used to calculate the weighted average effect size across studies, and also to assess the influence of covariates. The weighted average effect size is called the 'summary effect size', and is estimated with random-effects meta-analyses, assuming random differences in effect size among studies without including any covariates. The summary effect size is assumed to be normally distributed, and shows the difference between the effects of treatments on oviposition rate across all datasets. Because Hedges' g values are only informative on a relative scale, we facilitated their interpreta-

TABLE 2.2. Sets of collinear variables, their variance inflation factors, and if they were included in the final model. Pairwise correlations are given between brackets, and indicate the pairwise correlation between two variables at the time that one of them was removed from the model. Correlations may have varied during the collinearity analysis, because it was conducted in a step-by-step procedure that also involved pooling of datasets (see SUPPLEMENTARY NOTE S2 for a description of the stepwise removal of collinear variables). VIF = variance inflation factor, NA = not applicable.

Main variable[1]	Collinear variables (correlation[2])	VIF	Included in final model
***T. evansi* - control**			
Research group	Environment (0.76) - First author (0.56) First author - Plant variety (0.73), Proportion leaves infested (0.58) Plant variety - *T. evansi* host variety (1) Proportion leaves infested – Infestation level (0.63) Arena (0.68) - Separation method (0.84) Time in culture (*T. urticae*) (1) - *T. urticae* host species (1), *T. urticae* population (1)	NA	no (p = 0.196)
Species of secondary infestation	Oviposition duration (0.77) Number of females tested (0.52)	NA	no (p = 0.173)
Time in culture (*T. evansi*)		1.11	yes (p = 0.031)
***T. urticae* - control**			
Species of secondary infestation[3]	Oviposition duration (0.79) Number of females tested (0.55)	NA	no (p = 0.614)
Arena[3]	Separation method (0.79)	NA	no (p = 0.525)
Proportion leaves infested[3]	Infestation level (0.62) First author (0.61) - Plant variety (0.75) Plant variety - *T. evansi* host variety (0.79)	1.08	no (p = 0.283)
Time in culture (*T. urticae*)	Time in culture (*T. evansi*) (0.86)	1.09	yes (p = 0.013)
Environment	*T. urticae* host species (0.87) - *T. urticae* population (0.99) Research group (0.68)	NA	yes (p = 0.012)
T. evansi* - *T. urticae			
Species of secondary infestation	*T. evansi* population (0.85) Arena (0.66) - Separation method (0.90), *T. urticae* population (0.74), *T. evansi* host variety (0.70) *T. urticae* population - *T. urticae* host species (0.96), Environment (0.80) *T. evansi* lab host variety - Plant variety (0.73)	NA	no (p = 0.997)

TABLE 2.2. Continued

Main variable[1]	Collinear variables (correlation[2])	VIF	Included in final model
[*T. evansi* - *T. urticae*]			
Proportion leaves infested	Infestation level (0.48[4]) First author (0.50)	1.39	no (p = 0.808)
Number of females tested		1.23	no (p = 0.216)
Oviposition duration		1.31	no (p = 0.113)
Research group		NA	no (p = 0.138)
Time in culture (*T. urticae*)	Time in culture (*T. evansi*) (0.52)	1.47	yes (p = 0.023)

[1] The main variable is the variable of a collinear set with the highest statistical power. This variable was included in the backward model selection procedure as the representative of its collinear set.

[2] Depending on variable type (categorical or numerical), correlations represent polychoric, polyserial or Pearson correlations as calculated by the *polycor* package (Fox 2010).

[3] Even though the power to detect a minimally substantial effect was below the 80% threshold (TABLE 2.3), the non-significant sets of collinear variables were not included in the final model, because their p-values were larger than the significance level required to meet 80% power ($\alpha = 0.103$).

[4] Although this pairwise correlation was below the 0.5 threshold, the three variables in this set caused strong multicollinearity ($3.25 < VIF < 92.65$) and were therefore compiled into one collinear set.

tion by converting the estimated summary effects to Cohen's d values (see SUPPLEMENTARY NOTE S2). Because the majority (92%) of oviposition rate measurements were performed with *T. evansi* mites, these Cohen's d values can be interpreted on the scale of *T. evansi* oviposition rates. Gotoh et al. (2010) showed that adult *T. evansi* females lay on average 47.1 (sd = 6.94) eggs at 25 °C in the first five days of their oviposition period, which are the age and approximate temperature used for oviposition rate measurements in most included studies and most relevant for fitness (Sabelis 1991). Therefore, we multiplied the Cohen's d values with the standard deviation of the oviposition rate of *T. evansi* during the first five days of their oviposition period, obtaining an estimate of the summary effect size on an interpretable scale.

In accordance with the recommendations of Senior et al. (2016), we quantified the variation in effect size relative to the mean by calculating the standard deviation. In addition, the I^2 statistic (Higgins 2003) estimates which part of the observed variation in effect size can be attributed to differences among studies, rather than within-study sampling error, and thus gives an indication of the proportion of variation that can be explained by covariates. We confirmed the validity of the normality assumption for the summary effect size by performing approximate permutation tests (1000 iterations), and considered the assumption valid if the average permuted summary effect size and standard deviation were identical by four decimals to the parameters estimated by the meta-analytic model.

To assess the influence of covariates on effect size, we performed mixed-effects meta-analyses, assuming that differences in effect size are linearly affected by the selected set of covariates plus random variation among studies. In all cases, the among-study variance was estimated using restricted maximum likelihood estimation, coefficient estimates were adjusted with the Knapp & Hartung method, and calculations were performed using R package *metafor* (Viechtbauer 2010).

Influential case diagnostics

To investigate the sensitivity of our results to small changes in the dataset, we tried to identify influential datasets in both the random-effects meta-analyses that calculated the summary effects, as well as in the mixed-effects meta-analyses that assessed the influence of covariates. In meta-analysis, influential case diagnostics specify the change in model predictions after excluding a dataset from the analysis. Specifically, we calculated DFFITS values (Belsley et al. 1980), Cook's distances (Cook & Weisberg 1982), hat values (Viechtbauer & Cheung 2010) and DFBETAS (Belsley et al. 1980) for each dataset, and considered a dataset influential if any of these measures exceeded its cut-off as specified in the description of the *metafor* package (Viechtbauer 2010). We indicate influential datasets in figures, and report the change in model predictions following their exclusion.

Bias analysis

An integral part of meta-analysis is the investigation of bias (Borenstein et al. 2009, pp. 271-292). A meta-analysis can be considered 'biased' if the included studies differ systematically from all studies that should have been included based on the inclusion criteria but were left out due to an imperfect literature search (Borenstein et al. 2009, p. 291). Several processes can produce a biased collection of datasets, such as publication bias, language bias, citation bias and database bias (Egger & Smith 1998). We assessed if the effect sizes reported by the datasets included in our meta-analyses were biased by a procedure explained in SUPPLEMENTARY NOTE S4. All calculations were performed using R v.3.2.4 (R Core Team 2016).

Results

Summary effects

Oviposition rates of both *T. urticae* and *T. evansi* were higher on plants previously infested by *T. evansi* than on uninfested control plants, with an estimated summary effect size of 0.655 Hedges' g, which was significantly different from zero (FIGURE 2.1a, TABLE 2.3). Translated to the oviposition rates measured by Gotoh et al. (2010), this value would correspond to a difference in oviposition rate of 4.64 ($\pm$3.93 CI_{95}) eggs per 5 days. Oviposition rates on plants previously infested by *T. urticae* were lower than on uninfested control plants, with an estimated summary effect size of -0.614 Hedges' g (FIGURE 2.1b, TABLE 2.3), which corresponds to a difference in oviposition rate of 4.35 ($\pm$3.50 CI_{95}) eggs per 5 days. Overall, experiments in which these two treatments were compared directly confirm these results: oviposition rates were higher on plants previously infested by *T. evansi* than by *T. urticae*, with an estimated summary effect size of 0.781 Hedges' g (FIGURE 2.1c, TABLE 2.3), corresponding to a difference in oviposition rate of 5.52 ($\pm$3.47 CI_{95}) eggs per 5 days.

Variation in effect size and effects of covariates

All three comparisons revealed a large and significant amount of variation in effect size among studies (TABLE 2.3). This contributed for ~90% to the total variability in effect size, which shows that variation in effect size was mostly caused by differences among studies, and not by sampling error within studies. Covariates potentially explain some of this variation. Power analyses indicated that two of the three meta-analyses were sufficiently powered (power > 80%) to detect a minimally substantial effect in any of the sets of covariates selected after the collinearity analyses (TABLE 2.3).

After model simplification, the time that populations had been in culture significantly affected effect size in all three meta-analyses (TABLE 2.2). In comparisons between *T. evansi*-infested plants and control plants, effect sizes decreased with the time that *T. evansi* populations had been cultured ($T_{1,23}$ = -2.30, p = 0.031; FIGURE 2.2a), explaining

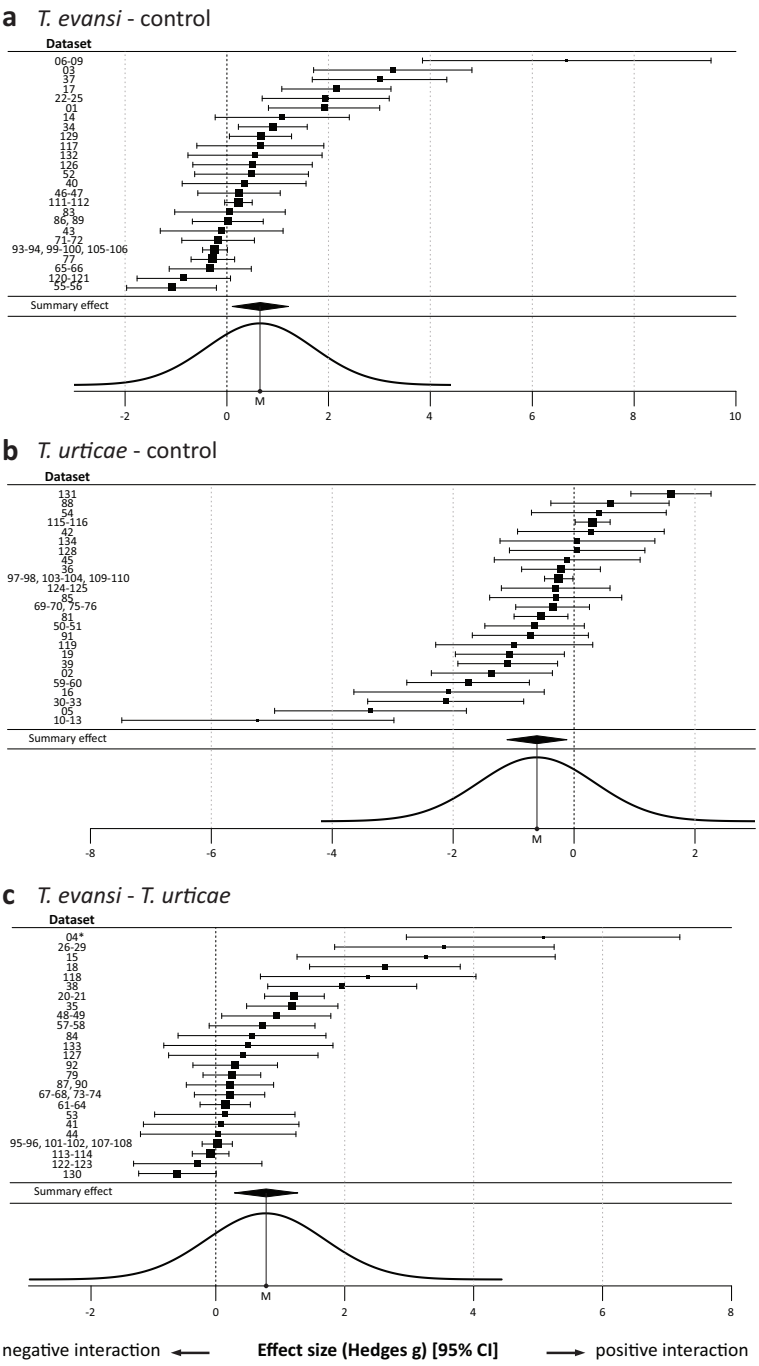

a T. evansi - control
Dataset
06-09
03
37
17
22-25
01
14
34
129
117
132
126
52
40
46-47
111-112
83
86, 89
43
71-72
93-94, 99-100, 105-106
77
65-66
120-121
55-56
Summary effect
M
-2
0
2
4
6
8
10
b T. urticae - control
Dataset
131
88
54
115-116
42
134
128
45
36
97-98, 103-104, 109-110
124-125
85
69-70, 75-76
81
50-51
91
119
19
39
02
59-60
16
30-33
05
10-13
Summary effect
M
-8
-6
-4
-2
0
2
c T. evansi - T. urticae
Dataset
04*
26-29
15
18
118
38
20-21
35
48-49
57-58
84
133
127
92
79
87, 90
67-68, 73-74
61-64
53
41
44
95-96, 101-102, 107-108
113-114
122-123
130
Summary effect
M
-2
0
2
4
6
8
negative interaction
Effect size (Hedges g) [95% CI]
positive interaction

TABLE 2.3. Summary effects, variation among studies, and power to detect effects of covariates for each meta-analysis. Confidence intervals (95%) for M, T and I^2 are given between brackets.

Statistic		*T. evansi* – control	*T. urticae* – control	*T. evansi* – *T. urticae*
Summary effect M (Hedges' g)		0.655 (± 0.55)	-0.614 (± 0.50)	0.781 (± 0.49)
Test of M ≠ 0	T (df=24) =	2.45	-2.55	3.28
	p =	0.022	0.018	0.003
Variation in effect size among studies: st. dev. T (Hedges' g)		1.050 (± 0.95)	0.960 (± 0.77)	0.925 (± 0.82)
Test of T ≠ 0	Q (df=24) =	124.27	122.00	118.85
	p =	< 0.001	< 0.001	< 0.001
Total variation (%) explained by differences among studies: I^2		89.90 (± 7.09)	87.96 (± 7.98)	89.39 (± 7.37)
Power to detect a minimally substantial effect in any of the (sets of) covariates (%)		85.71	69.45	98.00

24.49% of the heterogeneity among studies. For *T. urticae*-infested plants vs. controls, a collinear set consisting of the time in culture of *T. evansi* and *T. urticae* populations explained 28.45% of the heterogeneity among studies, and here too, effect size decreased with time in culture ($T_{1,22}$ = 2.69, p = 0.013; FIGURE 2.2b). Direct comparisons of oviposition rates on plants infested with *T. evansi* and *T. urticae* reflected these patterns: effect size also decreased with time in culture ($T_{1,23}$ = -2.44, p = 0.023; FIGURE 2.2c, 17.59% heterogeneity explained). Because directional selection towards smaller effect size in cultures would predict a concurrent decrease in effect size variance, we also assessed changes in variance over the time populations had been cultured, and found slightly decreasing but non-significant patterns (FIGURE S2.1).

In the comparison of *T. urticae*-infested plants vs. controls, effect size was also affected by the environment in which the experiments were performed (greenhouse or climate room) ($T_{1,22}$ = -2.75, p = 0.012; FIGURE S2.2), which was part of a collinear set of variables with *T. urticae* host species, *T. urticae* population and Research group. This collinear

FIGURE 2.1. Average effect sizes (and 95% CIs) of meta-analyses comparing spider mite oviposition rates on (**a**) *T. evansi*-infested plants vs. controls, (**b**) *T. urticae*-infested plants vs. controls, and (**c**) *T. evansi*-infested plants vs. *T. urticae*-infested plants. Datasets are ordered by their effect size from most negative (bottom) to most positive (top), where positive effect sizes indicate that spider mites oviposited more on the first treatment in the comparison, and negative effect sizes vice versa. The size of a square corresponds to the weight of this dataset in the meta-analysis. The estimated summary effect is represented by a diamond, with the vertical extremes indicating the summary effect size and the horizontal extremes the 95% confidence interval. The estimated distribution of effect sizes due to heterogeneity among studies is shown in the normal curve below each plot. See TABLE 2.3 for statistical details. Asterisks indicate datasets with strong influence on model predictions. Detailed information about individual datasets is given in TABLES S2.1-S2.4.

set explained 32.73% of the heterogeneity among studies. The remaining sets of covariates were not significant and therefore not included in the final model (TABLE 2.2).

Influential datasets and bias analysis

We identified datasets with a disproportionally large influence on model predictions by rerunning the meta-analytic models after removing individual datasets. No datasets were influential in the random-effects meta-analyses that calculated the summary effects, except for dataset 04 in the comparison between *T. evansi*-infested plants and *T. urticae*-infested plants (FIGURE 2.1c). Its removal would lead to an 18% reduction in the summary effect size, but only a negligible change in significance ($T_{1,23}$ = 3.26, p = 0.004). In the mixed-effects meta-analyses that assessed the effect of covariates, influential datasets largely reflected the time in culture effect: datasets with the shortest culture times had the largest influence, although the consequence of removing these datasets differed. In the comparison between *T. evansi*-infested plants and control plants, removing datasets 01 and 03 pushed the p-value of the time in culture effect to borderline significance ($T_{1,22}$ = -2.08, p = 0.050) or non-significance ($T_{1,22}$ = -1.53, p = 0.140), respectively (FIGURE 2.2a), whereas in the comparison between *T. evansi*-infested plants and *T. urticae*-infested plants, removing dataset 04 rendered the effect of time in culture non-significant ($T_{1,22}$ = -1.67, p = 0.109), but removing datasets 61-64 increased its significance ($T_{1,22}$ = -3.55, p = 0.002, FIGURE 2.2c).

Bias analyses indicated that the impact of bias, if any, is that we slightly underestimated the summary effect size in the comparison of *T. evansi*-infested plants versus controls, and in the comparison of *T. evansi*-infested plants versus *T. urticae*-infested plants (SUPPLEMENTARY NOTE S4).

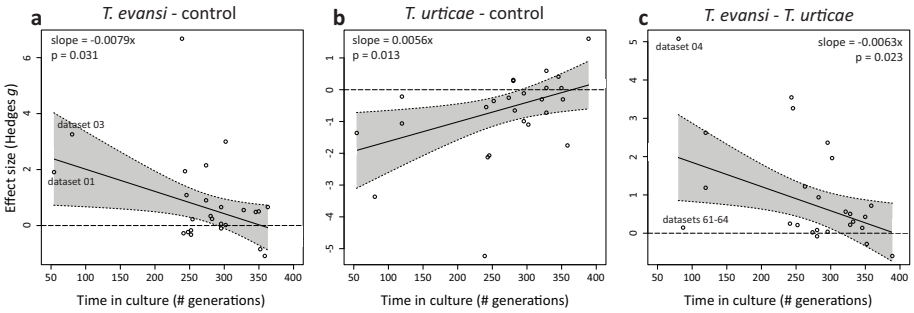


FIGURE 2.2. Effects of 'time in culture' on effect size. Solid lines indicate model fit, shaded areas delineate 95% confidence intervals, circles indicate observed effect size estimates, and dashed lines indicate zero effect size. The slope and significance of time in culture are given in the upper corners of each plot. Influential datasets are indicated with their name.

Discussion

The estimated summary effects in all three meta-analyses confirm previously published assertions: feeding on tomato by *T. evansi* enhances the performance of spider mites in subsequent infestations, whereas feeding by *T. urticae* decreases it (FIGURE 2.1). These summary effects translate into a difference in oviposition rate of spider mites in secondary infestations of around one egg per day, corresponding to an increase or decrease of 9-12% (Gotoh et al. 2010), which is substantial for species with exponential growth (Sabelis 1991). These effects are very variable, ranging from strongly negative or positive to none at all or even slightly opposite. Most of this variation was caused by differences among studies, rather than within-study sampling error, and we found that effect sizes significantly decreased with the time that spider mite populations had been cultured in the lab (FIGURE 2.2). In line with a diminishing effect over time in culture, datasets with the shortest culture times had the strongest influence on model predictions. Because the pattern of decreasing effect size was consistently found in all three comparisons, and because the consequences of removing influential datasets for the significance of the time in culture effect differed, we are convinced that the effects of time in culture are not artefacts of a small sample size at short culture times.

The reduction in effect size was observed over many generations, and therefore likely reflects a heritable phenotypic change of the mites in response to selection in lab cultures. This selection could have been directional because the variance in effect size decreased slightly, albeit non-significantly (FIGURE S2.1). Although genetic drift in lab populations may also account for changes in effect size and a concurrent decrease in variance (Halligan & Keightley 2009), we think this is unlikely because the effect size was consistent across populations. In lab cultures, reduced suppression of plant defence may be favourable if suppression is ineffective but nevertheless entails physiological costs, such as expression of salivary effectors (Jonckheere et al. 2016, Villarroel et al. 2016). Suppression may be ineffective in lab cultures because mite populations were always cultivated on detached leaves, and tomato leaves then readily turn purple from anthocyanin accumulation and clearly suffer from water stress and a severe mite infestation. This constant state of being stressed may cause leaves to be constitutively induced and consequently suppression may no longer be possible, potentially causing the defence suppression trait to erode (cf. Cooper & Lenski 2000).

Reduced induction of plant defence in lab cultures can also be favourable, e.g., if inducing mites are themselves susceptible to induced plant defences and hence suffer lower oviposition. This is even more likely, because the advantages that defence induction would normally provide in nature, namely a decreased performance of competitors, are absent because competitors are excluded from lab cultures. Literature indicates that mite-induced plant defence indeed reduces spider mite performance in controlled environments (Li et al. 2002, 2004, Ament et al. 2004, Kant et al. 2008, Zhurov et al. 2014,

Ataide et al. 2016, Villarroel et al. 2016), and is therefore likely adaptive for plants rather than herbivores. Another possibility is that the constantly stressed state of detached leaves in mite cultures may invoke selection for resistance to plant defence, resulting in smaller differences in mite performance between induced and undamaged leaves. We found some tentative support for the latter explanation in an analysis of absolute daily oviposition rates, which increased slightly, but not always significantly, on undamaged control plants and on *T. urticae*-infested plants, but not on *T. evansi*-infested plants (FIGURE S2.4).

In the comparison of *T. urticae*-infested plants with controls, effect size was not only affected by the time populations had been cultured, but also by a collinear set of variables consisting of the environment in which experiments were performed (greenhouse or climate room), the host plant species on which *T. urticae* populations were cultured, and the *T. urticae* population used (TABLE 2.2, FIGURE S2.2). In the other two comparisons, none of these variables had a significant effect on effect size, but roughly three quarters of the among-study heterogeneity was unexplained. One potential explanation for large variation in effect size is given by Anderson et al. (2009), who modelled transient dynamics of interactions between two herbivore populations mediated by plant quality. They conclude that differences in the extent or rate at which plants respond to herbivore infestation may cause strong variation in short-term herbivore performance measurements, such as those in the experiments reviewed here. Consequently, plasticity in plant defence induction may have been a source of variability in our datasets, e.g., depending on small differences in growing conditions such as nitrogen availability or disturbance (Hoffland et al. 2000). Additionally, because herbivores are often found to adjust their response to plant defence to the defence levels they experience (Després et al. 2007), defence suppression and induction by spider mites could also be subject to phenotypic plasticity. We therefore discuss the possibility that plasticity in defence response by plants and in defence suppression and induction by mites accounted for some of the observed variation in effect size.

Although defence induction is often seen as an adaptive response of the plant to reduce herbivory (Cipollini et al. 2014), plant resource allocation to defence actually depends on many factors, such as competition for light, the presence of predators or pathogens, and resource availability (Orrock et al. 2015, Hahn & Maron 2016). In addition, some herbivores use plant defence induction to their own advantage, e.g., by inducing defence responses that are antagonistic to the defences that would have been effective against them (Zarate et al. 2007). Therefore, environmental conditions, as well as defences being exploited by herbivores, can cause plants to adjust their level of defence. Herbivores, in turn, may also have adaptive potential for plasticity in defence suppression. For example, suppression of plant defence is often advantageous for herbivores in laboratory settings (Musser et al. 2002, Ataide et al. 2016), but may also have adverse

effects if it increases performance of competing herbivores (Sarmento et al. 2011b, Glas et al. 2014) or exposes offspring to increased predation (Ataide et al. 2016).

Indeed, there is evidence that herbivores monitor their environment, and adjust their physiology and behaviour accordingly (Dussourd 2017). *Tetranychus evansi*, for example, produced more web in the presence of cues associated with competitors, excluding them from their feeding sites (Sarmento et al. 2011b), interfered with their reproduction through heterospecific mating (Sato et al. 2014, 2016, Clemente et al. 2016, 2018), and oviposited more in the web than on the leaf surface in the presence of cues associated with predators (Lemos et al. 2010). In addition, spider mites can show transcriptional plasticity of detoxification genes (Dermauw et al. 2013, Wybouw et al. 2015), and suppression of plant defence by *T. evansi* is at least partly controlled by expression of effector proteins in their saliva (Jonckheere et al. 2016, Villarroel et al. 2016). Schimmel et al. (2017a, b) observed that *T. evansi* increases its expression of some of these putative defence-suppressing effector genes after exposure to plant-mediated interactions with defence-inducing *T. urticae*, which coincided with increased *T. evansi* oviposition rates. Together, these findings suggest that *T. evansi* is able to collect information from its environment, and uses it to adjust the degree to which it suppresses plant defence.

Both in laboratory experiments (Sarmento et al. 2001b) and in areas where *T. evansi* is invasive, it rapidly replaces *T. urticae* as the most abundant spider mite species (Ferragut et al. 2013, Azandémè-Hounmalon et al. 2015). This is in contrast with the results of the experiments reviewed here, which show that *T. evansi* facilitated *T. urticae*, whereas *T. urticae* had a negative plant-mediated effect on *T. evansi*. There are several possible explanations for this discrepancy. First, direct interactions, exploitative competition, and apparent competition were not included in the experiments reviewed here. However, previous research has shown that *T. evansi* and *T. urticae* interact directly through reproductive interference (Sato et al. 2014, 2016, Clemente et al. 2016, 2018) and by protecting feeding sites against competitors by depositing web on the leaf surface (Sarmento et al. 2011b). Second, *T. evansi* and *T. urticae* occur in diverse communities (Ferragut et al. 2013), and it is possible that the outcome of their interaction is different on host plants other than tomato, or modified by the presence of other herbivores and predators (Glas et al. 2014, Ataide et al. 2016). Third, we restricted the current analyses to studies investigating plant-mediated interactions that occur within tomato leaflets. However, induced plant responses differ among tissues (Orians et al. 2000) and can be further modulated by systemic effects (Biere & Goverse 2016) and timing of infestation (Alba et al. 2015, Schimmel et al. 2017). Therefore, the relative importance of plant-mediated, indirect interactions for spider mite performance remains to be demonstrated.

Our meta-analysis shows that intraspecific variation in *T. urticae* and *T. evansi* causes variation in the fitness consequences of plant-mediated interactions between these

species. It also suggests that mite populations evolve towards weaker plant-mediated interactions in culture conditions, which is an example of inadvertent selection in lab populations (Harshmann & Hoffman 2000). Previously, Kant et al. (2008) and Alba et al. (2015) demonstrated that different strains of *T. urticae* and *T. evansi* induced different responses in tomato, with consequences for the performance of other *T. urticae* mites. Furthermore, Kant et al. (2008) showed that *T. urticae* strains differ in their susceptibility to herbivore-induced tomato responses. We complement their findings by showing that plant-mediated interactions among spider mites are variable, but on average relevant for fitness, and that the net effect of suppression and induction of, and susceptibility to, plant defence is subject to phenotypic change over generations. Together, these results suggest that spider mites have the potential to adapt to plant-mediated ecological interactions, and thus constitute suitable study systems for eco-evolutionary dynamics of plant-herbivore interactions (Utsumi et al. 2011, Ohgushi 2016). Specifically, given their short generation time and considering the results of the present study, spider mites are especially amenable for experimental evolution designs (e.g., Magalhães et al. 2011, Wybouw et al. 2015). Future work could make use of these possibilities to select for spider mites that differ in induction and suppression of plant defences, or to investigate life-history trade-offs of induced plant responses for herbivores, e.g., in the presence or absence of competitors or under different environmental conditions.

Acknowledgements

This research was supported by grants from the Dutch Science Foundation (NWO): TOP-grants 854.11.005 (to JMA, BK and FL) and 864.10.005 (to ETK), ALW-OPEN grant 824.14.011 (to LMSA), and STW VIDI grant 13492 (to JMA, RC, MRK and BCJS); from the European Research Council: ERC Grant Agreement 335542 (to ETK); from the Brazilian Ministry of Education: CAPES (to LMSA, AMGB, CRD, FL, EFdO, PAdS, and NRdSJ); from the Brazilian National Council for Scientific and Technological Development: CNPq (to MVAD, FL, AP, FRR, PAdS, and NRdSJ); from the Foundation for Research Support of the Brazilian State of Minas Gerais: FAPEMIG (to AP, FRR, PAdS, and NRdSJ); from the Brazilian National Research Institute for Plant-Pest Interactions: INCTIPP (to NRdSJ); and from the Portuguese National Funding Agency for Science, Research and Technology: grant FCT PD/BD/114010/2015 (to DPG).

References

Agrawal, A.A. (1998) Induced responses to herbivory and increased plant performance. *Science*, **279**, 1201-1202.

Alba, J.M., Schimmel, B.C.J., Glas, J.J., Ataide, L.M.S., Pappas, M.L., Villarroel, C.A., Schuurink, R.C., Sabelis, M.W. & Kant, M.R. (2015) Spider mites suppress tomato defences downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist*, **205**, 828-840.

Ament, K., Kant, M.R., Sabelis, M.W., Haring, M.A. & Schuurink, R.C. (2004) Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiology*, **135**, 2025-2037.

Anderson, K.E., Inouye, B.D. & Underwood, N. (2009) Modeling herbivore competition mediated by inducible changes in plant quality. *Oikos*, **118**, 1633-1646.

Ataide, L.M.S., Pappas, M.L., Schimmel, B.C.J., Lopez-Orenes, A., Alba, J.M., Duarte, M.V.A., Pallini, A., Schuurink, R.C. & Kant, M.R. (2016) Induced plant-defenses suppress herbivore reproduction but also constrain predation of their offspring. *Plant Science*, **252**, 300-310.

Azandémè-Hounmalon, G.Y., Affognon, H.D., Assogba Komlan, F., Tamò, M., Fiaboe, K.K.M., Kreiter, S. & Martin, Th. (2015) Farmers' control practices against the invasive red spider mite, *Tetranychus evansi* Baker & Pritchard in Benin. *Crop Protection*, **76**, 53.58.

Belsley, D.A., Kuh, E. & Welsch, RE. (1980) *Regression Diagnostics*. Wiley, New York.

Bensoussan, N., Santamaria, M.E., Zhurov, V., Diaz, I., Grbić, M. & Grbić, V. (2016) Plant-herbivore interaction: dissection of the cellular pattern of *Tetranychus urticae* feeding on the host plant. *Frontiers in Plant Science*, **7**, 1105.

Benz, G. (1974) Negative Rückkoppelung durch Raum- und Nahrungskonkurrenz sowie zyklische Veränderung der Nahrungsgrundlage als Regelprinzip in der Populationsdynamik des Grauen Lärchenwicklers, *Zeiraphera diniana* (Guenée) (Lep., Tortricidae). *Zeitschrift für angewandte Entomologie*, **76**, 196-228.

Biere, A. & Goverse, A. (2016) Plant-mediated systemic interactions between pathogens, parasitic nematodes, and herbivores above-and belowground. *Annual Review of Phytopathology*, **54**, 499-527.

Bonato, O. (1999) The effect of temperature on life history parameters of *Tetranychus evansi* (Acari: Tetranychidae). *Experimental and Applied Acarology*, **23**, 11-19.

Borenstein, M., Hedges, L.V., Higgins, J.P.T. & Rothstein, H.R. (2009) *Introduction to Meta-Analysis*. John Wiley & Sons, Chichester.

Chester, K.S. (1933) The problem of acquired physiological immunity in plants. *The Quarterly Review of Biology*, **8**, 275-324.

Cipollini, D., Walters, D. & Voelckel, C. (2014) Costs of resistance in plants: from theory to evidence. *Annual Plant Reviews*, **47**, 263-308.

Clemente S.H., Rodrigues L.R., Ponce R., Varela S.A.M., Magalhães S. (2016). Incomplete species recognition entails few costs in spider mites, despite first-male precedence. *Behavioral Ecology and Sociobiology*, **70**, 1161-1170.

Clemente S.H., Santos I., Ponce R., Rodrigues L., Varela S.A.M., Magalhães S. (2018). Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly. *Behavioral Ecology*, **29**, 321-327.

Cook, J.L., Houseweart, M.W., Kulman, H.M. & Thompson, L.C. (1978) Foliar mineral differences related to sawfly defoliation of white spruce. *Environmental Entomology*, **7**, 780-781.

Cook, R.D. & Weisberg, S. (1982) *Residuals and Influence in Regression*. Chapman & Hall, New York.

Cooper, V.S. & Lenski, R.E. (2000) The population genetics of ecological specialization in evolving *Escherichia coli* populations. *Nature*, **407**, 736-739.

Denno, R.F. & Kaplan, I. (2007) Plant-mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. *Ecological communities: plant mediation in indirect interaction webs* (eds T. Ohgushi, T.P. Craig & P.W. Price), pp 19-50. Cambridge University Press, London.

Denno, R.F., McClure, M.S. & Ott, J.R. (1995) Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology*, **40**, 297-331.

de Oliveira, E.F., Pallini, A. & Janssen, A. (2016) Herbivores with similar feeding modes interact through the induction of different plant responses. *Oecologia*, **180**, 1-10.

Dermauw, W., Wybouw, N., Rombauts, S., Menten, B., Vontas, J., Grbić, M., Clark, R.M., Feyereisen, R. & Van Leeuwen, T. (2013) A link between host plant adaptation and pesticide resistance in the polyphagous spider mite Tetranychus urticae. *Proceedings of the National Academy of Sciences*, **110**, E113-22.

Després, L., David, J.-P. & Gallet, C. (2007) The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology and Evolution*, **22**, 298-307.

Dussourd, D.E. (2017) Behavioral sabotage of plant defenses by insect folivores. *Annual Review of Entomology*, **62**, 15-34.

Egas, M. & Sabelis, M.W. (2001) Adaptive learning of host preference in a herbivorous arthropod. *Ecology Letters*, **4**, 190-195.

Egger, M. & Smith, G.D. (1998) Bias in location and selection of studies. *The British Medical Journal*, **316**, 61-66.

Faeth, S.H. (1986) Indirect interactions between temporally separated herbivores mediated by the host plant. *Ecology*, **67**, 497-494.

Ferragut, F., Garzón-Luque, E. & Pekas, A. (2013) The invasive spider mite *Tetranychus evansi* (Acari: Tetranychidae) alters community composition and host-plant use of native relatives. *Experimental and Applied Acarology*, **60**, 321-341.

Fox, L.R. (1981) Defense and dynamics in plant-herbivore systems. *American Zoologist*, **21**, 853-864.

Gange, A.C. & Brown, V.K. (1989) Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia*, **81**, 38-42.

Glas, J.J., Alba, J.M., Simoni, S., Villarroel, C.A., Stoops, M., Schimmel, B.C.J., Schuurink, R.C., Sabelis, M.W. & Kant, M.R. (2014) Defence suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. *BMC Biology*, **12**, 98.

Gloss, A.D., Groen, S.C. & Whiteman, N.K. (2016) A genomic perspective on the generation and maintenance of genetic diversity in herbivorous insects. *Annual Review of Ecology, Evolution and Systematics*, **47**, 165-187.

Godinho, D.P., Janssen, A., Dias, T., Cruz, C. & Magalhães, S. (2016) Down-regulation of plant defence in a resident spider mite species and its effect upon con- and heterospecifics. *Oecologia*, **180**, 161-167.

Gotoh, T., Sugimoto, N., Pallini, A., Knapp, M., Hernandez-Suarez, E., Ferragut, F., Ho, C., Migeon, A., Navajas, M. & Nachman, G. (2010) Reproductive performance of seven strains of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae) at five temperatures. *Experimental and Applied Acarology*, **52**, 239-259.

Gould, F. (1979) Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* . *Evolution*, **33**, 791-802.

Green, T.R. & Ryan, C.A. (1972) Wound induced proteinase inhibitor in plant leaves: a possible defence mechanism against insects. *Science*, **175**, 776-777.

Hahn, P.G. & Maron, J.L. (2016) A framework for predicting intraspecific variation in plant defense. *Trends in Ecology and Evolution*, **31**, 646-656.

Halligan, D.L. & Keightley, P.D. (2009) Spontaneous mutation accumulation studies in evolutionary genetics. *Annual Review of Ecology, Evolution and Systematics*, **40**, 151-172.

Harshman, L.G. & Hoffman, A.A. (2000) Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends in Ecology and Evolution*, **15**, 32-36.

Hedges, L.V. (1981) Distribution theory for Glass's estimator of effect size and related estimators. *Journal of Educational Statistics*, **6**, 107-128.

Higgins, J.P.T. (2003) Measuring inconsistency in meta-analyses. *British Medical Journal*, **327**, 557-560.

Hoffland, E., Dicke, M., van Tintelen, W., Dijkman, H. & van Beusichem, M.L. (2000) Nitrogen availability and defense of tomato against two-spotted spider mite. *Journal of Chemical Ecology*, **26**, 2697-2711.

Inouye, B. & Stinchcombe, J.R. (2001) Relationships between ecological interaction modifications and diffuse coevolution: similarities, differences, and causal links. *Oikos*, **95**, 353-360.

Janzen, D.H. (1980) When is it coevolution? *Evolution*, **34**, 611-612.

Johnson, S.N., Clark, K.E., Hartley, S.E., Jones, T.H., McKenzie, S.W. & Koricheva, J. (2012) Aboveground–belowground herbivore interactions: a meta-analysis. *Ecology*, **93**, 2208-2215.

Jonckheere, W., Dermauw, W., Zhurov, V., Wybouw, N., Van den Bulcke, J., Villarroel, C.A., Greenhalgh, R., Grbić, M., Schuurink, R.C., Tirry, L., Baggerman, G., Clark, R.M., Kant, M.R., Vanholme, B., Menschaert, G. & Van Leeuwen, T. (2016) The salivary protein repertoire of the polyphagous spider mite *Tetranychus urticae*: a quest for effectors. *Molecular and Cellular Proteomics*, **15**, 3594-3613.

Kalisz, S. (1986) Selection on the timing of germination in *Collinsia verna* (Scrophulariaceae). *Evolution*, **40**, 479-491.

Kant, M.R., Jonckheere, W., Knegt, B., Lemos, F., Liu, J., Schimmel, B.C.J., Villarroel, C.A., Ataide, L.M.S., Dermauw, W., Glas, J.J., Egas, M., Janssen, A., Van Leeuwen, T., Schuurink, R.C., Sabelis, M.W. & Alba, J.M. (2015) Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany*, **115**, 1015-1051.

Kant, M.R., Sabelis, M.W., Haring, M.A. & Schuurink, R.C. (2008) Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proceedings of the Royal Society of Londen B*, **275**, 443-452.

Kaplan, I. & Denno, R.F. (2007) Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, **10**, 977-994.

Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*. Chicago University Press, Chicago, USA.

Kessler, A. & Halitschke, R. (2007) Specificity and complexity: the impact of herbivore-induced plant responses on arthropod community structure. *Current Opinion in Plant Biology*, **10**, 409-414.

Leimu, R. & Koricheva, J. (2006) A meta-analysis of genetic correlations between plant resistances to multiple enemies. *American Naturalist*, **168**, E15-E37.

Lemos, F., Sarmento, R.A., Pallini, A., Dias, C.R., Sabelis, M.W. & Janssen, A. (2010) Spider mite web mediates anti-predator behaviour. *Experimental and Applied Acarology*, **52**, 1-10.

Li, C., Williams, M.M., Loh, Y., Lee, G.I. & Howe, G.A. (2002) Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. *Plant Physiology*, **130**, 494-503.

Li, L., Zhao, Y., McCaig, B.C., Wingerd, B.A., Wang, J., Whalon, M.E., Pichersky, E. & Howe, G.A. (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell*, **16**, 126-143.

Magalhães, S., Blanchet, E., Egas, M. & Olivieri, I. (2011) Environmental effects on the detection of adaptation. *Journal of Evolutionary Biology*, **24**, 2653-2662.

Migeon, A. & Dorkeld, F. (2017) Spider Mites Web: a comprehensive database for the Tetranychidae. http://www.montpellier.inra.fr/CBGP/spmweb

Musser, R.O., Hum-Musser, S.M., Eichenseer, H., Pfeiffer, M., Ervin, G., Murphy, J.B. & Felton, G.W. (2002) Caterpillar saliva beats plant defences. *Nature,* **416**, 599-600.

Navajas, M., de Moraes, G.J., Auger, P. & Migeon, A. (2013) Review of the invasion of *Tetranychus evansi*: biology, colonization pathways, potential expansion and prospects for biological control. *Experimental and Applied Acarology*, **59**, 43-65.

Nykänen, H. & Koricheva, J. (2004) Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos*, **104**, 247-268.

Ohgushi, T. (2005) Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution and Systematics*, **36**, 81-105.

Ohgushi, T. (2016) Eco-evolutionary dynamics of plant-herbivore communities: incorporating plant phenotypic plasticity. *Current Opinion in Insect Science*, **14**, 40-45.

Orians, C.M., Pomerleau, J. & Ricco, R. (2000) Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. *Journal of Chemical Ecology*, **26**, 471-485.

Orrock, J.L., Sih, A., Ferrari, M.C.O., Karban, R., Preisser, E.L., Sheriff, M.J. & Thaler, J.S. (2015) Error management in plant allocation to herbivore defence. *Trends in Ecology and Evolution*, **30**, 441-445.

Poelman, E.H., Broekgaarden, C., van Loon, J.J.A. & Dicke, M. (2008) Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Molecular Ecology*, **17**, 3352-3365.

R Core Team (2016) R: A language and environment for statistical computing. Foundation for statistical computing, Vienna, Austria. URL: https://www.R-project.org/

Sabelis, M.W. (1991) Life-history evolution of spider mites. *The Acari: Reproduction, Development and Life-History Strategies* (eds R. Schuster & P.W. Murphy), pp. 23-49. Springer, London.

Sarmento, R.A., Lemos, F., Bleeker, P.M., Schuurink, R.C., Pallini, A., Oliveira, M.G.A., Lima, E.R., Kant, M.R., Sabelis, M.W. & Janssen, A. (2011a) A herbivore that manipulates plant defence. *Ecology Letters*, **14**, 229-236.

Sarmento, R.A., Lemos, F., Dias, C.R., Kikuchi, W.T., Rodrigues, J.C.P., Pallini, A., Sabelis, M.W. & Janssen, A. (2011b) A herbivorous mite down-regulates plant defence and produces web to exclude competitors. *PLoS One*, **6**, e23757.

Sato, Y., Alba, J.M. & Sabelis, M.W. (2014) Testing for reproductive interference in the population dynamics of two congeneric species of herbivorous mites. *Heredity*, **113**, 495-502.

Sato, Y., Staudacher, H. & Sabelis, M.W. (2016) Why do males choose heterospecific females in the red spider mite? *Experimental and Applied Acarology*, **68**, 21-31.

Schimmel B.C.J., Ataide, L.M.S., Chafi, R., Villarroel, C.A., Schuurink, R.C. & Kant, M.R. (2017a). Overcompensation of herbivore reproduction through hyper-suppression of plant defenses in response to competition. *New Phytologist*, **214**, 1688-1701.

Schimmel B.C.J., Ataide L.M.S., Kant M.R. (2017b). Spatiotemporal heterogeneity of tomato induced defense responses affects spider mite performance and behavior. *Plant Signaling & Behavior*, **12**, e1370526.

Schoener, T.W. (2011) The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science*, **331**, 426-429.

Senior, A.M., Grueber, C.E., Kamiya, T., Lagisz, M., O'Dwyer, K., Santos, E.S.A. & Nakagawa, S. (2016) Heterogeneity in ecological and evolutionary meta-analyses: its magnitude and implications. *Ecology*, doi: 10.1002/ecy.1591.

Siepielski, A.M., DiBattista, J.D. & Carlson, S.M. (2009) It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters*, **12**, 1261-1276.

Siepielski, A.M., Gotanda, K.M., Morrissey, M.B., Diamond, S.E., DiBattista, J.D. & Carlson, S.M. (2013) The spatial patterns of directional phenotypic selection. *Ecology Letters*, **16**, 1382-1392.

Stam, J.M., Kroes, A., Li, Y., Gols, R., van Loon, J.J.A., Poelman, E.H. & Dicke, M. (2014). Plant interactions with multiple insect herbivores: from community to genes. *Annual Review of Plant Biology*, **65**, 689-713.

Strauss, S.Y. & Irwin, R.E. (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution and Systematics*, **35**, 435-466.

Strauss, S.Y., Sahli, H. & Conner, J.K. (2005) Toward a more trait-centered approach to diffuse (co)evolution. *New Phytologist*, **165**, 81-89.

Utsumi, S. (2011) Eco-evolutionary dynamics in herbivorous insect communities mediated by induced plant responses. *Population Ecology*, **53**, 23-34.

Utsumi, S. (2015) Feeding evolution of a herbivore influences an arthropod community through plants: implications for plant-mediated eco-evolutionary feedback loop. *Journal of Ecology*, **103**, 829-839.

Utsumi, S., Ando, Y., Roininen, H., Takahashi, J. & Ohgushi, T. (2013) Herbivore community promotes trait evolution in a leaf beetle via induced plant response. *Ecology Letters*, **16**, 362-370.

Via, S. (1990) Adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. *Annual Review of Entomology*, **35**, 421-446.

Viechtbauer, W. (2010) Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, **36**, 1-48.

Viechtbauer, W. & Cheung, M.W.-L. (2010) Outlier and influence diagnostics for meta-analysis. *Research Synthesis Methods*, **1**, 112-125.

Villarroel, C.A., Jonckheere, W., Alba, J.M., Glas, J.J., Dermauw, W., Haring, M.A., Van Leeuwen, T., Schuurink, R.C. & Kant, M.R. (2016) Salivary proteins of spider mites suppress defences in *Nicotiana benthamiana* and promote mite reproduction. *The Plant Journal*, **86**, 119-131.

Watson, Th.F. (1964) Influence of host plant condition on population increase of *Tetranychus telarius* (Linnaeus) (Acarina: Tetranychidae). *Hilgardia*, **35**, 271-322.

Wise, M.J. & Rausher, M.D. (2013) Evolution of resistance to a multiple-herbivore community: genetic correlations, diffuse coevolution, and constraints on the plant's response to selection. *Evolution*, **67**, 1767-1779.

Wybouw, N., Zhurov, V., Martel, C., Bruinsma, K.A., Hendrickx, F., Grbić, V. & Van Leeuwen, T. (2015) Adaptation of a polyphagous herbivore to a novel host plant extensively shapes the transcriptome of herbivore and host. *Molecular Ecology*, **24**, 4647-4663.

Zangerl, A.R. & Berenbaum, M.R. (1990) Furanocoumarin induction in wild parsnip: genetics and populational variation. *Ecology*, **71**, 1933-1940.

Zarate, S.I., Kempema, L.A. & Walling, L.L. (2007) Silverleaf whitefly induces salicylic acid defences and suppresses effectual jasmonic acid defences. *Plant Physiology*, **143**, 866-875.

Zhurov, V., Navarro, M., Bruinsma, K.A., Arbona, V., Santamaria, M.E., Cazaux, M., Wybouw, N., Osborne, E.J., Ens, C., Rioja, C., Vermeirssen, V., Rubio-Samoza, I., Krishna, P., Diaz, I., Schmid, M., Gómez-Cadenas, A., Van de Peer, Y., Grbić, M., Clark, R.M., Van Leeuwen, T. & Grbić, V. (2014) Reciprocal responses in the interaction between Arabidopsis and the cell-content-feeding chelicerate herbivore spider mite. *Plant Physiology*, **164**, 384-399.

Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, **1**, 3-14.

Supplementary tables and figures

TABLES S2.1-S2.4: see http://www.bred.nl/bram_knegt_thesis_ch2/Supplementary tables S1-S4.xlsx
[Table_S1_complete_dataset,
Table_S2_Tevansi_control,
Table_S3_Turticae_control,
Table_S4_Tevansi_Turticae]

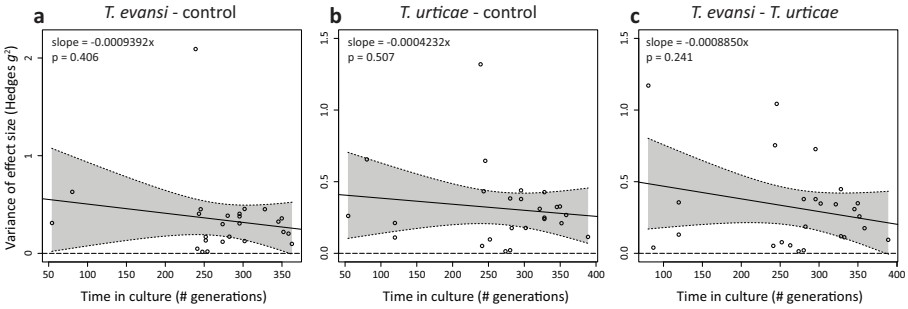


FIGURE S2.1. Effects of 'time in culture' on effect size variance, as assessed using linear models with Gaussian error distribution. Solid lines indicate model predictions, shaded areas delineate 95% confidence intervals, and circles indicate observed effect size estimates. The slope and significance of the 'time in culture' factor are given in the upper corner of each plot.

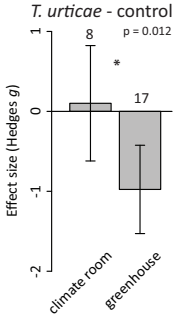


FIGURE S2.2. Effects of 'environment' (climate room, greenhouse) on effect size. Error bars indicate 95% confidence intervals. Numbers of datasets are given above each bar. The significance of the factor is given in the upper corner of the plot (* $p < 0.05$). Because the factor 'environment' was collinear with the factors '*T. urticae* lab host species', '*T. urticae* population' and 'research group' (TABLE 2.2), its effects can be caused by any of these factors.

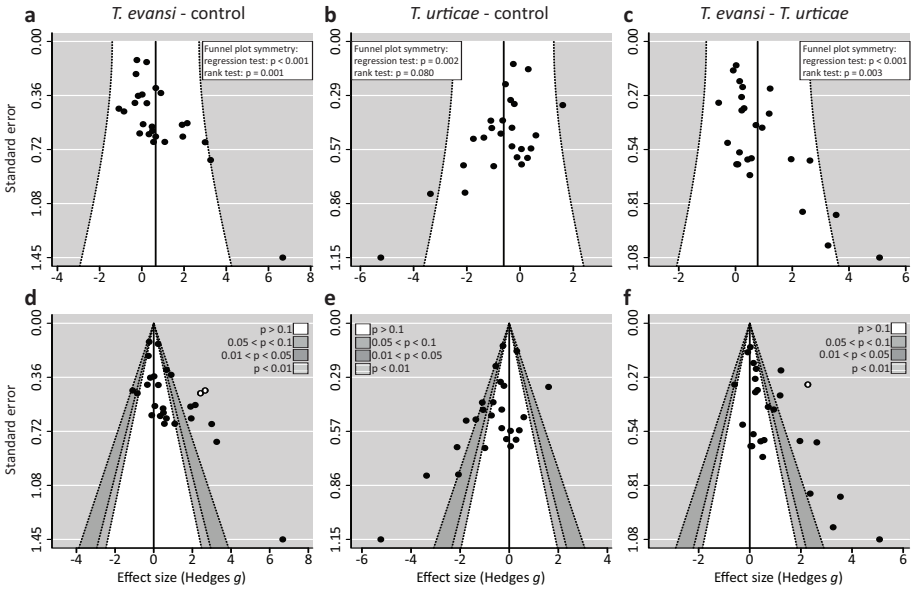


FIGURE S2.3. Bias analysis. (**a-c**) Funnel plots of effect sizes and their standard errors. Vertical lines indicate the estimated summary effect, circles indicate datasets, and dotted lines delineate 95% pseudo-confidence intervals (i.e., $M \pm 1.96 \sqrt{(SE^2 + T^2)}$). The results of two tests for funnel plot symmetry are given in the upper corner of each plot. (**d-f**) Contour-enhanced funnel plots of effect sizes and their standard errors. Vertical lines indicate zero effect size, filled circles indicate observed datasets, open circles indicate estimated 'missing' studies from the trim and fill method to restore funnel plot symmetry, differently shaded areas indicate different levels of statistical significance for individual studies under the null hypothesis that Hedges' g = 0.

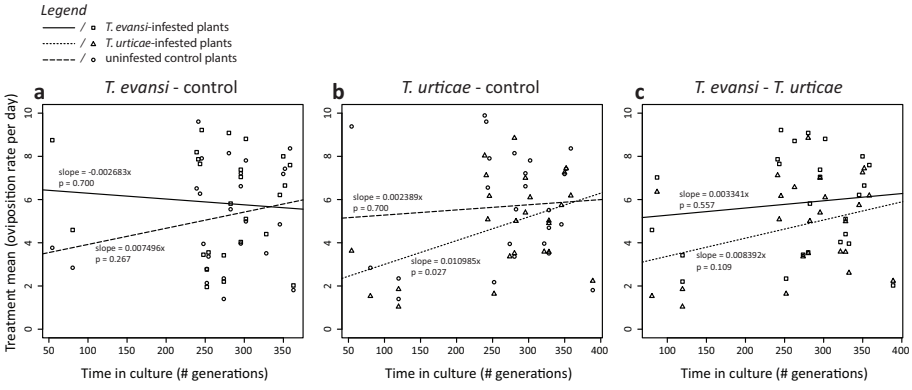


FIGURE S2.4. Effects of 'time in culture' on mean oviposition rates per day, as assessed using linear models with a Gaussian error distribution. We could not apply weighted meta-analytic models, because some datasets provided oviposition rates and variance only per 2, 3 or 4 days, prohibiting the calculation of the variance of oviposition rate per day. Lines indicate model predictions. Each graph has two lines, one for each of the two treatments under comparison. The slope and significance of the 'time in culture' factor are given with each model prediction.

Supplementary note S1

Complex data structures and effect size computation

Many datasets contained dependency structures that required addressing before effect sizes could be computed. We encountered three types of complex data structure: (1) experiments that were performed in multiple blocks in time, (2) experiments that compared multiple experimental treatments to the same control treatment, and (3) experiments that measured oviposition rates on multiple leaves of the same plant. In our effect size calculations we accounted for data dependency following the recommendations of Borenstein et al. (2009, pp. 214-245). Specifically, when experiments were performed in blocks, we first calculated the effect size and variance per block, and then aggregated these by performing a fixed effects meta-analysis across blocks, obtaining an overall effect size and variance. When multiple treatments were compared to the same control, or when measurements were performed on different leaves of the same plant, we obtained the overall effect size by taking the average of the effect sizes of each treatment comparison or leaf, and the overall variance by applying the formula

$$\mathrm{var}\left(\frac{1}{m}\sum_{i=1}^{m} Y_i\right) = \left(\frac{1}{m}\right)^2 \left(\sum_{i=1}^{m} V_i + \sum_{i \neq j} \left(r_{ij}\sqrt{V_i}\sqrt{V_j}\right)\right)$$

(Borenstein et al. 2009, p. 228), where 'var' stands for variance, Y_i and V_i are the effect size and variance of treatment comparison or leaf i, m is the total number of treatment comparisons or leaves, and r_{ij} is the correlation between the effect sizes of treatment comparison or leaf i and j. In the case of multiple comparisons to the same control treatment, r_{ij} equals 0.5, but when measurements were performed on different leaves of the same plant, r_{ij} was estimated by taking the intraclass correlation of oviposition rate measurements among leaves of the same plant (*ICC*[2,*k*] *sensu* Shrout & Fleiss 1979, calculated using R package *irr* [Gamer et al. 2012]), averaged across all datasets weighted by the number of plants per dataset.

Supplementary note S2

Collinearity analysis

When covariates correlate, the variables are collinear, and their influence on the effect size cannot be assessed independently. Collinearity also confounds statistical tests, which can be avoided by removing collinear variables from a statistical model (Legendre & Legendre 1998). Removed variables are not meaningless, but an effect of a remaining variable can be attributed to any variable in the collinear set. Further dissemination of the independent contributions of each variable in a collinear set requires new experimentation. We assessed collinearity among covariates by calculating pairwise correlations among all variables, using R package *polycor* (Fox 2010). We considered covariates to be collinear when their pairwise correlation was larger than 0.5, or when they had a variance inflation factor larger than 2 (Zuur et al. 2010). Because calculation of variance inflation factors requires fitting regression models with the covariates from the main analysis as response variables, variance inflation factors can only be calculated for numerical covariates. For simplicity we calculated unweighted correlations, ignoring differences in precision (1 / std. error of effect size) among studies.

When we found a pair of covariates to be collinear, we removed the variable with the lowest statistical power from our statistical model. To calculate statistical power of individual variables we conducted a power analysis, making assumptions about the minimally substantial influence on the summary effect size and the among-study heterogeneity. We considered the effect of a covariate on the summary effect size to be substantial if the range of the variable (for continuous variables) or the differences between its levels (for categorical variables) upheld a variation of at least 0.5 standard deviation in effect size, i.e., a Cohen's d of 0.5. We converted this into a Hedges' g value using the formula

$$Hedges'\ g = Cohen'\ s\ d \times \left(1 - \frac{3}{4df - 1}\right)$$

with df = 2 × average sample size – 2 (Borenstein et al. 2009, p. 27). For numerical variables, we calculated the minimally substantial coefficient, β, as Hedges' g / (max – min), where max and min are the maximum and minimum of the observed range of the covariate. For categorical variables, we used Hedges' g as the minimally substantial difference between levels. Because we could not find earlier meta-analyses investigating plant-mediated herbivore interactions within one system, we applied the convention described in Hedges & Pigott (2001), and assumed a moderate degree of heterogeneity among studies, equal to two thirds of the average within-study variance. We calculated σ^*, the variance of coefficient β, using the matrix approach described by Hedges & Pigott (2004). For numerical variables, the statistical power for detecting a minimally substantial coefficient, assuming two-sided tests, is then given by

$$power = 1 - \Phi\left(C_{\alpha} - \frac{\beta}{\sqrt{\sigma *}}\right) + \Phi\left(-C_{\alpha} - \frac{\beta}{\sqrt{\sigma *}}\right)$$

(Hedges & Pigott 2004), where $\Phi(x)$ is the standard normal cumulative distribution function, and C_{α} is the critical value for the significance test, given significance level $\alpha = 0.05$, thus $C_{\alpha} = \Phi(1 - 0.5 \times \alpha) = 1.96$. For categorical variables, power is given by

$$power = 1 - \Phi\left(C_{\alpha} - \frac{Hedges'g}{\sqrt{\sigma *}}\right) + \Phi\left(-C_{\alpha} - \frac{Hedges'g}{\sqrt{\sigma *}}\right).$$

Supplementary note S3

Model selection

After the collinearity analysis, we removed covariates from the final model with a backward model simplification procedure. We first specified full models that contained all covariates that remained after the collinearity analysis, and performed a mixed-effects meta-analysis using R package *metafor* (Viechtbauer 2010). We did not include interaction effects among covariates because the number of datasets was too small for interactions to be properly investigated. In a step-by-step process we omitted non-significant variables one by one until only significant variables remained. To avoid omitting variables because of a lack of power, we calculated the power for detecting a non-zero coefficient in any of the variables. To do so, we estimated minimally substantial effect sizes and heterogeneity among studies as explained in SUPPLEMENTARY NOTE S2, but because more than one variable is involved, power is given by

$$power = 1 - H\left(C_\alpha \middle| q; \lambda\right),$$

where H is the cumulative distribution function of the non-central chi-square distribution with q degrees of freedom and non-centrality parameter λ (Hedges & Pigott 2004). We considered a power of 80% or more to be sufficient.

Supplementary note S4

Bias analysis

To investigate if the effect sizes reported by the datasets were biased, we constructed funnel plots (Egger et al. 1997). Funnel plots show the relation between effect size and standard error of each dataset. In the absence of bias, the data points in a funnel plot form a symmetrical funnel around the summary effect, whereas biased datasets produce asymmetrical funnels where the most precise studies, with the smallest standard error, have effect sizes that are either consistently smaller or larger than the summary effect (Egger et al. 1997, Egger & Smith 1998). We tested for funnel plot symmetry using Egger's regression tests (Egger et al. 1997) and rank correlation tests (Begg & Mazumdar 1994). When we found significant funnel plot asymmetry, we investigated the impact of this bias using the trim and fill approach (Duval & Tweedie 2000). With this method, 'missing' studies are estimated with effect size and standard error chosen such that funnel plot symmetry is restored. We then repeated the meta-analysis including the estimated 'missing' studies, and assessed how the new summary effect differed from the original estimation.

Funnel plots displayed some asymmetry in all three comparisons (FIGURES S2.3a-c). Restoring funnel symmetry with the trim and fill method led to the inclusion of extra 'missing' studies in the comparison of *T. evansi*-infested plants versus controls, and in the comparison of *T. evansi*-infested plants versus *T. urticae*-infested plants. Including the missing studies raised the estimated summary effect sizes by 25 and 10%, respectively, showing that the impact of bias, if any, is that we slightly underestimated the summary effect size in these two comparisons.

We minimized the potential effects of publication bias by including unpublished datasets into our analyses. To investigate if this was effective, we constructed contour-enhanced funnel plots (Peters et al. 2008) and assessed if the results of the 'missing' studies from the trim and fill method would have been significantly different from a nil effect size. If so, then any observed bias in our final collection of datasets (published and unpublished) is not likely caused by publication bias. We found that the effect sizes of 'missing' studies would have been significantly different from zero (FIGURES S2.3d-f), indicating that publication bias is not a likely cause of bias in our collection of datasets.

References in supplementary notes

Begg, C.B. & Mazumdar, M. (1994) Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088-1101.

Borenstein, M., Hedges, L.V., Higgins, J.P.T. & Rothstein, H.R. (2009) *Introduction to Meta-Analysis*. John Wiley & Sons, Chichester.

Duval, S. & Tweedie, R. (2000) A nonparametric 'trim and fill' method of accounting for publication bias in meta-analysis. *Journal of the American Statistical Association*, **95**, 89-98.

Egger, M. & Smith, G.D. (1998) Bias in location and selection of studies. *The British Medical Journal*, **316**, 61-66.

Egger, M., Smith, G.D., Schneider, M. & Minder, C. (1997) Bias in meta-analysis detected by a simple, graphical test. *British Medical Journal*, **315**, 629-634.

Fox, J. (2010) polycor: polychoric and polyserial correlations. R package version 0.7-8. https://CRAN.R-project.org/package=polycor.

Gamer, M., Lemon, J. & Singh, I.F.P. (2012) irr: various coefficients of interrater reliability and agreement. R package version 0.84. https://CRAN.R-project.org/package=irr.

Graham, M.H. (2003) Confronting multicollinearity in ecological multiple regression. *Ecology*, **84**, 2809-2815.

Hedges, L.V. & Pigott, T.D. (2001) The power of statistical tests in meta-analysis. *Psychological Methods*, **6**, 203-217.

Hedges, L.V. & Pigott, T.D. (2004) The power of statistical tests for moderators in meta-analysis. *Psychological Methods*, **9**, 426-445.

Legendre, P. & Legendre, L. (1998) *Numerical Ecology*. Elsevier, Amsterdam.

Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R. & Rushton, L. (2008) Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *Journal of Clinical Epidemiology*, **61**, 991-996.

Shrout, P.E. & Fleiss, J.L. (1979) Intraclass correlations: uses in assessing rater reliability. *Psychological Bulletin*, **86**, 420-428.

Viechtbauer, W. (2010) Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, **36**, 1-48.

Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, **1**, 3-14.

3

Tetranychus evansi spider mite populations suppress tomato defences to varying degrees

Bram Knegt, Tomas T. Meijer, Merijn R. Kant,
E. Toby Kiers & Martijn Egas

Abstract

Plant defence suppression is an offensive strategy of herbivores, in which they manipulate plant physiological processes to increase their performance. Paradoxically, defence suppression does not always benefit the defence-suppressing herbivores, because lowered plant defences can also enhance the performance of competing herbivores and can expose herbivores to increased predation. Suppression of plant defence may therefore entail considerable ecological costs depending on the presence of competitors and natural enemies in a community. Hence, we hypothesize that local differences in community composition select for different optimal magnitudes of suppression. To investigate this, we studied defence suppression across populations of *Tetranychus evansi* spider mites, a herbivore from South America that is an invasive pest of solanaceous plants including cultivated tomato, *Solanum lycopersicum*, in other parts of the world. We measured the level of expression of defence marker genes in tomato plants after infestation with mites from eleven different *T. evansi* populations. These populations were chosen across a range of native (South American) and non-native (other continents) environments and from different host plant species. We found significant variation at three out of four defence marker genes, demonstrating that *T. evansi* populations suppress jasmonic acid- and salicylic acid-dependent plant signalling pathways to varying degrees. While we found no indication that this variation in defence suppression was explained by differences in host plant species, invasive populations tended to suppress plant defence to a smaller extent than native populations. We speculate that this may reflect the absence of specialized natural enemies in invasive *T. evansi* populations.

A revised version is accepted for publication in *Ecology & Evolution*.

Introduction

Plants and herbivores share a 420 million year history of antagonistic coevolution (Labandeira 1998). Over this time, these adversaries have been in an arms race of adaptations and counter-adaptations. This has resulted in the evolution of elaborate plant defence mechanisms, such as two-component toxins (Matile 1980) and recruitment of natural enemies with plant volatiles (Baldwin & Schultz 1983, Heil 2014). In response, herbivores have evolved offensive traits that enable them to consume plant tissues more efficiently, such as mechanisms to detoxify defensive plant compounds (Smith 1955, Heckel 2014). Over the last decade, herbivores were also found to suppress plant defence by manipulating plant physiological processes, thereby promoting herbivore performance (Musser et al. 2002, Kant et al. 2015). Whiteflies, for example, normally induce a defence response in their host plants that is regulated by the plant hormone jasmonic acid (JA) (van de Ven et al. 2000, Walling 2000). *Bemisia tabaci* silverleaf whiteflies, however, hijack defence regulation of their *Arabidopsis thaliana* hosts by inducing salicylic acid (SA) dependent defence signalling (Zarate et al. 2007). Induced SA levels suppress JA levels through hormonal cross-talk (Thaler et al. 2012), and hence protect silverleaf whiteflies from JA-dependent defences. More than twenty arthropod herbivore species suppress plant defences (Kant et al. 2015), and a majority are crop pest species, such as the corn earworm (*Helicoverpa zea*) (Musser et al. 2002), the Colorado potato beetle (*Leptinotarsa decemlineata*) (Lawrence et al. 2007) and the two-spotted spider mite (*Tetranychus urticae*) (Kant et al. 2008).

Understanding why defence suppression is a successful herbivore offense strategy requires insight into its evolutionary costs and benefits (Blaazer et al. 2018). A benefit of defence suppression for herbivores is that it prevents expression of plant defence, which would otherwise have reduced herbivore performance (Musser et al. 2002, Kant et al. 2015). At the same time, however, defence suppression creates a hospitable, nutritious plant (i.e., a public good, Rankin et al. 2007) from which competitors and natural enemies can also benefit (Alba et al. 2011, Kant et al. 2015). This introduces new costs. A prime example of such costs can be found in *Tetranychus evansi* spider mites (Blaazer et al. 2018). By suppressing tomato defence, *T. evansi* increase not only their own performance but also that of competing *Tetranychus* spp. spider mites (Sarmento et al 2011a, b, Alba et al. 2015, Godinho et al. 2016). In addition, defence suppression by *T. evansi* exposes their offspring to increased predation by *Phytoseiulus longipes* predatory mites, possibly due to reduced transfer of defensive compounds from plants through spider mites into their eggs (Ataide et al. 2016). Defence suppression may therefore entail considerable costs depending on the biotic environment in which it is employed (Glas et al. 2014, Schimmel et al. 2017a, b).

To assess the role of biotic interactions in the evolution of defence suppression, it is pivotal to quantify variation in defence suppression across different biotic environments.

In *Leptopilina boulardi* parasitoid wasps, for example, genotypes from different localities were found to suppress the immune system of their *Drosophila* hosts to varying degrees, depending on the abundance of specific host species (Dupas & Boscaro 1999). Yet, in herbivores, variation in defence suppression has hitherto been investigated only scarcely (Alba et al. 2015). Here, we aim to quantify variation in the magnitude of suppression of plant defence among populations of the defence-suppressing spider mite *T. evansi*, and secondarily to explore whether differences relate to characteristics of their biotic environments. Specifically, we assessed if variation was explained by the host plant from which populations were sampled, and by the presence or absence of natural enemies.

The tomato red spider mite *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae) is a herbivorous spider mite from South America, feeding mainly from solanaceous host plants such as tomato, potato and eggplant (Navajas et al. 2013, Migeon and Dorkeld 2018). Spider mites are cell content-feeders, piercing plant parenchyma cells with their stylets, sucking up the contents and leaving behind empty cells that are visible as white feeding scars (Bensoussan et al. 2016). *Tetranychus evansi* occurs mostly in tropical, subtropical and Mediterranean climates, and can reach fast intrinsic rates of population increase due to short generation times (<15 days), especially at high temperatures (Bonato 1999, Gotoh et al. 2010). Over the last twenty years, *T. evansi* has become invasive in many areas with subtropical and Mediterranean climates, such as sub-Sahara Africa, the Mediterranean region and East-Asia (Navajas et al. 2013). In its invasive range *T. evansi* replaces *T. urticae* and other indigenous spider mite species as the dominant species in spider mite communities and colonizes new host plant species (Ferragut et al. 2013, Azandémè-Hounmalon et al. 2015).

The mechanism of defence suppression by *T. evansi* has mostly been studied on cultivated tomato plants, *Solanum lycopersicum*. In tomato, the induced defence response against spider mites is orchestrated by the plant hormones JA and SA (Li et al. 2002, Ament et al. 2004, Kant et al. 2004). These hormones set in motion internal signalling cascades leading to the production of defence-associated compounds such as proteinase inhibitors (PIs) and polyphenol oxidases (PPOs) (Kant et al. 2004, Martel et al. 2015, Arnaiz et al. 2018). *Tetranychus evansi* suppress tomato defence downstream of plant hormone accumulation, such that the plant's expression of defence-associated genes is downregulated to the benefit of the herbivore (Sarmento et al. 2011a, Alba et al. 2015, Ataide et al. 2016). Defence suppression by spider mites is most likely exerted by salivary effectors injected into the host plant (Jonckheere et al. 2016, Villarroel et al. 2016, Schimmel et al. 2017a), independent of herbivore-associated bacteria (Staudacher et al. 2017).

Host plants are a major determinant of herbivore biotic environments. Arthropod communities, for example, differ among locations due to variation in secondary metabolites of their host plants (Bangert et al. 2006, Poelman et al. 2008, Randlkofer et al. 2010, Richards et al. 2015, Bálint et al. 2016, Glassmire et al. 2016). Plant identity and diversi-

ty therefore affect interactions between plants and herbivores (Agrawal et al. 2006). The host range of *T. evansi* includes more than a hundred plant species, mainly from the Solanaceae family (Migeon and Dorkeld 2018), with considerable variation in secondary metabolites and resistance to herbivory (Wink 2003, Fridman et al. 2005, Spooner et al. 2005). The *T. evansi* populations investigated in this study were sampled from four host plant species, all belonging to the *Solanum* genus (TABLE 3.1). *Solanum* species produce different levels of glycoalkaloids and proteinase inhibitors that differentially affect herbivore performance, are therefore likely to harbour different arthropod communities (Tingey 1984, Cipollini and Levey 1997, Girard et al. 2007, Nohara et al. 2007, Hartl et al. 2010, Jared et al. 2016), and may expose defence-suppressing herbivores to different levels of competition and predation. Consequently, we explored if the level of defence suppression by *T. evansi* varied among populations collected from these host plants.

We also explored if the level of defence suppression differed between native (South-American) and invasive (other continents) *T. evansi* populations. The predatory mite *Phytoseiulus longipes* and the entomopathogenic fungus *Neozygites floridiana* are able to severely reduce *T. evansi* populations in their native range (Ribeiro et al. 2009, da Silva et al. 2010), but are absent in areas where *T. evansi* is invasive (Ferragut et al. 2013). Defence suppression by *T. evansi* entails costs in the presence of natural enemies, such as increased egg predation by *P. longipes* (Ataide et al. 2016). The lack of natural enemies in areas where *T. evansi* is invasive may therefore reduce such ecological costs, allowing *T. evansi* to suppress plant defence more strongly.

We sampled *T. evansi* mites from eleven locations across its native and invasive range (FIGURE 3.1), and measured their magnitude of defence suppression with two approaches. First, we measured the expression of a reporter gene for defence induction in *pLAP-A1:GUS* tomato plants. In these plants, the promoter of the *β-glucuronidase* (GUS) reporter gene is fused to the JA-dependent promoter of the plant defence-associated gene *leucine aminopeptidase A1* (*LAP-A1*) (Chao et al. 1999). When plant defence is induced, *LAP-A1* is activated, and thereby also the GUS reporter, of which its activity can be determined in a fluorimetric assay (Jefferson et al 1987). Because these assays were more variable than expected, we also measured the level of gene expression of four defence-associated tomato genes through quantitative reverse transcription polymerase chain reaction (qRT-PCR). *Tetranychus evansi* suppresses these genes in tomato (Sarmento et al. 2011a, Alba et al. 2015, Schimmel et al. 2017a). In all experiments, we included a treatment where tomato plants were infested with a defence-inducing *T. urticae* genotype as a benchmark for defence induction. For those genes where we observed different levels of expression among populations, we investigated if this variation was affected by host plant species or geographical range (invasive or native). We also verified that variation was not due to the identity of their host plants in the laboratory, or the time that populations had been maintained there. Last, we investigated

genetic differentiation among the *T. evansi* populations by determining to which genetic lineage (Gotoh et al. 2009, Boubou et al. 2012) each population belonged through sequencing a part of the mitochondrial *cytochrome oxidase subunit 1* gene (CO1).

Materials and methods

Spider mite populations and culture conditions

We obtained eleven *T. evansi* populations that had been collected by other research groups from several locations across South America, Europe, Africa and Asia (FIGURE 3.1, TABLE 3.1). Because a change in host plant can have drastic consequences for genetic and phenotypic variation within a population (Magalhães et al. 2009, Dermauw et al. 2013, Wybouw et al. 2015), we maintained them on the same host plant as they had been on in the research group from which we obtained these populations, i.e., ached *S. nigrum* leaves or *S. lycopersicum* leaflets. We placed leaves and leaflets with their abaxial side facing upwards on wet cotton wool in open plastic trays in a controlled environment (25 °C; 16: 8 h light: dark photoperiod; 60% relative humidity). We grew plants in a greenhouse (25: 18 °C; 16: 8 h light: dark photoperiod; 50–60% relative humidity) for 4-5 weeks before leaves were used to feed the mite cultures.

Infestation treatments and sampling

We obtained *pLAP-A1:GUS* seeds from Linda Walling (University of California, Riverside, CA, USA) and grew these and untransformed UC82 tomato plants in a greenhouse (25: 18 °C; 16: 8 h light: dark photoperiod; 50–60% relative humidity) for 11-14 days, and then transferred them to a climate room (25 °C; 16: 8 h light: dark photoperiod; 60% relative humidity) to acclimatize for 7-10 days, such that plants were exactly 21 days old at the start of the experiments. We only used plants with three or four

FIGURE 3.1. Sampling locations of *Tetranychus evansi* populations used in this study.

TABLE 3.1. Collection records of *Tetranychus evansi* populations used in this study.

Population	Lab host	Field host	Location	Range	Latitude	Longitude	Date	Collector	Reference
Algarrobo-1	*S. lycopersicum* cv. Castlemart	*S. nigrum*	Andalucía, Spain	invasive	36° 45' N	4° 02' W	2011	J.M. Alba	Alba et al. (2015)
Carangola-1	*S. lycopersicum* cv. Santa Clara	*S. lycopersicum*	Minas Gerais, Brazil	native	20° 44' S	42° 02' W	2013	J. Mencalha	this study
Chiyoda-1	*S. nigrum*	*S. nigrum*	Tokyo, Japan	invasive	35° 40' N	139° 45' E	Sep 2010	T. Gotoh	this study
JT	*S. nigrum*	*S. nigrum*	Tokyo, Japan	invasive	35° 35' N	139° 36' E	Nov 2006	T. Gotoh, Y. Kitashima	Gotoh et al. (2009)
Kagoshima-1	*S. nigrum*	*S. nigrum*	Kagoshima, Japan	invasive	31° 34' N	130° 30' E	Jul 2009	Y. Sakamaki	Ikeshima et al. (2009)
KM	*S. nigrum*	*S. lycopersicum*	Makueni County, Kenya	invasive	01° 42' S	37° 25' E	Mar 2001	M. Knapp	Gotoh et al. (2009)
SC	*S. nigrum*	*S. lycopersicum*	Canary Islands, Spain	invasive	28° 23' N	16° 33' W	Dec 2006	E. Hernandez-Suarez	Gotoh et al. (2009)
Sde Eliyahu-1	*S. lycopersicum defenseless-1*[b]	*S. tuberosum* + *S. melongena*	Mo'atza Azorit Emeq Hamaayanot, Israel	invasive	32° 26' N	35° 30' E	Jun 2013	A. Tabic	this study
SV	*S. nigrum*	*S. lycopersicum*	Valencia, Spain	invasive	39° 29' N	0° 20' W	Jan 2007	F. Ferragut	Gotoh et al. (2009)
TW	*S. nigrum*	*S. nigrum*	Wufeng, Taiwan	invasive	24° 04' N	121° 42' E	Dec 2006	C.-C. Ho	Gotoh et al. (2009)
Viçosa-1[a]	*S. lycopersicum* cv. Castlemart	*S. lycopersicum*	Minas Gerais, Brazil	native	20° 45' S	42° 52' W	2002	A. Pallini	Sarmento et al. (2007)

[a] This population was referred to as 'BP' by Gotoh et al. (2009, 2010), and as 'Vicoça-1' by Alba et al. (2015). We choose to use the latter name, because it was first collected in Viçosa and described by Sarmento et al. (2007).

[b] Population Sde Eliyahu-1 was collected from potato (*S. tuberosum*) and eggplant (*S. melongena*) in a field where also tomato was grown, but none of the tomato plants were infested with *T. evansi*. We reasoned that this lack of preference for tomato could potentially be caused by a different defence suppression phenotype, which we preferred not to select against in lab cultures. However, we did not have potato or eggplant leaves available at the time this population arrived, and therefore chose the tomato mutant *defenseless-1* (*def-1*), which does not accumulate JA after spider mite feeding (Li et al. 2002), as a host. If the lack of preference for tomato in this population was caused by an inability to suppress JA-dependent tomato defence, then we prevented selection for more potent suppressors by using *def-1* host plants.

expanded leaves, and included this difference as a variable in our analyses. We infested *pLAP-A1:GUS* plants with 45 age-synchronized (14 days after oviposition and thus 2-4 days old) *T. evansi* females for 1 day, by manually transferring individual mites with a fine brush to three leaflets of three different leaves per plant, such that each leaflet received 15 mites. We prepared a lanolin barrier around the petiole at the base of each infested leaflet to confine mites to the infested leaflets. We included a benchmark treatment for defence induction by infesting *pLAP-A1:GUS* plants with mites from a defence-inducing *T. urticae* genotype (previously called 'KMB' in Kant et al. 2008, renamed to 'Santpoort-2' by Alba et al. 2015), as well as uninfested *pLAP-A1:GUS* plants and uninfested, untransformed UC82 plants as negative controls. Uninfested *pLAP-A1:GUS* and UC82 control plants also received lanolin, as well as a mock infestation through gently touching leaflets with a clean brush.

Because a pilot experiment indicated that differences among suppression and induction benchmarks for GUS activity were most pronounced after 1 day of infestation, we harvested infested leaflets after 1 day. We digitally scanned them (HP Scanjet G3110, Hewlett-Packard, Palo Alto, USA) to determine leaf damage (see next section) and flash-froze them within 2 minutes after harvest in 15 mL tubes in liquid nitrogen for storage at -80 °C. We performed the experiments in five blocks in time, such that all 14 treatments, 11 *T. evansi* populations, the induction benchmark treatment, plus 2 controls, had a sample size of 10 to 15 plants evenly distributed across blocks (TABLE 3.2).

Leaf damage quantification

We quantified the damaged area of each infested leaflet using ImageJ v.1.49 (Rasband 2016). We transformed RGB-coloured scans of damaged leaflets to black and white images using the Type tool, and distinguished damaged from non-damaged leaf area by applying a colour threshold typical for spider mite leaf damage using the Adjust Threshold tool. After this step, leaf damage appears as black spots while undamaged leaf surface was white. The background was automatically transformed to dark and ignored during the measurements. We then selected the damaged area within the leaf with the Selection tool, and measured damaged leaf area in mm^2 by using the Analyze Particles tool. We averaged leaf areas across the three damaged leaflets into one value per plant. Each scan included a piece of millimetre paper to ensure accurate scaling of leaf size and damaged surface area.

Plants usually respond in a dose-dependent manner to spider mite damage (Gols et al. 2003, Horiuchi et al. 2003) and herbivory in general (Agrawal 2004, Niinemets et al. 2013). Therefore, we normalised our measurements of tomato gene expression (GUS assays and qRT-PCR measurements) to the absolute amount of leaf damage, to correct for variation due to differences in damaged tissue. We also present non-normalised averages to allow comparison to the uninfested control treatments which have no feeding damage.

TABLE 3.2. Treatment details and sample size (number of plants per treatment in this study).

Treatment	Mite species	Mite population	Plant genotype	Sample size
Algarrobo-1	*Tetranychus evansi*	Algarrobo-1	*pLAP-A1:GUS*	10
Carangola-1	*Tetranychus evansi*	Carangola-1	*pLAP-A1:GUS*	11
Chiyoda-1	*Tetranychus evansi*	Chiyoda-1	*pLAP-A1:GUS*	12
JT	*Tetranychus evansi*	JT	*pLAP-A1:GUS*	12
Kagoshima-1	*Tetranychus evansi*	Kagoshima-1	*pLAP-A1:GUS*	12
KM	*Tetranychus evansi*	KM	*pLAP-A1:GUS*	10
SC	*Tetranychus evansi*	SC	*pLAP-A1:GUS*	10
Sde Eliyahu-1	*Tetranychus evansi*	Sde Eliyahu-1	*pLAP-A1:GUS*	12
SV	*Tetranychus evansi*	SV	*pLAP-A1:GUS*	11
TW	*Tetranychus evansi*	TW	*pLAP-A1:GUS*	12
Viçosa-1	*Tetranychus evansi*	Viçosa-1	*pLAP-A1:GUS*	11
T. urticae	*Tetranychus urticae*	Santpoort-2	*pLAP-A1:GUS*	12
Control	-	-	*pLAP-A1:GUS*	11
UC82	-	-	UC82	15

Protein extraction and total protein quantification

We ground frozen leaf material in 15 mL tubes by vortexing for 15 s while using two slim metal rods to crush the leaflets. We repeated this step four times. We then transferred leaf material to 2 mL Eppendorf tubes and manually ground it to fine powder using a sterile pestle for 15 s, and repeated manual grinding three times. During both grinding methods, we kept our samples frozen, and afterwards stored them at -80 °C. We extracted total protein by adding 300 µL extraction buffer (50 mM $NaPO_4$ (pH 7.2), 1 mM EDTA, 0.1% v/v Triton x-100 and 0.1% v/v Sarcosyl) to each tube, mixed the samples with a sterile pestle for 10-15 s and then centrifuged them at 4 °C and 13,000 rpm for 2 min. We transferred 200 µL of the protein-rich supernatant to new Eppendorf tubes and stored these at -80 °C. To assess the total amount of protein extracted from each plant tissue sample, we transferred 199 µL miliQ water to a 96 wells plate, after which we added 1 µl protein extract. We then added 50 µL BIO RAD protein dye concentrate (BIO RAD, München, Germany) and mixed samples carefully in the tip of a pipette. We added calibration curves samples, containing 0, 0.1, 0.3, 0.5 and 0.7 mg/mL bovine serum albumin (Sigma-Aldrich, St. Louis, USA), and then incubated the plate for 2 minutes at room temperature after which we measured absorbance at 595 nm using a plate reader (Tecan infinite F50, Tecan Group, Männedorf, Switzerland).

GUS activity assay

pLAP-A1:GUS tomato plants have the *GUS* gene fused to a copy of the promoter (and part of the 5' untranslated region) of the endogenous *LAP-A1* gene, such that when the endogenous *LAP-A1* is expressed, *GUS* enzyme is produced in parallel (Chao et al. 1999). Because (young) tomato plants have no intrinsic GUS activity (Hu et al. 1990), the amount of GUS activity in *pLAP-A1:GUS* plants is proportional to the expression of the endoge-

nous *LAP-A1* gene. As a glycosidase, GUS catalyzes the breakdown of carbohydrates. GUS activity can therefore be determined in a fluorimetric assay where non-fluorescent 4-methylumbelliferyl-ß-D-glucuronide (MUG) is transformed by GUS into fluorescent 4-methylumbelliferone (MU) (Jefferson et al 1987). We transferred 25 µL protein-rich plant extract to 96 wells microtiter plates, after which we added 25 µL reaction buffer (1 mM MUG, 20 mM ß-mercaptoethanol) and mixed the samples in the tip of a pipette. We covered the microtiter plate with saran wrap and incubated it at 37 °C for 90 min. We then added 50 µL stop buffer (0.2 M $Na_2CO_3.10H_20$) to stop the reaction and added our calibration curve samples (0.0, 0.05, 0.1, 0.15, 0.2 and 0.3 mM MU) to the plate. We measured fluorescence with a plate reader (Biotek synergy MX, Biotek Instruments, Winooski, VT, USA) at wavelengths of 360 nm (excitation) and 460 nm (emission).

RNA extraction and cDNA synthesis

Of all treatments (TABLE 3.2) we extracted total plant RNA from ground, frozen leaf tissue using the hot phenol method of Verwoerd et al. (1989). We diluted RNA samples such that they reached the concentration of the lowest, and then performed a DNAse treatment using an Ambion TURBO DNAse kit (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, we added DNAse mastermix (2.0 µL 10x DNAse buffer and 0.5 µL DNAse) to 17.5 µL RNA solution, incubated the tubes at 37 °C for 40 min, added 2 µL DNAse inactivation reagent, mixed the samples gently at room temperature for 5 min, centrifuged them at 13,000 rpm for 5 min, and then transferred 12.5 µL of the supernatant to new tubes. Next, we synthesized cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). We first added 1 µL oligo $(dT)_{18}$ primer and incubated samples at 70 °C for 5 min. Then, we added 6.5 µL reverse transcriptase (RT) mastermix (4.0 µL 5x RT buffer, 2.0 µL dNTPs, 0.5 µL RT), synthesized cDNA at 42 °C for 60 min and inactivated the RT enzyme at 70 °C for 10 min. We diluted the resulting cDNA solutions 5 times.

Gene expression assays (qRT-PCR)

To investigate the degree to which the *T. evansi* populations suppressed tomato defence, we measured expression of the defence-associated tomato genes *LAP-A1*, *polyphenol oxidase-D* (*PPO-D*), *proteinase inhibitor IIc* (*PI-IIc*) and *pathogenesis-related protein 1a* (*PR-1a*) in all treatments (TABLE 3.2). We used the tomato *actin* gene as a housekeeping reference (Løvdal & Lillo 2009). Expression of *LAP-A1* was demonstrated to depend on JA defence signalling (Chao et al. 1999), and JA-dependent regulation is likely for *PPO-D* and *PI-IIc*, since tomato JA accumulation mutants have no *polyphenol oxidase-F* or any *PI-II* expression (Li et al. 2004). *PR-1a* is associated with SA signalling, because tomato *PR1a* expression increases upon exogenous application of SA (van Kan et al. 1995), and tomato *PR-1a* is highly similar to *PR-1a* in tobacco (van Kan et al. 1992), which is regulated by SA (Niki et al. 1998).

We performed qRT-PCR on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA), and prepared samples such that all genes for the same samples were run on the same plate, in duplo. The PCR program, quality control, and calculation of relative expression are explained in APPENDIX NOTE S3.1. We normalized relative expression to absolute feeding damage through dividing it by the damaged area in mm^2.

Statistics

We used R v.3.2.4 (R Core Team 2016) for all statistical analyses. First, to investigate variation among *T. evansi* populations for feeding damage, GUS activity and relative transcript abundance of tomato genes, we defined statistical models with *T. evansi* population (categorical, 11 levels) and plant stage (categorical, 2 levels) as fixed factors, and experimental block (categorical, 5 levels) as a random factor. Because the response variables are on a continuous scale, we assumed Gaussian error distributions and implemented these models using package *lme4* (Bates et al. 2015). We square root-transformed GUS activity and relative transcript abundance to meet assumptions of normality, homogeneity of variance, independence and absence of negative fitted values. We assessed the significance of the *T. evansi* population factor using approximate F tests with a Kenward-Roger approximation as implemented in the package *pbkrtest* (Halekoh and Højsgaard 2014). This approximation estimates the denominator degrees of freedom in the F test, producing decimal values. We calculated pairwise post-hoc contrasts between treatments using the package *multcomp* (Hothorn et al. 2008) and corrected for multiple testing with Holm's method.

Because our main purpose was to investigate variation in defence suppression among *T. evansi* populations, in all figures we report the results of tests from which the treatment with the defence-inducing *T. urticae* had been excluded. However, to assess if tomato responses were more induced in the *T. urticae* treatment than in the *T. evansi* treatments, as a verification of defence suppression, we separately analysed models where *T. urticae* was included as a treatment, and report their outcomes in the text of the Results section. In addition, as explained above, we normalised GUS activity and qRT-PCR results to differences in feeding damage among samples, precluding comparisons with uninfested control treatments. However, to assess if tomato defence expression differed between infested and uninfested treatments, we also analysed models where GUS activity and relative transcript abundance of defence-associated tomato genes had not been normalised, and report their outcomes in APPENDIX FIGURES S3.2 and S3.3.

Next, to explore which factors correlate with variation in tomato defence expression, we defined models with damage-corrected relative transcript abundance as a response variable (continuous), experimental block (categorical, 5 levels) and *T. evansi* population (categorical, 11 levels) as crossed random factors, and either range (categorical, 2 levels),

lab host plant (categorical, 2 levels), field host plant (categorical, 3 levels) or time in culture (continuous) as a fixed factor, as well as plant stage (categorical, 2 levels). We expressed the time that populations had been cultured in lab environments in an estimated number of generations, assuming a generation time of 14 days at 25 °C (Bonato 1999). We square root-transformed relative transcript abundance to meet model assumptions, and assessed the significance of terms using approximate F tests with a Kenward-Roger approximation.

CO1 sequencing

To determine the genetic lineage (Gotoh et al. 2009, Boubou et al. 2012) to which the *T. evansi* populations used in this study belong, we sequenced a part of the mitochondrial CO1 gene (APPENDIX NOTE S3.2). CO1 sequences were deposited in GenBank under accession numbers (to be submitted upon acceptance).

Phylogeny construction

We edited, assembled and aligned DNA sequences (900 bp) in Codoncode Aligner (v.5.0.2, Codoncode Corporation, Dedham, MA, USA). We removed primers and low quality reads and verified our contigs by using nucleotide blasts (National Centre of Biotechnology Information, USA, http://blast.ncbi.nlm.nih.gov/Blast.cgi) after which we clipped them to remove gaps at terminal sites and realigned them in MEGA v.7.0.25 (Kumar et al. 2015) using MUSCLE (Edgar 2004). This alignment consisted of 127 sequences (868 bp), plus 7 reference sequences from GenBank: a *CO1* sequence of *T. urticae* (accession number: NC_010526, Van Leeuwen et al. 2008) as an outgroup and six *T. evansi CO1* sequences (accession numbers: FJ440675, FJ440676, FJ440677 and FJ440678 [Gotoh et al. 2009] and KF447575 and KF447576 [Alba et al. 2015]). We then used jModelTest v.2.1.10 (Darriba et al. 2012) to select the General Time Reversible model (Tavaré 1986) with substitution rate variation among sites (GTR + G, gamma shape = 0.2376) as the optimal nucleotide substitution model, and constructed a maximum likelihood phylogenetic tree with 5000 bootstraps using MEGA (Hall 2013).

Results

To investigate variation in defence suppression among the *T. evansi* populations, we first quantified differences in feeding damage, and assessed the magnitude of the JA-responses via measuring GUS activity in *pLAP-1A:GUS* plants. We found significantly different amounts of damage (7 to 28 mm^2 of leaf tissue per leaflet) among populations ($F_{10,112} = 3.99$, $p < 0.001$, FIGURE S3.1). When normalised for feeding damage, GUS activity was highly variable but not significantly different among populations ($F_{10,112} = 0.78$, $p = 0.644$, FIGURE S3.2). We also observed low levels of fluorescence in some of the control treatments (FIGURE S3.2B), which could be an indication of enzymatic activ-

ity in the absence of GUS. Possibly, the activity of tomato glycosidases other than GUS introduced some background variability in our measurements (Gu et al. 1996).

To obtain more specific insight into the activation of tomato defences due to feeding by our different *T. evansi* populations, we used qRT-PCR analysis to investigate expression of the JA-responsive defence-marker genes *LAP-A1*, *PPO-D* and *PI-IIc,* and the SA-dependent gene *PR-1a*. Except for *PI-IIc,* we found significantly different expression of all three marker genes among tomatoes infested with the different *T. evansi* populations (FIGURE 3.2). Populations JT and Viçosa-1 suppressed *LAP-A1*, *PPO-D* and

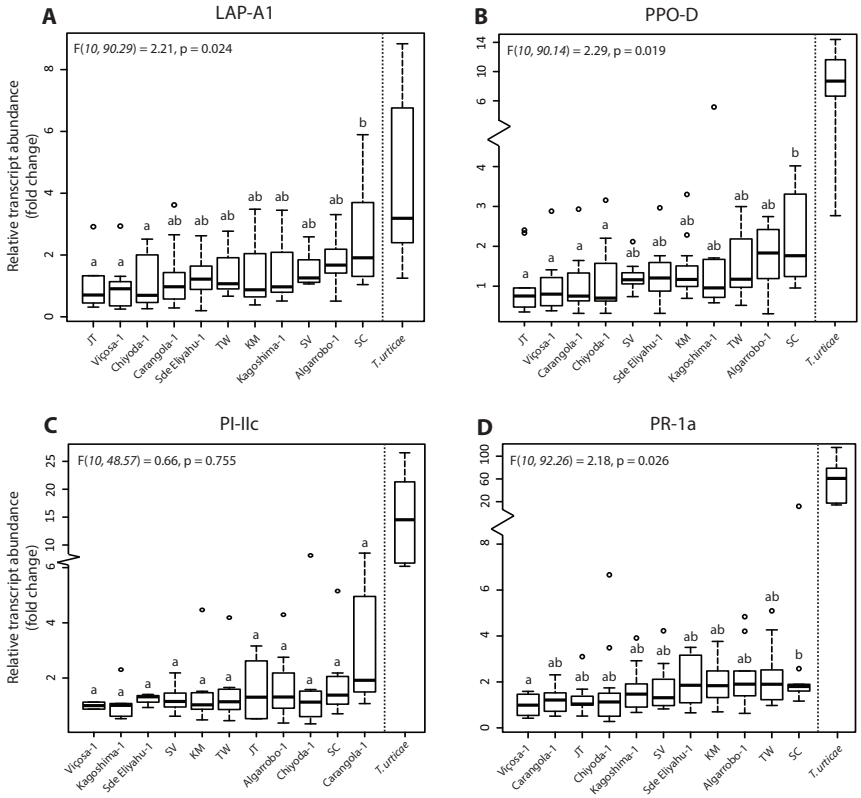


FIGURE 3.2. Expression of the plant defence-associated marker genes *LAP-A1* (**A**), *PPO-D* (**B**), *PI-IIc* (**C**) and *PR-1a* (**D**) in *LAP:GUS* tomato plants after 1 day of infestation with adult *Tetranychus evansi* or *T. urticae* females from different populations. Gene expression was measured using qRT-PCR and expressed in transcript abundance relative to that of *actin*, corrected for differences in feeding damage, and normalized to the lowest treatment mean. Details of statistical tests for differences among *T. evansi* populations are given in the upper left corners of each graph. Gene expression of plants infested by a defence-inducing *T. urticae* population is shown on the right end of each graph, but was not included in statistical tests. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences between treatments as assessed through Holm-adjusted post hoc contrasts.

PR-1a the strongest, whereas population SC allowed the strongest induction in tomato. These patterns did not correlate with differences in feeding damage, because Pearson correlations between damaged area and corrected relative transcript abundances were below 0.2 and non-significant ($p > 0.1$) for all defence marker genes.

The *T. urticae* genotype Santpoort-2, our benchmark treatment for defence induction, induced higher expression than any of the *T. evansi* populations for all marker genes (all pairwise comparisons $p < 0.05$), except for *LAP-A1* expression, which was similar between tomatoes infested with SC and Santpoort-2 ($p = 1.00$). Although *T. evansi* was previously found to sometimes suppress tomato defence expression significantly below control levels (Sarmento et al. 2011a, de Oliveira et al. 2016, Godinho et al. 2016), we found expression levels to be similar to the levels in control plants or to be slightly higher for *PPO-D*, *PI-IIc* and *PR-1a*, and to be significantly higher for *LAP-A1* (FIGURE S3.3).

To further explore the observed variation in defence suppression among *T. evansi* populations, we assessed the correlation between marker gene expression levels and either geographical range, host plant, or time in culture. We found that invasive populations tended to suppress tomato defence less strongly than native populations, and this pattern was significant for the level of *PR-1a* expression (FIGURE 3.3A). Expression levels did not correlate with the host plant species from which the *T. evansi* populations had been collected (FIGURE 3.3B). Likewise, expression levels were similar among plants infested with *T. evansi* populations cultured on *S. lycopersicum* or *S. nigrum* (FIGURE 3.3C), and did not correlate with the time that populations had been maintained in lab environments (FIGURE 3.3D).

To determine to which of the two genetically differentiated *T. evansi* lineages (Gotoh et al. 2009, Boubou et al. 2012) our populations belonged, we sequenced a part of the mitochondrial *CO1* gene, and found that all invasive populations belonged to lineage I and all native populations to lineage II (FIGURE 3.4). Geographical range and genetic lineage are therefore completely collinear variables in our dataset, which precludes disentangling their effects on variation in defence suppression among *T. evansi* populations. Within lineage II we found further differentiation within the Carangola-1 population, and our samples from the Viçosa-1 population belonged to a different haplotype than previously archived *CO1* sequences from the same population (KF447575, Alba et al. 2015).

Discussion

Multiple arthropod herbivore species suppress the defences of their hosts plants to prevent exposure to harmful plant defence and enhance herbivore performance (Musser et al. 2002, Kant et al. 2015). Lowered plant defences, however, may also increase the performance of competing herbivores and promote predation (Kant et al. 2008, Sarmento et al. 2011a, Glas et al. 2014, Ataide et al. 2016, Schimmel et al. 2017a, b). Biotic interactions among defence-suppressing herbivores and competitors or predators may there-

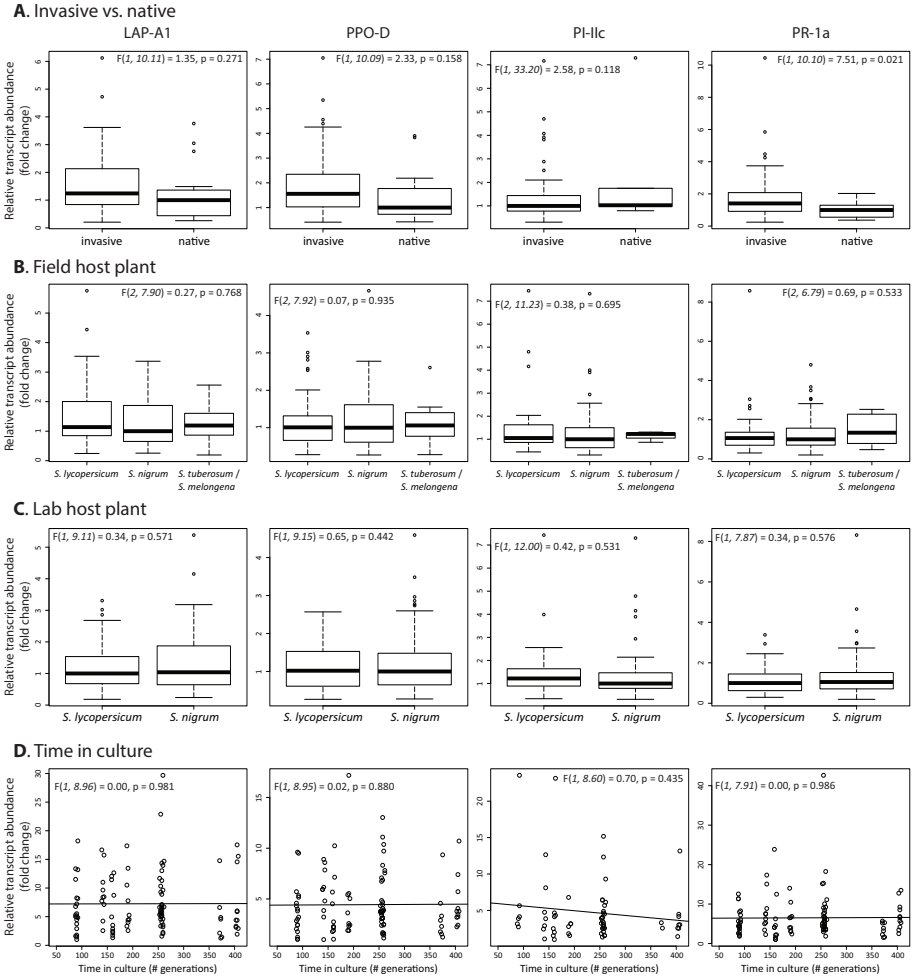


FIGURE 3.3. Variation in expression of defence-associated tomato genes compared between native and invasive populations (**A**), among field host plant species (**B**), lab host plant species (**C**), and depending on the time the populations have been cultured in the laboratory (**D**). Gene expression of *LAP-A1*, *PPO-D*, *PI-IIc* and *PR-1a* was measured using qRT-PCR and expressed in transcript abundance relative to that of *actin*, and corrected for differences in feeding damage. Details of statistical tests for differences in relative transcript abundance are given in the upper corners of each graph. In panels A-C values were normalised to the lowest treatment median. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height) and circles indicate outliers. In panel D, values were normalised to the smallest individual relative expression. Circles indicate data points and lines indicate linear model predictions for relative transcript abundance over time in culture.

fore give rise to ecological costs associated with defence suppression, and may vary among locations. To understand the role of biotic interactions in the evolution of defence suppression it is necessary to quantify variation in defence suppression across different biotic environments.

The purpose of this study was to investigate intraspecific variation in defence suppression among *T. evansi* populations from eleven locations, and secondarily to explore if suppression differed across host plant species and native or non-native ranges. We found significant variation in expression of the JA-responsive marker genes *LAP-A1* and *PPO-D*, and at the SA-dependent locus *PR-1a* (FIGURE 3.2). This shows that *T. evansi* populations suppress the two hormonal signalling pathways that regulate tomato defence expression against spider mites to varying degrees. Although the effect size of this variation was small relative to the magnitude of induction by the *T. urticae* genotype Santpoort-2, small differences in defence gene expression still can correlate with significant differences in spider mite performance (Alba et al. 2015). For example, low levels of JA-dependent defence induction reduce *T. evansi* performance considerably, but stronger induction does not reduce *T. evansi* fecundity any further (Ataide et al. 2016).

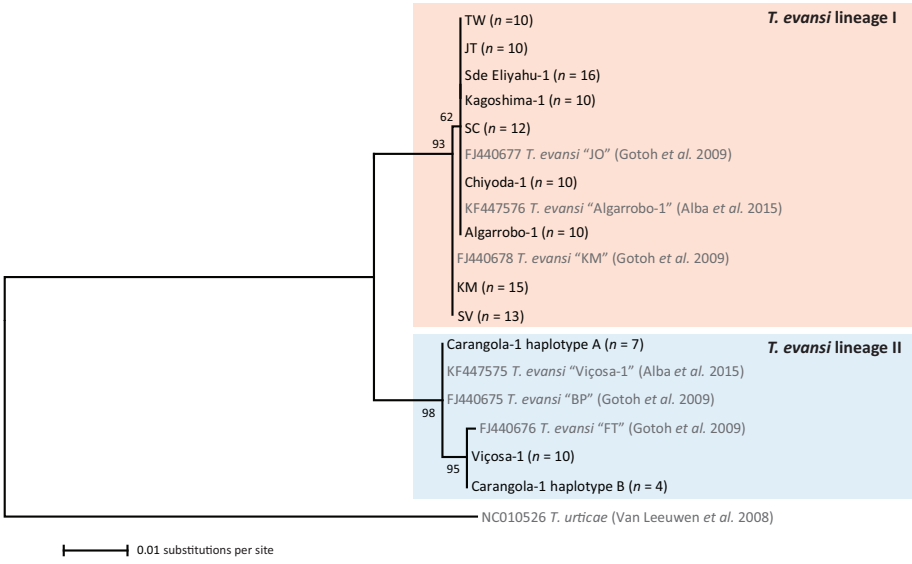


FIGURE 3.4. Phylogenetic relationships between *Tetranychus evansi* populations based on mitochondrial *CO1* gene sequences (868 bp). Relationships were inferred using the maximum likelihood method and the general time reversible model plus substitution rate variation among sites. Nucleotide positions with gaps or missing data (8.8%) were excluded. Branch support based on 5000 bootstraps is indicated above each node. Populations of which *CO1* sequences were collected in this study are shown in black font along with their sample size, and reference sequences are indicated by their GenBank accession numbers and shown in grey. The naming of the two differentiated *T. evansi* lineages is as in Boubou et al. (2012).

Because the observed variation in the level to which tomato defences are suppressed by our *T. evansi* populations likely falls within this lower range of tomato defence induction, these differences can have substantial consequences for *T. evansi* performance.

We assessed if the observed variation in defence suppression among *T. evansi* populations correlated with their geographical range or with the identity of their host plant species. We expected invasive populations to suppress plant defence more strongly than native populations, because the absence of natural enemies in invasive populations alleviates ecological costs, such as increased predation by *P. longipes* predatory mites (Ataide et al. 2016). On the contrary, we found a trend that invasive *T. evansi* populations suppress tomato defences less strongly in their invasive range than populations that are endemic to their habitat (FIGURE 3.3A). Possibly, *T. evansi* and *P. longipes* are engaged in an arms race (Dawkins and Krebs 1979) over plant defence signalling. Under this scenario, *T. evansi* is selected to suppress tomato defence to prevent detection by *P. longipes*. Because *P. longipes* is absent in areas where *T. evansi* is invasive, *T. evansi* may evolve a lower degree of defence suppression through antagonistic pleiotropy (Cooper and Lenski 2000), or it may erode through genetic drift (Halligan & Keightley 2009). To obtain more insight into the effect of enemy release (Jeffries and Lawton 1984, Colautti et al. 2004) on *T. evansi* defence suppression, future research could investigate which kind and which amounts of volatiles *P. longipes* needs to detect *T. evansi*-infested tomato plants (Sarmento et al. 2011a). Insight into how suppression of plant defence affects the recruitment and performance of other natural enemies, such as *N. floridiana* fungi (Elliot et al. 2000, Hountondji et al. 2005) also awaits further study.

Geographical range and genetic lineage are completely collinear variables in our dataset, and we cannot disentangle their effects on variation in defence suppression among *T. evansi* populations. Although morphologically similar (Gotoh et al. 2009), the two *T. evansi* lineages are partly reproductively isolated (Gotoh et al. 2009, Knegt et al. 2017). Differentiation between these lineages likely preceded invasion of areas outside South America, but among the invasive populations lineage I is more prevalent than lineage II (Boubou et al. 2012, Meynard et al. 2013). Previous studies have found that lineage I tolerates colder temperatures than lineage II (Migeon et al. 2015), and has higher expression of digestive proteases (Santamaría et al. 2018). Our results complement these findings by showing that lineage II tends to suppress tomato defences more strongly than lineage I (FIGURE 3.3A), as all our invasive *T. evansi* populations belonged to lineage I and all native populations to lineage II. Therefore, another possible explanation for the observed trend in defence suppression between native and invasive populations is that already in South America differences among the habitats of the two *T. evansi* lineages selected for different levels of defence suppression. Future work could confirm this hypothesis by characterising more *T. evansi* populations from their native South-American habitats.

The four *Solanum* host plant species used in this study vary in their defensive metabolites, and may therefore harbour different arthropod communities (Tingey 1984, Cipollini and Levey 1997, Girard et al. 2007, Nohara et al. 2007, Hartl et al. 2010, Jared et al. 2016). Because the costs of defence suppression by *T. evansi* depend on biotic interactions with competitors and predators in these communities, we hypothesized that this variation could select *T. evansi* to suppress plant defences to different degrees. However, we found no indication that host plant species identity explained variation in defence suppression among *T. evansi* populations (FIGURE 3.3B). Future work could aim to characterise arthropod communities on these host plants in nature, to be able to assess their interactions with *T. evansi* and their potential effects on defence suppression in more detail.

The tomato genes assayed in this study constitute marker genes of tomato defence induction. This does not imply direct causal relationships between their gene products and spider mite performance. Although expression of PI genes and PI activity, for example, increase upon infestation with defence-inducing *T. urticae* (Sarmento et al. 2011a, de Oliveira et al. 2016, Godinho et al. 2016, Ataide et al. 2016), and a weak negative correlation between PI activity and *T. urticae* (but not *T. evansi*) performance was observed (de Oliveira et al. 2016), the efficiency of these compounds as digestive inhibitors has been questioned because spider mite guts may lack their enzymatic targets (Santamaría et al. 2012, Arnaiz et al. 2018). Similarly, plant PPOs have been hypothesized to react with plant phenolic compounds in the herbivore gut after ingestion to produce quinones, which subsequently damage enzymes, membranes and DNA (Constabel and Barbehenn 2008), thus decreasing herbivore performance. However, because these processes might not be effective in spider mite guts due to their acidity (Erban and Hubert 2010, Martel et al. 2015), the defensive role of PPOs against spider mites also awaits experimental confirmation. Since it is not known which tomato genes have a causal relationship with spider mite performance, these defence marker genes may paint an incomplete quantitative picture, and possibly we overlook relevant defences with different induction and suppression kinetics. It would for example be interesting to also investigate the accumulation of steroidal glycoalkaloids, since these correlate with resistance of nightshades to *T. evansi* (Jared et al. 2016). Nevertheless, because *T. evansi* was previously shown to be sensitive to the magnitude of JA-defences (Ataide et al. 2016), while *PPO-D* and *PI-IIc* have been shown to be reliable markers for the magnitude of this defence (Alba et al. 2015), our results must be largely relevant.

Suppression of plant defence by herbivorous arthropods is an intriguing phenomenon due to its complex ecological consequences (Kant et al. 2015). Biotic interactions with competitors and natural enemies may shape the costs associated with defence suppression (Sarmento et al. 2011a, b, Glas et al. 2014, Ataide et al. 2016), and we found variation in defence suppression among *T. evansi* populations from various locations,

potentially related to their varying biotic environments. Notably, however, *T. evansi* is not helpless against biotic threats. In response to the presence of competing *T. urticae* mites, *T. evansi* increases its web production to secure feeding sites (Sarmento et al. 2011b), and increases fecundity to promote population growth (Schimmel et al. 2017a). Additionally, *T. evansi* males actively interfere with the reproduction of *T. urticae* females (Sato et al. 2014, 2016, Clemente et al. 2016, 2018). Moreover, in the presence of cues associated with *P. longipes*, *T. evansi* females choose to more often oviposit in their web, where their eggs are less prone to predation by *P. longipes* than on the leaf surface (Lemos et al. 2010). Although these traits may also entail costs (e.g., web production), they provide protection against competitors and natural enemies, and thus 'buffer' (Frank 2007) *T. evansi* against the negative biotic consequences of defence suppression (Blaazer et al. 2018). In this context, future research could investigate if the degree to which *T. evansi* populations engage into such buffering behaviour correlates with the variation in defence suppression observed in this study, because this may point towards ecological costs of defence suppression.

Acknowledgements

We thank dr. Arne Janssen and dr. Felipe Lemos for sending *T. evansi* population Carangola-1, Arnon Tabic for sending population Sde Eliyahu-1, prof. Tetsuo Gotoh for sending populations Chiyoda-1, JT, Kagoshima-1, KM, SC, SV and TW, prof. Linda Walling for providing *pLAP-A1:GUS* tomato seeds, and dr. Juan M Alba for help with lab analyses. This work was supported by the Dutch Science Foundation (NWO): TOP-grants 854.11.005 (to BK) and 864.10.005 (to ETK).

References

Agrawal AA (2004) Plant defense and density dependence in the population growth of herbivores. Am Nat 164:113-120.

Agrawal AA, Lau JA, Hambäck PA (2006) Community heterogeneity and the evolution of interactions between plants and insect herbivores. Q Rev Biol 81:349-376.

Alba JM, Glas JJ, Schimmel BCJ, Kant MR (2011) Avoidance and suppression of plant defenses by herbivores and pathogens. J Plant Interact 6:221-227.

Alba JM, Schimmel BCJ, Glas JJ, Ataide LMS, Pappas ML, Villarroel CA, Schuurink RC, Sabelis MW, Kant MR (2015) Spider mites suppress tomato defences downstream of jasmonate and salicylate independently of hormonal crosstalk. New Phytol 205:828-840.

Ament K, Kant MR, Sabelis MW, Haring MA, Schuurink RC (2004) Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. Plant Physiol 135:2025-2037.

Arnaiz A, Talavera-Mateo L, Gonzalez-Melendi P, Martinez M, Diaz I, Santamaria ME (2018) *Arabidopsis* Kunitz trypsin inhibitors in defense against spider mites. Front Plant Sci 9:1-16.

Ataide LMS, Pappas ML, Schimmel BCJ, Lopez-Orenes A, Alba JM, Duarte MVA, Pallini A, Schuurink RC, Kant MR (2016) Induced plant-defenses suppress herbivore reproduction but also constrain predation of their offspring. Plant Sci 252:300-310.

Azandémè-Hounmalon GY, Affognon HD, Assogba Komlan F, Tamò M, Fiaboe KKM, Kreiter S, Martin T (2015) Farmers' control practices against the invasive red spider mite, *Tetranychus evansi* Baker and Pritchard in Benin. Crop Prot 76:53.58.

Baldwin IT, Schultz JC (1983) Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. Science 221:277-279.

Bangert RK, Turek RJ, Rehill B, Wimp GM, Schweitzer JA, Allan GJ, Bailey JK, Martinsen GD, Keim P, Lindroth RL, Whitham TG (2006) A genetic similarity rule determines arthropod community structure. Mol Ecol 15:1379-1391.

Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67. doi: 10.18637/jss.v067.i01.

Bálint J, Zytynska SE, Veronika Salamon R, Mehrparvar M, Weisser WW, Schmitz OJ, Benedek K, Balog A (2016) Intraspecific differences in plant chemotype determine the structure of arthropod food webs. Oecologia 180:797-807.

Bensoussan N, Santamaria ME, Zhurov V, Diaz I, Grbić M, Grbić V. (2016) Plant-herbivore interaction: dissection of the cellular pattern of *Tetranychus urticae* feeding on the host plant. Front Plant Sci 7:1105.

Blaazer CJH, Villacis-Perez EA, Chafi R, Van Leeuwen T, Kant MR, Schimmel BCJ (2018) Why do herbivorous spider mites suppress plant defenses? Front Plant Sci 9:1-16.

Bonato O (1999) The effect of temperature on life history parameters of *Tetranychus evansi* (Acari: Tetranychidae). Exp Appl Acarol 23:11-19.

Boubou A, Migeon A, Roderick GK, Auger P, Cornuet J-M, Magalhães S, Navajas M (2012) Test of colonisation scenarios reveals complex invasion history of the red tomato spider mite *Tetranychus evansi*. PLoS One 7:e35601.

Chao WS, Gu Y-Q, Pautot V, Bray EA, Walling LL (1999) Leucine aminopeptidase RNAs, proteins, and activities increase in response to water deficit, salinity, and the wound signals systemin, methyl jasmonate, and abscisic acid. Plant Physiol 120:979-992.

Cipollini ML, Levey DJ (1997) Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. Am Nat 150:346-372.

Clemente SH, Rodrigues LR, Ponce R, Varela SAM, Magalhães S (2016) Incomplete species recognition entails few costs in spider mites, despite first-male precedence. Behav Ecol Sociobiol 70:1161-1170.

Clemente SH, Santos I, Ponce R, Rodrigues L, Varela SAM, Magalhães S (2018) Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly. Behav Ecol 29:321-327.

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721-733.

Constabel CP, Barbehenn R (2008) Defensive roles of polyphenol oxidase in plants. In: Karban R, Baldwin T (eds) Induced responses to herbivory. Chicago University Press, Chicago, pp253-269.

Cooper VS, Lenski RE (2000) The population genetics of ecological specialization in evolving *Escherichia coli* populations. Nature 407:736-739.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772.

da Silva FR, de Moraes GJ, Gondim jr MGC, Knapp M, Rouam SL, Paes JLA, de Oliveira GM (2010) Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screenhouse tomatoes. Neotrop Entomol 39:991-995.

Dawkins R, Krebs JR (1979) Arms races between and within species. Proc R Soc Lond B Biol Sci 205:489-511.

de Oliveira EF, Pallini A, Janssen A (2016) Herbivores with similar feeding modes interact through the induction of different plant responses. Oecologia 180:1-10.

Dermauw W, Wybouw N, Rombauts S, Menten B, Vontas J, Grbić M, Clark RM, Feyereisen R, Van Leeuwen T (2013) A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. Proc Natl Acad Sci U S A 110:E113-22.

Dupas S, Boscaro M (1999). Geographic variation and evolution of immunosuppressive genes in a *Drosophila* parasitoid. Ecography 22:284-291.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-1797.

Elliot SL, Sabelis MW, Janssen A, van der Geest LPS, Beerling EAM, Fransen J (2000) Can plants use entomopathogens as bodyguards? Ecol Lett 3:228-235.

Erban T, Hubert J (2010) Determination of pH in regions of the midguts of acaridid mites. J Insect Sci 10:1-12.

Ferragut F, Garzón-Luque E, Pekas A (2013) The invasive spider mite *Tetranychus evansi* (Acari: Tetranychidae) alters community composition and host-plant use of native relatives. Exp Appl Acarol 60:321-341.

Fowler, J.H., Narváez-Vásquez, J., Aromdee, D.N., Pautot, V., Holzer, F.M. & Walling, L.L. (2009) Leucine aminopeptidase regulates defense and wound signaling in tomato downstream of jasmonic acid. Plant Cell 21:1239-1251.

Frank SA (2007) Maladaptation and the paradox of robustness in evolution. PLoS One 2:e1021.

Fridman E, Wang J, Iijima Y, Froehlich JE, Gang DR, Ohlrogge J, Pichersky E (2005) Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. Plant Cell 17:1252-1267.

Gadea, J., Mayda, M.E., Conejero, V. & Vera, P. (1996) Characterization of defense-related genes ectopically expressed in viroid-infected tomato plants. Mol Plant Microbe In 9:409-415.

Girard C, Rivard D, Kiggundu A, Kunert K, Gleddie SC, Cloutier C, Michaud D (2007) A multicomponent, elicitor-inducible cystatin complex in tomato, *Solanum lycopersicum*. New Phytol 173:841-851.

Glas JJ, Alba JM, Simoni S, Villarroel CA, Stoops M, Schimmel BCJ, Schuurink RC, Sabelis MW, Kant MR (2014) Defense suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. BMC Biol 12:98.

Glassmire AE, Jeffrey CS, Forister ML, Parchman TL, Nice CC, Jahner JP, Wilson JS, Walla TR, Richards LA, Smilanich AM, Leonard MD, Morrison CR, Simbaña W, Salagaje LA, Dodson CD, Miller JS, Tepe EJ, Villamarin-Cortez S, Dyer LA (2016) Intraspecific phytochemical variation shapes community and population structure for specialist caterpillars. New Phytol 212:208-219.

Godinho DP, Janssen A, Dias T, Cruz C, Magalhães S (2016) Down-regulation of plant defence in a resident spider mite species and its effect upon con- and heterospecifics. Oecologia 180:161-167.

Gols R, Roosjen M, Dijkman H, Dicke M (2003) Induction of direct and indirect plant responses by jasmonic acid, low spider mite densities, or a combination of jasmonic acid treatment and spider mite infestation. J Chem Ecol 29:2651-2666.

Gotoh T, Araki R, Boubou A, Migeon A, Ferragut F, Navajas M (2009) Evidence of co-specificity between *Tetranychus evansi* and *Tetranychus takafujii* (Acari: Prostigmata, Tetranychidae): comments on taxonomic and agricultural aspects. Int J Acarol 35:485-501.

Gotoh T, Sugimoto N, Pallini A, Knapp M, Hernandez-Suarez E, Ferragut F, Ho C, Migeon A, Navajas M, Nachman G (2010) Reproductive performance of seven strains of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae) at five temperatures. Exp Appl Acarol 52:239-259.

Gu, Y-Q, Pautot V, Holzer FM, Walling LL (1996) A complex array of proteins related to the multimeric leucine aminopeptidase of tomato. Plant Physiol 110:1257-1266.

Halekoh U, Højsgaard S (2014) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models – the R package pbkrtest. J Stat Softw 59. doi: 10.18637/jss.v059.i09.

Hall BG (2013) Building phylogenetic trees from molecular data with MEGA. Mol Biol Evol 30:1229-1235.

Halligan DL, Keightley PD (2009) Spontaneous mutation accumulation studies in evolutionary genetics. Annu Rev Eco Evol S 40:151-172.

Hartl M, Giri AP, Kaur H, Baldwin IT (2010) Serine protease inhibitors specifically defend *Solanum nigrum* against generalist herbivores but do not influence plant growth and development. Plant Cell 22:4158-4175.

Heckel DG (2014) Insect detoxification and sequestration strategies. Annu Plant Rev 47:77-114.

Heil M (2014) Herbivore-induced plant volatiles: targets, perception and unanswered questions. New Phytol 204:297-306.

Horiuchi J-I, Arimura G-I, Ozawa R, Shimoda T, Takabayashi J, Nishioka T (2003) A comparison of the responses of *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae) to volatiles emitted from lima bean leaves with different levels of damage made by *T. urticae* or *Spodoptera exigua* (Lepidoptera: Noctuidae). Appl Entomol Zool 38:109-116.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346-363.

Hountondji FCC, Sabelis MW, Hanna R, Janssen A (2005) Herbivore-induced plant volatiles trigger sporulation in entomopathogenic fungi: the case of *Neozygites tanajoae* infecting the cassava green mite. J Chem Ecol 31:1003-1021.

Hu C-Y, Chee PP, Chesney RH, Zhou JH, Miller PD, O'Brien WT (1990) Intrinsic GUS-like activities in seed plants. Plant Cell Rep 9:1-5.

Ikeshima K, Sakamaki Y, Miyagi A, Ohno S, Fukuda T, Tsuda K (2009) Occurrence of the spider mite *Tetranychus takafujii* Ehara & Ohashi in Kyushu and Okinawa. Kyusku Pl Prot Res 55:136-140.

Jared JJ, Murungi LK, Wesonga J, Torto B (2016) Steroidal glycoalkaloids: chemical defence of edible African nightshades against the tomato red spider mite, *Tetranychus evansi* (Acari: Tetranychidae). Pest Manag Sci 72:828-836.

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901-3907.

Jeffries MJ, Lawton JH (1984) Enemy free space and the structure of ecological communities. Biol J Linn Soc Lond 23:269-286.

Jonckheere W, Dermauw W, Zhurov V, Wybouw N, Van den Bulcke J, Villarroel CA, Greenhalgh R, Grbić M, Schuurink RC, Tirry L, Baggerman G, Clark RM, Kant MR, Vanholme B, Menschaert G, Van Leeuwen T (2016) The salivary protein repertoire of the polyphagous spider mite *Tetranychus urticae*: a quest for effectors. Mol Cell Proteomics 15:3594-3613.

Kant MR, Ament K, Sabelis MW, Haring MA, Schuurink RC (2004) Differential timing of spider mite-induced direct and indirect defenses in tomato plants. Plant Physiol 135:483-495.

Kant MR, Jonckheere W, Knegt B, Lemos F, Liu J, Schimmel BCJ, Villarroel CA, Ataide LMS, Dermauw W, Glas JJ, Egas M, Janssen A, Van Leeuwen T, Schuurink RC, Sabelis MW, Alba JM (2015) Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. Ann Bot 115:1015-1051.

Kant MR, Sabelis MW, Haring MA, Schuurink RC (2008) Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. Proc R Soc Lond B Biol Sci 275:443-452.

Knegt B, Potter T, Pearson NA, Sato Y, Staudacher H, Schimmel BCJ, Kiers ET, Egas M (2017) Detection of genetic incompatibilities in non-model systems using simple genetic markers: hybrid breakdown in the haplodiploid spider mite *Tetranychus evansi*. Heredity 118:311-321.

Kumar S, Stecher G, Tamura K (2015) MEGA7: molecular evolutionary genetics analysis v.7.0 for bigger datasets. Mol Biol Evol 33:1870-1874.

Labandeira CC (1998) Early history of arthropod and vascular plant associations. Annu Rev Earth Planet Sci 26:329-377.

Lawrence SD, Novak NG, Blackburn MB (2007) Inhibition of proteinase inhibitor transcripts by *Leptinotarsa decemlineata* regurgitant in *Solanum lycopersicum*. J Chem Ecol 33:1041-1048.

Lemos F, Sarmento RA, Pallini A, Dias CR, Sabelis MW, Janssen A (2010) Spider mite web mediates anti-predator behaviour. Exp Appl Acarol 52:1-10.

Li C, Williams MM, Loh Y, Lee GI, Howe GA (2002) Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. Plant Physiol 130:494-503.

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell 16:126-143.

Løvdal T, Lillo C (2009) Reference gene selection for quantitative real-time PCR normalization in tomato subjected to nitrogen, cold, and light stress. Anal Biochem 387:238-242.

Magalhães S, Blanchet E, Egas M, Olivieri I (2009) Are adaptation costs necessary to build up a local adaptation pattern? BMC Evol Biol 9:182.

Martel C, Zhurov V, Navarro M, Martinez M, Cazaux M, Auger P, Migeon A, Santamaria ME, Wybouw N, Diaz I, Van Leeuwen T, Navajas M, Grbić M, Grbić V (2015) Tomato whole genome transcriptional response to *Tetranychus urticae* identifies divergence of spider mite-induced responses between Tomato and Arabidopsis. Mol Plant Microbe Interact 28:343-361.

Matile P (1980) Die Senfolbombe: zur Kompartimentierung des Myrosinasesystems. Biochem Physiol Pflanzen 175:722-731.

Meynard CN, Migeon A, Navajas M (2013) Uncertainties in predicting species distributions under climate change: a case study using *Tetranychus evansi* (Acari: Tetranychidae), a widespread agricultural pest. PLoS One 8:e66445.

Migeon A, Auger P, Hufbauer R, Navajas M (2015) Genetic traits leading to invasion: plasticity in cold hardiness explains current distribution of an invasive agricultural pest, *Tetranychus evansi* (Acari: Tetranychidae). Biol Inv 17:2275-2285.

Migeon A, Dorkeld F (2018) Spider Mites Web: a comprehensive database for the Tetranychidae. http://www.montpellier.inra.fr/CBGP/spmweb. Accessed 28 June 2017.

Musser RO, Hum-Musser SM, Eichenseer H, Pfeiffer M, Ervin G, Murphy JB, Felton GW (2002) Caterpillar saliva beats plant defences. Nature 416:599-600.

Navajas M, de Moraes GJ, Auger P, Migeon A (2013) Review of the invasion of *Tetranychus evansi*: biology, colonization pathways, potential expansion and prospects for biological control. Exp Appl Acarol 59:43-65.

Newman, S.M., Eannetta, N.T., Yu, H., Prince, J.P., de Vicente, M.C., Tanksley, S.D. & Steffens, J.C. (1993) Organization of the tomato polyphenol oxidase gene family. Plant Mol Biol 21:1035-1051.

Niinemets Ü, Kännaste A, Copolovici L (2013) Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. Front Plant Sci 4:262.

Niki T, Mitsuhara I, Seo S, Ohtsubo N, Ohashi Y (1998) Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol 39:500-507.

Nohara T, Ikeda T, Fujiwara Y, Matsushita S, Noguchi E, Yoshimitsu H, Ono M (2007) Physiological functions of solanaceous and tomato steroidal glycosides. J Nat Med 61:1-13.

Pluthero FG (1993). Rapid purification of high-activity *Taq* DNA polymerase. Nucleic Acids Res 21:4850-4851.

Poelman EH, van Loon JJA, Dicke M (2008) Consequences of variation in plant defense for biodiversity at higher trophic levels. Trends Plant Sci 13:534-541.

R Core Team (2016) R: A language and environment for statistical computing. Foundation for statistical computing, Vienna. https://www.R-project.org/

Randlkofer B, Obermaier E, Hilker M, Meiners T (2010) Vegetation complexity – the influence of plant species diversity and plant structures on plant chemical complexity and arthropods. Basic Appl Ecol 11:383-395.

Rankin DJ, Bargum K, Kokko H (2007) The tragedy of the commons in evolutionary biology. Trends Ecol Evol 22:643-651.

Rasband WS (1997-2016). ImageJ. U S National Institutes of Health, Bethesda. http://imagej.nih.gov/ij/

Ribeiro AEL, Gondim MGC jr, Calderan E, Delalibera I jr (2009) Host range of *Neozygites floridana* isolates (Zygomycetes: Entomophthorales) to spider mites. J Invertebr Pathol 102:196-202.

Richards LA, Dyer LA, Forister ML, Smilanich AM, Dodson CD, Leonard MD, Jeffrey CS (2015) Phytochemical diversity drives plant-insect community diversity. Proc Natl Acad Sci 112: 10973–10978.

Ruijter JM, Ramakers C, Hoogaars WMH, Karlen Y, Bakker O, van den Hoff MJB, Moorman AFM (2009). Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res 37:e45.

Santamaría ME, Auger P, Martínez M, Migeon A, Castañera P, Díaz I, Navajas M, Ortego F (2018) Host plant use by two distinct lineages of the tomato red spider mite, *Tetranychus evansi*, differing in their distribution range. J Pest Sci 91:169-179.

Santamaría ME, Cambra I, Martinez M, Pozancos C, Gonzáles-Melendi P, Grbić V, Castañera P, Ortego F, Diaz I (2012) Gene pyramiding of peptidase inhibitors enhances plant resistance to the spider mite *Tetranychus urticae*. PLoS One 7: e43011.

Sarmento RA, Lemos F, Bleeker PM, Schuurink RC, Pallini A, Oliveira MGA, Lima ER, Kant MR, Sabelis MW, Janssen A (2011a) A herbivore that manipulates plant defence. Ecol Lett 14:229-236.

Sarmento RA, Lemos F, Dias CR, Kikuchi WT, Rodrigues JCP, Pallini A, Sabelis MW, Janssen A (2011b) A herbivorous mite down-regulates plant defence and produces web to exclude competitors. PLoS One 6:e23757.

Sarmento RA, Venzon M, Pallini A, Oliveira EE, Janssen A (2007) Use of odours by *Cycloneda sanguinea* to assess patch quality. Entomol Exp Appl 124:313-318.

Sato, Y., Alba, J.M. & Sabelis, M.W. (2014) Testing for reproductive interference in the population dynamics of two congeneric species of herbivorous mites. Heredity 113:495-502.

Sato, Y., Staudacher, H. & Sabelis, M.W. (2016) Why do males choose heterospecific females in the red spider mite? Exp Appl Acarol 68:21-31.

Schimmel BCJ, Ataide LMS, Chafi R, Villarroel CA, Schuurink RC, Kant MR (2017a) Overcompensation of defense suppression by herbivores in response to competition. New Phytol 214:1688-1701.

Schimmel BCJ, Ataide LMS, Kant MR (2017b) Spatiotemporal heterogeneity of tomato induced defense responses affects spider mite performance and behavior. Plant Signal Behav 12:e1370526.

Smith J (1955). Detoxification mechanisms in insects. Biol Rev 30:455-475.

Spooner DM, Peralta IE, Knapp S (2005) Comparison of AFLPs with other markers for phylogenetic inference in wild tomatoes [*Solanum* L. section *Lycopersicon* (Mill.) Wettst.]. Taxon 54:43-61.

Staudacher H, Schimmel BCJ, Lamers MM, Wybouw N, Groot AT, Kant MR (2017) Independent effects of a herbivore's bacterial symbionts on its performance and induced plant defences. Int J Mol Sci 18:182.

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17:57-86.

Thaler JS, Humphrey PT, Whiteman NK (2012) Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci 17:260-270.

Tingey WM (1984) Glycoalcaloids as pest resistance factors. Am Potato J 61:157-167.

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635-641.

van de Ven WTG, LeVesque CS, Perring TM, Walling LL (2000) Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding. Plant Cell 12:1409-1423.

Van Kan JAL, Cozijnsen T, Danhash N, de Wit PJGM (1995) Induction of tomato stress protein mRNAs by ethephon, 2,6-dichloroisonicotinic acid and salicylate. Plant Mol Biol 27:1205-1213.

Van Kan JAL, Joosten MHAJ, Wagemakers CAM, van den Berg-Velthuis GCM, de Wit PJGM (1992) Differential accumulation of mRNAs encoding extracellular and intracellular PR proteins in tomato induced by virulent and avirulent races of *Cladosporium fulvum*. Plant Mol Biol 20:513-527.

Van Leeuwen T, Vanholme B, Van Pottelberge S, Van Nieuwenhuyse P, Nauen R, Tirry L, Denholm I (2008) Mitochondrial heteroplasmy and the evolution of insecticide resistance: non-Mendelian inheritance in action. Proc Natl Acad Sci U S A 105:5980-5985.

Verwoerd TC, Dekker BMM, Hoekema A (1989) A small-scale procedure for the rapid isolation of plant RNAs. Nucleic Acids Res 17:2362.

Villarroel CA, Jonckheere W, Alba JM, Glas JJ, Dermauw W, Haring MA, Van Leeuwen T, Schuurink RC, Kant MR (2016) Salivary proteins of spider mites suppress defences in *Nicotiana benthamiana* and promote mite reproduction. Plant J 86:119-131.

Walling LL (2000) The myriad plant responses to herbivores. J Plant Growth Regul 19:195-216.

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochem 64:3-19.

Wybouw N, Zhurov V, Martel C, Bruinsma KA, Hendrickx F, Grbić V, Van Leeuwen T (2015) Adaptation of a polyphagous herbivore to a novel host plant extensively shapes the transcriptome of herbivore and host. Mol Ecol 24:4647-4663.

Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol 143:866-875.

Appendix Note S1

PCR program, quality control, and calculation of relative expression in qRT-PCR measurements

We performed qRT-PCR on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, USA) in 20 µL reactions containing 7 µL cDNA solution, 4 µL EvaGreen (Solis BioDyne, Tartu, Estonia), 1 µL of both primers (5 µM, TABLE S3.1) and 7 µL H_20. We prepared samples such that all genes for the same samples were run on the same plate, in duplo, thus running 8 samples and 5 genes per plate. PCR conditions were initial heating at 50 °C for 2 min and 95 °C for 15 min, then 35 cycles of denaturation at 95 °C for 15 s and annealing / extension at 60 °C for 1 min, followed by a melt curve program. We inspected the amplification process of each individual reaction, and excluded reactions with a non-sigmoidal increase in fluorescence or with alternative melting curve peaks that were equally high or higher than the peak of the target amplicon (i.e., drops in fluorescence during melting curve analysis more than 2 °C different from the expected melting temperature of the target amplicon). These quality checks excluded 420 of 1336 (31%) reactions. We determined the reaction efficiency per target gene by taking the average of individual reaction efficiencies as calculated by LinRegPCR (Ruijter et al. 2009), while specifying a common threshold ^{10}log fluorescence of 4.889 (average software-determined threshold across plates, standard error = 0.005) and a window-of-linearity per target gene. We then determined the cycle threshold values (C_t-values) of each reaction by specifying the same threshold fluorescence, and averaged the two technical replicates into one C_t-value per gene per sample, through taking

$$^{2}\log\left(\frac{2^{C_t(replicate1)}+2^{C_t(replicate2)}}{2}\right).$$

For each sample, we used this C_t-value to calculate the expression of each gene relative to the expression of the *actin* housekeeping gene, as

$$\frac{efficiency\ locus^{-C_t(locus)}}{efficiency\ actin^{-C_t(actin)}}.$$

Appendix Note S2

CO1 sequencing

We extracted spider mite DNA from individual adult females of each population according to Walsh et al. (1991), by placing them in 100 µL 5% Chelex solution (Chelex 100 sodium form, Sigma-Aldrich, S*T*. Louis, USA) together with four zirconium beads, disrupting their tissues in a Precellys 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6000 rpm for 30 s, adding 5 µL proteinase K (20 mg/mL) to remove any nucleases from the solution, and incubating the samples at 56 °C for 60 min followed by denaturation at 95 °C for 8 min. Then, we amplified a part of the *CO1* gene through PCR in 25 µL solutions (13.5 µL H_2O, 2.5 µL 10x PCR buffer [H T Biotechnology, Cambridge, UK], 2.5 µL dNTPs [1 mM each], 1.2 µL bovine serum albumin [Sigma-Aldrich, St. Louis, USA], 0.5 µL of both primers [10 µM, TABLE S3.1], 0.3 µL Taq polymerase, home-made according to Pluthero [1993], and 4 µL DNA extract), through initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 20 s, annealing at 50 °C for 30 s, and extension at 72 °C for 55 s, followed by final extension at 72 °C for 10 min and cooling at 10 °C for 10 min, using a Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, USA). We confirmed PCR amplification on ethidium bromide-stained agarose gels, and then sent 10 µL solutions (1 µL PCR product, 1 µL primer (10 µM) and 8 µL H_2O) for sequencing to the Macrogen EZ-seq service (Macrogen Europe, Amsterdam, Netherlands).

TABLE S3.1. Specifications of primers used for qRT-PCR and *CO1* sequencing.

Target locus	Primer	Sequence (5' → 3')	Reference
actin	forward	TTAGCACCTTCCAGCAGATGT	Tomato Genome
	reverse	AACAGACAGGACACTCGCACT	Consortium (2012)
LAP-A1	forward	ATCTCAGGTTTCCTGGTGGAAGGA	Fowler et al. (2009)
	reverse	AGTTGCTATGGCAGAGGCAGAG	
PPO-D	forward	GCCCAATGGAGCCATATC	Newman et al. (1993)
	reverse	ACATTCGATCCACATTGCTG	
PI-IIc	forward	CAGGATGTACGACGTGTTGC	Gadea et al. (1996)
	reverse	GAGTTTGCAACCCTCTCCTG	
PR-1a	forward	TGGTGGTTCATTTCTTGCAACTAC	van Kan et al. (1992)
	reverse	ATCAATCCGATCCACTTATCATTTTA	
CO1	forward	GGAGGATTTGGAAATTGATTAGTTCC	Gotoh et al. (2009)

Costs and benefits of plant defence suppression by *Tetranychus evansi* spider mites

PhD Thesis – Bram Knegt – 2019 – University of Amsterdam

Errata

Supplementary figures and references

Chapter 3: *Tetranychus evansi* spider mite populations suppress tomato defences to varying degrees

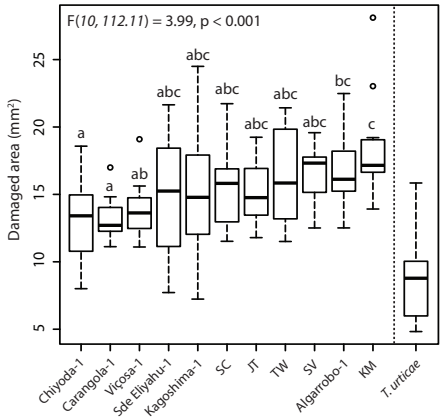


FIGURE S3.1. Leaf area damaged by *T. evansi* or *T. urticae* spider mites from different populations after 1 day of feeding. Details of a statistical test for differences among *T. evansi* populations are given in the upper left corner. Leaf area damaged by the defence-inducing *T. urticae* population is shown on the right but was not included in the statistical test. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences between treatments as assessed through Holm-adjusted post hoc contrasts.

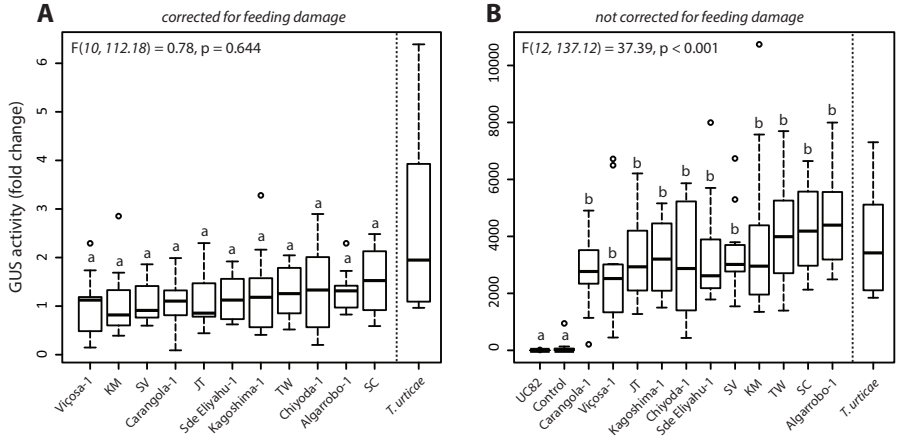


FIGURE S3.2. GUS activity in *LAP-A1:GUS* tomato plants after 1 day of infestation with *T. evansi* or *T. urticae* spider mites from different populations. GUS activity was measured in a fluorimetric assay, corrected for the total amount of protein extracted, and normalized to the lowest treatment mean. In panel **A** GUS activity was also corrected for differences in feeding damage between samples, whereas in panel **B** this correction was not performed to allow comparisons to uninfested ('Control') and uninfested, untransformed ('UC82') treatments. Details of statistical tests for differences among treatments are given in the upper left corners of each graph. GUS activity in plants after infestation by a defence-inducing *T. urticae* population is shown on the right end of each graph, but was not included in the statistical test. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences between treatments as assessed through Holm-adjusted post hoc contrasts.

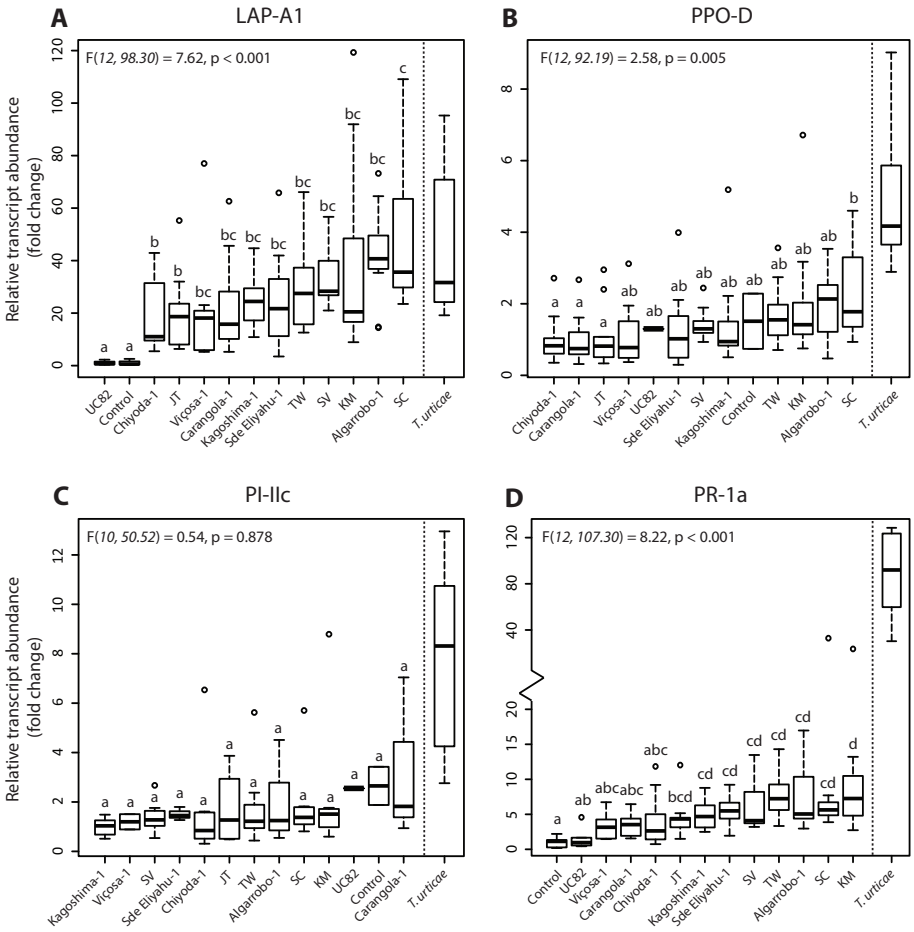


FIGURE S3.3. Expression of the plant defence-associated marker genes *LAP-A1* (A), *PPO-D* (B), *PI-IIc* (C) and *PR-1a* (D) in *LAP-A1:GUS* tomato plants after 1 day of infestation with adult *T. evansi* or *T. urticae* females from different populations. Gene expression was measured using qRT-PCR and expressed in transcript abundance relative to that of *actin* and normalized to the lowest treatment mean. These values were not corrected for differences in feeding damage among treatments, to allow comparisons to uninfested ('Control') and uninfested, untransformed ('UC82') treatments. Details of statistical tests for differences among *T. evansi* populations are given in the upper left corners of each graph. Gene expression of plants infested by a defence-inducing *T. urticae* population is shown on the right end of each graph, but was not included in statistical tests. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences between treatments as assessed through Holm-adjusted post hoc contrasts.

Literature in supplementary information

Fowler, J.H., Narváez-Vásquez, J., Aromdee, D.N., Pautot, V., Holzer, F.M. & Walling, L.L. (2009) Leucine aminopeptidase regulates defense and wound signaling in tomato downstream of jasmonic acid. *The Plant Cell*, **21**, 1239-1251.

Gadea, J., Mayda, M.E., Conejero, V. & Vera, P. (1996) Characterization of defense-related genes ectopically expressed in viroid-infected tomato plants. *Molecular Plant-Microbe Interactions*, **9**, 409-415.

Gotoh, T., Araki, R., Boubou, A., Migeon, A., Ferragut, F. & Navajas, M. (2009) Evidence of co-specificity between *Tetranychus evansi* and *Tetranychus takafujii* (Acari: Prostigmata, Tetranychidae): comments on taxonomic and agricultural aspects. *International Journal of Acarology*, **35**, 485-501.

van Kan, J.A.L., Joosten, M.H.A.J., Wagemakers, C.A.M., van den Berg-Velthuis, G.C.M. & de Wit, P.J.G.M. (1992) Differential accumulation of messenger-RNAs encoding extracellular and intracellular PR proteins in tomato induced by virulent and avirulent races of *Cladosporium fulvum*. *Plant Molecular Biology*, **20**, 513-527.

Newman, S.M., Eannetta, N.T., Yu, H., Prince, J.P., de Vicente, M.C., Tanksley, S.D. & Steffens, J.C. (1993) Organization of the tomato polyphenol oxidase gene family. *Plant Molecular Biology*, **21**, 1035-1051.

Pluthero FG (1993). Rapid purification of high-activity *Taq* DNA polymerase. *Nucleic Acids Research*, **21**, 4850-4851.

Ruijter JM, Ramakers C, Hoogaars WMH, Karlen Y, Bakker O, van den Hoff MJB, Moorman AFM (2009). Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, **37**, e45.

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, **485**, 635-641.

4

Detection of genetic incompatibilities in non-model systems using simple genetic markers: hybrid breakdown in the haplodiploid spider mite *Tetranychus evansi*

Bram Knegt, Tomos Potter, Nigel Pearson, Yukie Sato,
Heike Staudacher, Bernardus C.J. Schimmel,
E. Toby Kiers & Martijn Egas

Abstract

When two related species interbreed, their hybrid offspring frequently suffer from reduced fitness. The genetics of hybrid incompatibility are described by the Bateson-Dobzhansky-Muller (BDM) model, where fitness is reduced by epistatic interactions between alleles of heterospecific origin. Unfortunately, most empirical evidence for the BDM model comes from a few well-studied model organisms, restricting our genetic understanding of hybrid incompatibilities to limited taxa. These systems are predominantly diploid and incompatibility is often complete, which complicates the detection of recessive allelic interactions and excludes the possibility to study viable or intermediate stages. Here, we advocate research into non-model organisms with haploid or haplodiploid reproductive systems and incomplete hybrid incompatibility because (1) dominance is absent in haploids and (2) incomplete incompatibility allows comparing affected to unaffected individuals. We describe a novel two-locus statistic specifying the frequency of individuals for which two alleles co-occur. This approach to studying BDM incompatibilities requires genotypic characterization of hybrid individuals, but not genetic mapping or genome sequencing. To illustrate our approach, we investigated genetic causes for hybrid incompatibility between differentiated lineages of the haplodiploid spider mite *Tetranychus evansi*, and show that strong but incomplete hybrid breakdown occurs. In addition, by comparing the genotypes of viable hybrid males and inviable hybrid male eggs for eight microsatellite loci, we show that nuclear and cytonuclear BDM interactions constitute the basis of hybrid incompatibility in this species. Our approach opens up possibilities to study BDM interactions in non-model taxa, and may give further insight into the genetic mechanisms behind hybrid incompatibility.

Published as: Knegt et al. (2017) Detection of genetic incompatibilities in non-model systems using simple genetic markers: hybrid breakdown in the haplodiploid spider mite *Tetranychus evansi*. *Heredity* 118: 311-321.

Introduction

When two related species interbreed, they can form hybrid offspring. Hybrids often have reduced fitness compared to their non-hybrid siblings, typically caused by either a loss in fertility, a loss in viability, or both. This hybrid incompatibility is explained by negative fitness effects of interacting loci, called Bateson-Dobzhansky-Muller (BDM) incompatibilities (Bateson 1909, Dobzhansky 1936, Muller 1942, Maheshwari & Barbash 2011). Under the BDM model, epistatic interactions among alleles of heterospecific origin result in decreased fitness. Evolutionary biologists are interested in BDM incompatibilities because understanding the underlying genetics provides insight into the evolutionary processes shaping differentiation between populations. For example, it is currently not known which evolutionary forces are the major cause of hybrid incompatibilities (Maheswari & Barbash 2011), what the fate is of hybrid incompatibilities upon renewed contact between previously separated populations (Lindtke & Buerkle 2015), and to what extent populations harbour polymorphisms for BDM interactions (Cutter 2012).

Several cases of hybrid incompatibility have been genetically investigated in *Drosophila* and other model systems (reviewed in Rieseberg & Blackman 2010, Presgraves 2010, Maheshwari & Barbash 2011, Chae et al. 2014). However, this reliance on model organisms is unfortunate for two reasons. First, most well-documented cases of hybrid incompatibility involve diploid species; only three out of the 35 studies cited in the reviews mentioned above investigate non-diploid hybrids. Diploids have an inherent disadvantage for detecting incompatible allelic interactions in hybrids, because dominant allelic interactions can mask recessive interactions. Given that both theory (Charlesworth et al. 1987) and empirical work (Tao & Hartl 2003) suggest that incompatibility alleles are mostly recessive, dominance thus complicates the detection of BDM interactions in diploids. One possible solution to the problem of dominance would be to extend crossing by one generation, i.e., sampling second-generation (F2) hybrids. Recombination between parental chromosomes in the F1 generation breaks up coadapted gene complexes and detrimental interactions are therefore more likely to be expressed in the F2. Known as 'hybrid breakdown', F2 hybrids are generally more strongly affected by detrimental epistatic interactions than F1 hybrids (Stebbins 1958). In many study systems, however, the parental species have diverged to an extent that hybrid breakdown is complete and no F2 can be obtained (Orr & Presgraves 2000). Consequently, sampling F2 hybrids for the detection of BDM incompatibilities is only possible in an early stage of speciation, when hybrid incompatibility is incomplete.

The second major problem with using model organisms for detection of BDM incompatibilities is that sampling inviable individuals for genetic analysis may be difficult, because their development is often aborted at an early stage (Xu & He 2011). With sophisticated genomic tools, inviable individuals can sometimes still be sampled in

model organisms, but without such methods researchers have to infer inviable genotypes by their absence in viable individuals (e.g., Matute et al. 2010), or study hybrid sterility instead. However, hybrid sterility and inviability can have different genetic causes (Xu & He 2011), necessitating the study of both. In addition, inferring inviable genotypes from their absence in viable individuals overlooks the possibility of segregation distortion, i.e., segregation of alleles deviating from expected Mendelian ratios, whereas hybrids might be especially vulnerable to unleashed segregation distorting elements due to the breaking up of coadapted gene complexes (Johnson 2010).

Which systems are not vulnerable to the problems above, and are promising model systems for hybridization genetics? Ideally, research effort should be focused on (1) identifying hybrid incompatibilities that are *incomplete*, and (2) in sexual species with *haploid* life stages.

Incomplete or partial incompatibility occurs either when not all hybrid individuals are affected, or when hybrids have reduced fitness but are still viable and fertile. For example, Hou et al. (2015) found that hybrid incompatibilities in the yeast *Saccharomyces cerevisiae* are condition-specific, and growing them on different media allowed sampling of otherwise inviable individuals. By comparing genomes of viable and inviable individuals, these authors directly identified a two-locus BDM incompatibility, whose existence was previously disputed in yeast. The advantage of haploidy for hybridization genetics is that dominance effects are absent: any incompatibility will be expressed directly (FIGURE 4.1). Therefore, studying hybrid incompatibilities in haploid life stages does not require introgression designs or extensive hybrid F2 sampling to expose recessive deleterious interactions. In sexual eukaryotes, haploid multicellular life stages occur in a variety of organisms, including red, brown and green algae, mosses, ferns, and fungi, and at least 20% of all animal species are estimated to show haplodiploid reproduction (Crozier & Pamilo 1996). Given that most hybridization research has focused on diploids, extending the search for hybrid incompatibilities to eukaryotes with a partly haploid life cycle has the simultaneous advantage of allowing generalizations about the genetic drivers of BDM incompatibilities across a wider taxonomic diversity.

Here, we demonstrate the advantages of studying hybrid incompatibility in haploid life stages of species with incomplete incompatibility. We investigated hybrid breakdown and its genetic causes in a haplodiploid animal, the tomato red spider mite *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae), a specialist herbivore on Solanaceae. Two genetic lineages of *T. evansi* have been described on the basis of differentiation of both nuclear and mitochondrial loci (Gotoh et al. 2009). It is unknown if these genetic lineages occur sympatrically in their native range, or if they occupy different ecological niches, but interlineage hybrids have been recorded in the field (Boubou et al. 2012), providing evidence that the two genetic lineages hybridize in nature. By performing controlled crosses between the genetic lineages, we show that strong but incomplete hybrid

breakdown occurs. In addition, through sampling viable hybrid males and unhatched inviable hybrid male eggs, and by comparing their genotypes for eight microsatellite loci, we show that BDM incompatibilities underlie hybrid breakdown in this species. Finally, using 16S bacteriome sequencing, we show that it is unlikely that microbial endosymbionts are responsible for the observed reproductive incompatibilities.

Materials and methods

Mite populations and rearing conditions

Tetranychus evansi Algarrobo-1 was collected near Malaga, Spain, on a single *Solanum nigrum* (Solanaceae) plant in 2011 (36°45.487' N, 4°02.407' W). Based on mitochondrial *CO1* sequencing, this population was previously shown to belong to 'lineage I' (Alba et al. 2015). We adhere to lineage names as given in Boubou et al. (2012). *Tetranychus evan-*

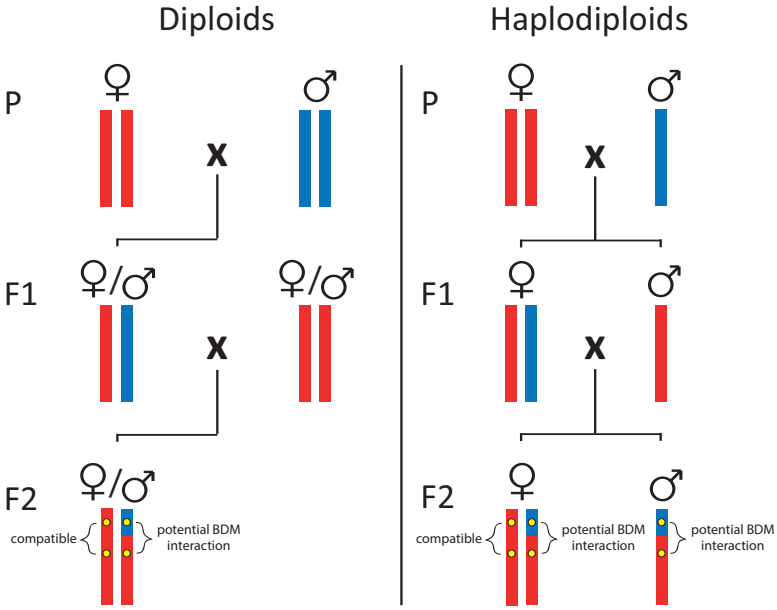


FIGURE 4.1. Detecting recessive Bateson-Dobzhansky-Muller (BDM) incompatibilities in diploids vs. haplodiploids. In the parental generation (P) a female from one species is crossed with a male from another, related species. In diploids, the offspring generation (F1) then consists of hybrid, non-recombinant males and females. In haplodiploids, F1 females also have hybrid, non-recombinant genotypes, but F1 males have non-hybrid, 'pure' maternally derived genotypes. When F1 females are backcrossed, diploid F2 offspring will have two haploid chromosome sets, one (maternally derived) recombinant set and one (paternally derived) non-recombinant set. In haplodiploids, the situation is the same for F2 females, but F2 males are haploid and carry only one recombinant chromosome set. If we assume that the BDM incompatibility shown in the F2 generation is recessive, then it will only be expressed in haplodiploid F2 males. Vertical bars represent haploid chromosome sets, coloured according to their species of origin. In the F2 generation only one of many theoretically possible genotypes is shown, depending on the number of recombination events and their locations in the genome.

si Viçosa-1 was collected in a glasshouse at the Federal University of Viçosa, Brazil, on *Solanum lycopersicum* cv. Santa Clara (Solanaceae) in 2002 (20°45.473' S, 42°52.163' W), and reared on detached leaves of the same tomato cultivar. Based on *CO1* sequencing, this population belongs to 'lineage II' (Alba et al. 2015). Mass cultures from both populations were established in our laboratory in Amsterdam in 2011 and 2010, respectively, and remained in culture for at least two years before the start of the experiments. *Tetranychus evansi* Viçosa-1 was exported from Brazil under export permit 03/2010/UTRA-VIÇ/DT-MG (National Plant Protection Organization of Brazil; register number BR-334), and imported into the Netherlands under declaration number 2010/016 (Plant Protection Service of the Netherlands). We maintained the cultures on detached leaves of *S. lycopersicum* cv. Castlemart plants (hereafter 'tomato'). Tomato plants were grown in a greenhouse (25: 18 °C; 16: 8 h photoperiod; 50-60% relative humidity) for four to six weeks, after which leaves were detached and put flat (adaxial side up) on wet cotton wool to keep them hydrated and to prevent mites from escaping. Detached leaves with mites were kept in a climate room (25 °C; 16: 8 h light: dark photoperiod; 60% relative humidity; 300 μmol m^{-2} s^{-1} light intensity).

Crosses

Prior to crossing, we transferred adult females from both mass cultures to fresh, detached tomato leaflets placed on wet cotton wool with their abaxial side up, 30 females per leaflet, and allowed them to oviposit for two days before being removed, thereby generating a cohort of offspring. After 10-12 days, the resultant offspring entered the quiescent teleiochrysalis stage, and females and males could be distinguished by their idiosomal width. As mating occurs only after adult emergence, teleiochrysalid females are thus virgin. We transferred teleiochrysalid females to fresh tomato leaflets, 25 females per leaflet, to ensure that they remained unmated. Subsequently, reciprocal crosses between, and control crosses within the two populations (in total 4 treatments) were obtained by allowing adult males from the mass cultures, 25 males per leaflet, to mate with newly emerged adult females over a period of two days.

Reproductive incompatibility

To assess reproductive incompatibility between our populations, we measured oviposition rate, hatch rate and sex ratio over two generations in all four cross treatments. We placed 40 mated females of each cross (160 in total) individually on tomato leaf discs (Ø = 14 mm) three days after adult emergence, and allowed them to oviposit. To avoid crowding in the F1 generation, we transferred each adult female to a fresh leaf disc three times (on days 2, 4, and 7) before removing the female (on day 9). We counted oviposition rate directly after female removal, and only included oviposition scores of females that survived until we transferred or removed them. Previous research estimated that *T.*

evansi eggs hatch after an average of 4.1 (SE = 0.09) days following oviposition, with a standard deviation of 0.5 days (Bonato 1999). Therefore, we determined the F1 hatch rate eight days after removing the adult female (i.e., eight standard deviations above the mean), and considered eggs inviable if they were still unhatched. Another six days later, i.e., two weeks after removing the adult females, we recorded the gender of the surviving F1 individuals and transferred teleiochrysalid F1 females to fresh leaf discs. These F1 females thus remained unmated and produced only haploid male offspring. To assess whether fertilization could 'rescue' hybrid breakdown, we also allowed virgin F1 females, 30 in each cross, to mate with adult males of either parental line in the same way as described above, thus performing backcrosses in both directions. Because adult females require on average one day to emerge from teleiochrysalis and another two days before they lay their first egg (Bonato 1999), we measured F1 oviposition between three and five or between four and six days after adult emergence. We measured F2 hatch rate as described above, i.e., eight days after oviposition.

Statistical analysis

We used R v.3.0.1 (R Development Core Team 2013) for statistical analyses. We analysed oviposition rate (eggs / female / day) assuming Gaussian distribution, and hatch rates and sex ratios assuming binomial distribution. In all models the variable of interest was cross (4 levels), which we included as a fixed term. We included leaf disc (4 levels) and mother (4 × 40 levels) as random terms in the models for parental oviposition, F1 hatch rate and F1 sex ratio, and oviposition period (3-5 or 4-6 days) and fertilization (mated, unmated) as fixed terms in the models for F1 oviposition and F2 hatch rate. We inspected model results by plotting residuals against fitted values and against all model variables, and confirmed the absence of negative fitted values, residual patterns, and, in the case of Gaussian models, non-normality and heteroscedasticity. For binomial models we confirmed the absence of overdispersion by dividing the sum of squared residuals by the residual degrees of freedom, and considered values > 1.5 to be inappropriate. We fitted mixed models using package *lme4* (Bates et al. 2013). We assessed the significance of cross treatments using F tests or likelihood ratio tests, while evaluating contrasts between treatments using package *multcomp* (Hothorn et al. 2008) or, in the case of mixed binomial models, by pooling factor levels until only significant contrasts remained (Crawley 2013).

Sampling for genetic analysis

The aim of our genetic analysis was to describe genotypic differences between viable and inviable F2 males. Therefore, we performed reciprocal interlineage crosses (2 treatments, FIGURE 4.2) as described above, and allowed groups of mated females, 25 females per leaflet, to oviposit on tomato leaflets for two days. We transferred

teleiochrysalid females of the resulting F1 generation to new leaflets, keeping them unmated, and allowed them to oviposit for four days. To be sure that unhatched F2 eggs were indicative of death rather than delayed development, as above, we sampled the resulting F2 individuals 8-10 days after oviposition. Thus, we sampled both adult F2 males and inviable F2 eggs, representing viable and inviable recombinant offspring of the two reciprocal crosses. We divided this dataset into four groups according to their phenotype (viable or inviable) and cytotype (lineage I or lineage II): viable males with a lineage I cytotype (*viable I*, n = 92), viable males with a lineage II cytotype (*viable II*, n = 94), inviable males with a lineage I cytotype (*inviable I*, n = 134), and inviable males with a lineage II cytotype (*inviable II*, n = 134) (FIGURE 4.2).

DNA extraction

After sampling, we stored adult males individually in 96% ethanol for preservation. Prior to DNA extraction, we evaporated the ethanol, and then transferred the mites into

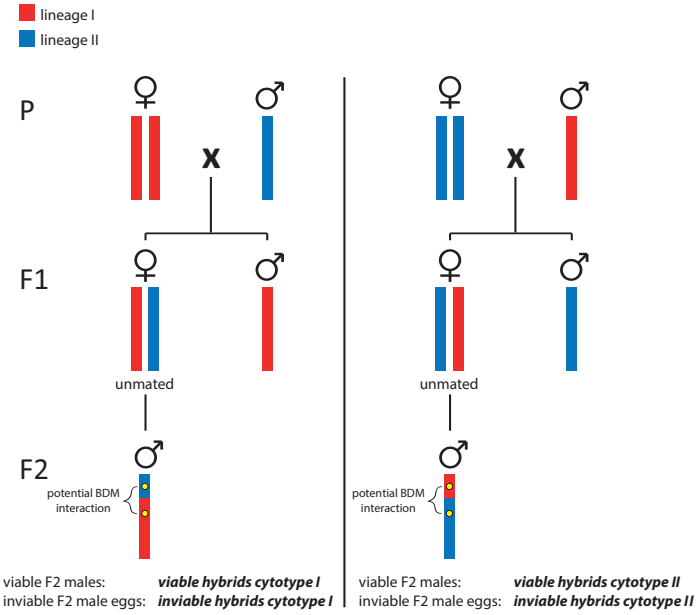


FIGURE 4.2. Production of recombinant F2 males via reciprocal interlineage crosses. In the parental generation (P) females from one lineage are crossed with males from the other lineage and *vice versa* (left and right panels show reciprocal crosses). Offspring (F1) consist of hybrid, non-recombinant females and non-hybrid males. F1 females are kept unmated to produce only hybrid, recombinant sons (F2). Vertical bars represent haploid chromosome sets, coloured according to their lineage of origin. In the F2 generation only one of many theoretically possible genotypes is shown, depending on the number of recombination events and their locations in the genome. The different hybrid groups from each cross that are included in the genetic analysis are indicated at the bottom of the figure.

1.5 mL tubes containing 50 µL of 5% Chelex solution (Bio-Rad Laboratories, MA, USA) and 3-4 zirconium beads. We homogenized samples for 20 s using a Precellys24 tissue homogenizer (Bertin Technologies, MD, USA), and added 2.5 µL of proteinase K (20 mg/mL) to each sample, followed by incubation at 56 °C for 60 minutes, then denaturation of the proteinase at 95 °C for 8 minutes. We centrifuged samples at 14,000 rpm for 2 minutes, and stored them at -20 °C until amplification. We collected individual inviable eggs using an ethanol- and flame-sterilized pin, and crushed them in 10 µL of 5x Phire buffer (Thermo Fisher Scientific, MA, USA) in a PCR tube. Samples were centrifuged at 4,000 rpm for 2 minutes and then stored at -20 °C. Prior to amplification, we pipetted the buffer-egg mix up and down to facilitate mixing of buffer and sample. To generate control samples for the PCR process, we followed the above processes without transferring a sample into the reaction medium.

Amplification by PCR

For all samples, we amplified 16 microsatellite markers in two multiplex sets of 8 microsatellites per set (Boubou et al. 2012), modified for use with Phire II Hot Start polymerase (Thermo Fisher Scientific): for adult samples, multiplex PCR was carried out in 10 µL reactions containing 2 µL 5x Phire buffer, 2 µL dNTPs (1 mM each), 0.6 µL MgCl2 (50 mM), 0.2 µL of each forward and reverse primer (10 µM each), 0.08 µL of Phire Hot Start II DNA polymerase, 0.12 µL ddH_2O and 2 µL of extracted DNA per sample. For egg samples, we replaced 5x Phire buffer with ddH_2O, and 2 µL of buffer-egg mix instead of extracted DNA. Each set of PCR reactions included a negative control: for adult samples, we used ddH_2O instead of extracted DNA; for egg samples, we used 5x Phire buffer instead of buffer-egg mix. Initial denaturation was at 98 °C for 30 s, then 35 cycles of denaturation at 98 °C for 10 s, annealing at 59 °C for 10 s and extension at 72 °C for 10 s. Final extension was at 72 °C for 60 s. PCR products were stored at -20 °C until analysis.

Genotyping

For each sample, we loaded 2 µL of PCR product with 9.66 µL of HiDi formamide (Applied Biosystems, CA, USA) and 0.34 µL of -500 LIZ size standard (Applied Biosystems) in a 96-well reaction plate, which we heated for 1 minute at 96°C. Two negative controls were run on each plate: the ABI control contained 2 µL of HiDi formamide instead of a PCR product; the PCR control contained 2 µL of control PCR product, as described previously. Plated samples were analysed by capillary electrophoresis in a 3130 Genetic Analyzer (Applied Biosystems). We used GeneMapper v.4.1 to analyse the output. In some instances we observed multiple peaks at one locus. Given that samples are haploid, this effect is most likely due to a stutter peak, i.e., a very strong signal at an adjacent allele size, or other artefact of the PCR procedure. In such

instances, we scored the largest peak as the allele size for that sample, but only if its peak area was at least double that of all other peaks at that locus. If alleles could not be distinguished by this rule, no allele was scored. Additionally, as a cut-off for background noise, we also scored no allele if the peak area was lower than 400 units.

Marker selection

To identify diagnostic markers, we genotyped 20 adult males of each parental population. Of the 16 loci, 8 markers (TABLE S4.1) were selected for further use because they were fixed for different alleles in the parental populations and had consistent success of amplification. Hybrid individuals with no allele scored at more than two out of these eight markers were excluded from genetic analyses.

Genetic analysis

Since we selected microsatellite markers for which the parental populations were fixed for different alleles, haploid F2 males have one of two possible alleles at each locus. We contrasted genotypes among the hybrid groups *viable I*, *viable II*, *inviable I* and *inviable II* (FIGURE 4.2) in three comparisons (see below). The results of this analysis are unaffected by linkage, because if there is linkage between our loci, it will affect the genotypes of each hybrid group to the same extent. Consequently, linkage may affect the genotypic composition of each hybrid group individually, but any genotypic differences among the groups cannot be caused by linkage. Similarly, genotypic comparisons between hybrid groups also do not assume Mendelian segregation of alleles, because non-random segregation would affect each hybrid group identically.

Genetic analysis: cytonuclear interactions

In order to assess cytonuclear BDM interactions, we compared *viable I* to *viable II* (comparison 1). The difference between the viable groups is their cytotype; hence differences in allele frequency would indicate that some nuclear loci interact with the cytoplasm to affect viability. We contrasted allele frequencies using Fisher's exact tests of independence for each marker separately, and adjusted the resulting p-values using a sequential Bonferroni correction procedure.

Genetic analysis: nuclear interactions

For assessing strictly nuclear BDM interactions we compared viable and inviable hybrid groups within each of the two cytotypes (comparisons 2 and 3). Since BDM interactions involve interactions between at least two loci, contrasting patterns of single allele frequencies is insufficient. Instead, we calculated a two-locus statistic specifying the frequency of individuals for which two alleles co-occur. This is a novel approach to studying BDM incompatibilities, and we therefore describe its application and interpretation in detail.

The eight markers that we studied form 28 unique marker pairs. Given that all loci have two possible alleles, for each marker pair there are four possible combinations: both alleles derived from lineage I (*I,I*), both alleles derived from lineage II (*II,II*), first allele derived from lineage I and second allele derived from lineage II (*I,II*) and first allele derived from lineage II and second allele derived from lineage I (*II,I*). Here, we refer to these allele combinations as 'haplotypes'; hence the frequency with which each allele combination occurs within a hybrid group is the haplotype count of that allele combination in that group. *I,I* and *II,II* are parental haplotypes, whereas *I,II* and *II,I* are recombinant haplotypes. Importantly, allele frequencies and haplotype counts are composed of the same data, and thus correlated. For example, if *viable I* has a lack of lineage I alleles at marker A compared to *inviable I*, then at marker pair AB it will most likely have a lack of *I,I* and *I,II* haplotypes and a surplus of *II,II* and *II,I* haplotypes. On the other hand, if in addition at marker B it has a surplus of lineage I alleles, then the marker A bias might cancel out for *I,I* and *II,II* but be even stronger for *I,II* and *II,I*. These correlations will produce a linear relation between allele frequency and haplotype count; any non-linear effect, such as epistatic interactions between loci, will obscure the relation. Therefore, deviations of the linear relation between allele frequency and haplotype counts indicate allelic interactions between loci.

In order to explicitly assess the relation between allele frequency and haplotype count, we first tested the overall difference between viable and inviable hybrid groups across haplotypes using generalized Cochran-Mantel-Haenszel tests, while adjusting p-values with a sequential Bonferroni correction. Subsequently, we subjected both the allele frequencies and the haplotype counts to a contingency table analysis, calculating the adjusted residuals of each cell (APPENDICES S4.1 and S4.2). Adjusted residuals are Pearson residuals divided by the standard deviation of all residuals, thus obtaining z-scores that follow a Gaussian distribution with zero mean and unit standard deviation (Haberman 1973). These residuals take into account both the sample size and the distribution of alleles or haplotypes for each locus or marker pair. Out of 224 adjusted haplotype residuals, one could not be computed because expected values equalled zero. In order to regress the adjusted haplotype residuals against some allele frequency metric, we defined an 'allele indicator' statistic, which is the sum of two adjusted residual allele frequencies (APPENDIX S4.3). Next, we defined a linear model that regresses adjusted haplotype residuals (continuous) on allele indicator (continuous) and an interaction between marker pair (28 levels) and haplotype (2 levels: parental, recombinant), and assessed significance of terms using likelihood ratio tests. For significant interactions, we evaluated post hoc contrasts using package *phia* (De Rosario-Martinez 2015). Under the BDM model, we expect a significant interaction between marker pair and haplotype, and specifically such that viable individuals have more parental and fewer recombinant haplotypes than inviables. The opposite pattern, where viable individuals have more recombinant haplotypes and fewer parental haplotypes, is

indicative of heterosis. Because this regression includes allele indicator as a term, any correlation between haplotypes due to differences in allele frequency is controlled for.

Mite-associated bacteria

Hybrid breakdown is not only caused by genetic mechanisms, but also by microbes such as gut (Brucker & Borderstein 2013) or endosymbiotic bacteria (Vala et al. 2000). We screened the parental Algarrobo-1 and Viçosa-1 populations for bacterial infections using two approaches. First, we assessed whether the two populations were infected with *Wolbachia*, *Cardinium*, and/or *Spiroplasma*, the most prevalent reproductive parasites among arthropods (Duron et al. 2008). To achieve this, we extracted DNA of eight adult females of each population using the Chelex protocol described above, and subsequently amplified bacterial loci using specific primers (TABLE S4.2). We included positive and negative controls for each locus, and confirmed successful DNA extraction by amplification of a 166 bp long fragment of the spider mite *β*-actin gene. We performed PCR reactions in 10 μL solutions containing 2 μL 5x Phire Buffer (Thermo Fisher Scientific), 2 μL DNTPs (1 mM each), 0.5 μL of each primer (10 μM), 4.4 μL ddH_2O, 0.1 μL Phire II Hot Start polymerase and 0.5 μL DNA sample. Initial denaturation was at 98 °C for 30 s, then 35 cycles of denaturation at 98 °C for 5 s, annealing at 57 °C (*Cardinium*), 52 °C (*Wolbachia* and *Spiroplasma*) or 58 °C (*β*-actin) for 5 s and extension at 72 °C for 10 s. Final extension was at 72 °C for 60 s. We checked amplification on 1% agarose gels using ethidium bromide staining.

Mite-associated bacteria: 16S sequencing and analysis

Second, to assess infection with other potential reproductive parasites, we performed a 16S rDNA metagenomic survey of the bacterial community in and on the parental Algarrobo-1 and Viçosa-1 populations. We Chelex-extracted DNA from 10 adult females per population, pooled into two samples of 5 individuals each. To avoid problems in downstream processing due to pollution, we diluted each sample 20 times. Subsequently, bacterial DNA was amplified at LGC Genomics (Berlin, Germany), using universal 16S primers (TABLE S4.2). Company guidelines were followed, with the exception that PCRs were run for 35 cycles. After purification and barcoding a 2 × 250 bp paired-end read library was constructed, which was sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). We analysed the output fastq files using Qiime software (Caporaso et al. 2010a), and first joined forward and reverse reads with the join_paired_ends.py algorithm. Next, we pooled the reads from the two Viçosa-1 samples, but excluded the second Algarrobo-1 sample (see Results below), and then quality-filtered our sequences with the split_libraries_fastq.py algorithm. We applied a default base call Phred threshold of 20, allowing maximum three low-quality base calls before truncating a read, including only reads with >75% consecutive high-quality base

calls, and excluding reads with ambiguous (N) base calls. Subsequently, we derived Operational Taxonomic Units (OTUs) through a uclust screen (Edgar 2010) on the GreenGenes database (DeSantis et al. 2006) with the pick_open_reference_otus.py command and a similarity cut-off of 97%. Reads with no reference in the database were clustered *de novo*. The most abundant reads from each *de novo* cluster were aligned using PyNAST (Caporaso et al. 2010b) and also included in the OTU table. We removed chimeric sequences with Chimera Slayer (Haas et al. 2011) using the identify_chimeric_seqs.py command. Finally, we manually removed reads identified as chloroplasts and mitochondria, as well as global singletons.

Mite-associated bacteria: 16S data interpretation

For reproductive parasites to have an effect on hybrid breakdown between host populations, it is assumed that hosts should be infected with different strains or species or with different densities of the same species (Vala et al. 2000). We used two methods to assess this. First, we calculated the relative abundance of infection for each OTU, and listed OTUs for which the difference between Algarrobo-1 and Viçosa-1 was more than 0.5%. Second, to avoid overlooking differences for species with low densities, we also listed OTUs for which the difference in absolute read number between Algarrobo-1 and Viçosa-1 was larger than 100. For all listed OTUs we gathered as much information as possible on the taxa in the resulting list of OTUs by searching available literature and GenBank references, and summarized this into a proposed function of each OTU within its environment. We judged the possibility of each OTU to function as a reproductive parasite in spider mites based on two criteria: if it has been found in association with arthropods, and if it has been shown to affect reproductive isolation in any species. If one of these criteria was met, we discussed the likelihood of this OTU to function as a reproductive parasite in spider mites specifically.

Results

Reproductive incompatibility

Parental females laid on average 4.48 (SE = 0.13) eggs per day, irrespective of cross treatment ($\chi^2(3) = 3.04$, $p = 0.39$). Of these eggs, 86% hatched, also independent of treatment ($\chi^2(3) = 2.37$, $p = 0.50$). Typically, *T. evansi* populations consist for 20% of males (Bonato 1999), but we found variable sex ratios among the treatments in the F1 generation, ranging from 15% males up to 36% males ($\chi^2(3) = 42.92$, $p < 0.001$, FIGURE S4.1). F1 females laid fewer eggs (3.02, SE = 0.13) than their mothers during the same part of their lives, but F1 oviposition was similar among crosses ($F_{3,294} = 0.17$, $p = 0.91$). However, fertilization increased F1 oviposition by about 8% ($F_{1,297} = 22.10$, $p < 0.001$). In the second generation of offspring (F2), we found strong but incomplete hybrid inviability: hatch rate of non-hybrids was ~91%, but only ~5% of the hybrid F2

eggs hatched ($\chi^2(3) = 1\,679.79$, $p < 0.001$, FIGURE 4.3). Fertilization did not affect these hatch rates ($\chi^2(1) = 1.19$, $p = 0.27$). Means and sample sizes for all measurements in this section are provided in supplemental TABLE S4.3.

Genetic analysis

After excluding samples with more than two missing alleles, the dataset contained the following samples: $n_{viable\ I} = 88$, $n_{viable\ II} = 80$, $n_{inviable\ I} = 68$, and $n_{inviable\ II} = 60$, with overall 9% missing data. Pooled across all hybrid groups, 46.0% (SE = 1.1%) of all alleles were derived from lineage I, and the percentages varied per marker from 32% (SE = 2.9%) to 68% (SE = 2.8%). Within each marker pair we observed all possible haplotypes at least once, confirming that the hybrids sampled in this study have recombined genomes, and that all eight microsatellite loci are nuclear (TABLE S4.4).

Cytonuclear interactions

Viable I and *viable II* had similar allele frequencies at six microsatellite loci, ranging from 0.3 to 0.8 lineage I-derived alleles (FIGURE S4.2). At loci F and G, however, allele frequencies differed significantly between the two viable lines, indicating that these loci, or loci linked to it, might interact with the cytoplasm and affect viability. Remarkably, the direction of difference was opposite for these two loci, with more lineage I alleles for

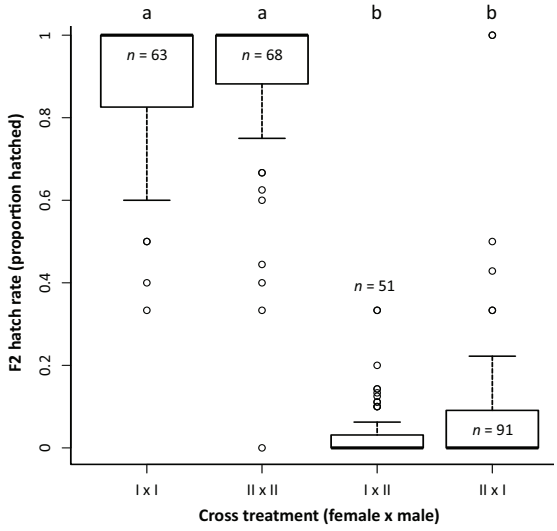


FIGURE 4.3. Effects of cross treatment on F2 hatch rate. Thick lines indicate treatment median; boxes encompass data from first to third quartile; whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height); circles indicate outliers; and different letters indicate significant differences between treatments (post hoc contrasts assessed by pooling factor levels until only significant contrasts remain, with $p < 0.05$). Sample sizes are indicated within or above each box.

viable I at locus G, but more lineage II alleles at locus F. This indicates that having lineage II-derived alleles in a lineage I cytotype background, and vice versa, can have either a positive or a negative effect on viability.

Nuclear interactions

We found that haplotype counts across all marker pairs differed significantly between *viable I* and *inviable I* (generalized Cochran-Mantel-Haenszel test: $M^2 = 108.62$, df = 3, $p < 0.001$) as well as between *viable II* and *inviable II* (generalized Cochran-Mantel-Haenszel test: $M^2 = 28.41$, df = 3, $p < 0.001$). This shows that nuclear loci affect the viability of F2 hybrid males.

In order to assess whether these effects could have been caused by interactions between nuclear loci, as predicted by the BDM model, we regressed adjusted haplotype residuals on allele indicator across all marker pairs. In the absence of BDM interactions, adjusted haplotype residuals are expected to correlate linearly with allele indicator. In contrast, if BDM interactions affect the viability of individuals, then an interaction between marker pair and adjusted haplotype residuals is expected, such that viable individuals have more parental and fewer recombinant haplotypes than inviables. In the *viable I – inviable I* comparison, we found a significant interaction between marker pair and haplotype ($F_{27,55} = 33.61$, $p < 0.001$). Post hoc tests indicated that 16 out of 28 marker pairs show significant differences between parental and recombinant haplotypes (FIGURE 4.4A), of which 6 conformed with the BDM model and 10 showed an opposite, heterotic pattern (TABLE 4.1). Similarly, in the *viable II – inviable II* comparison we also found a significant interaction between marker pair and haplotype ($F_{27,54} = 23.08$, $p < 0.001$), and 13 out of 28 marker pairs were significantly different between parental and recombinant haplotypes (FIGURE 4.4B). Of these, two followed the predictions of the BDM model, and 11 marker pairs showed the opposite pattern (TABLE 4.1). Taken together, these results show that BDM incompatibilities between some loci affect viability, but recombination between other loci had positive effects.

Reproductive parasites

We did not detect any of the known reproductive parasites *Wolbachia*, *Cardinium* or *Spiroplasma* in our *T. evansi* populations using standard PCR techniques (FIGURE S4.3).

TABLE 4.1. Number and type of interactions found across all 28 marker pairs for comparisons between viable and inviable hybrid groups.

Comparison	BDM interaction	No effect	Heterosis
Viable hybrids cytotype I – inviable hybrids cytotype I	6	12	10
Viable hybrids cytotype II – inviable hybrids cytotype II	2	15	11

The 16S sequencing procedure yielded more than 10 000 reads for three of the four samples (TABLE S4.5). One Algarrobo-1 sample, however, produced only 151 forward and reverse reads which is unsatisfactory even for a qualitative analysis, and we therefore excluded this sample from further analysis. Joining and quality filtering reduced the remaining dataset by ~30%. The uclust search against the GreenGenes database yielded 509 operational taxonomic units (OTUs), of which 105 were identified as chimeric, 9 as chloroplasts and 2 as mitochondria, and another 165 were global singletons. After removing these clusters, the final dataset consisted of 228 OTUs, of which 40 were clustered *de novo*. Both populations contributed equally to this final dataset, with 48 955 reads from Algarrobo-1 and 47 598 from Viçosa-1.

We found 11 OTUs with more than 0.5% difference in relative abundance between Algarrobo-1 and Viçosa-1 (TABLE 4.2), and 19 OTUs for which the difference in absolute read abundance between Algarrobo-1 and Viçsoa-1 was more than 100 (TABLE 4.3). Among these OTUs we found one, *Lactococcus* sp., an uncultured glucose ferment-

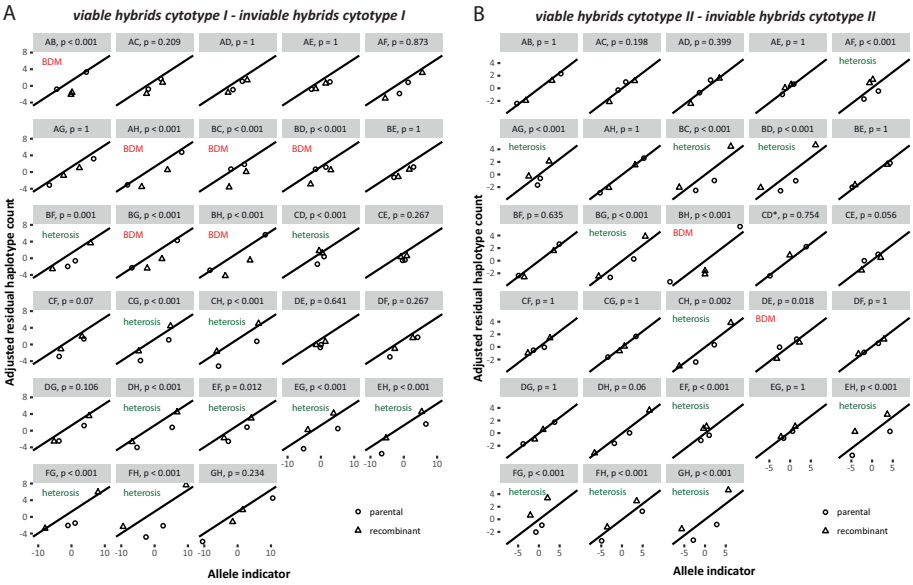


FIGURE 4.4. Assessment of allelic interactions at all 28 marker pairs in the *viable I – inviable I* comparison (**A**) and the *viable II – inviable II* comparison (**B**). Circles indicate parental haplotypes (*I.I* and *II.II*); triangles indicate recombinant haplotypes (*I.II* and *II.I*); and straight lines indicate predicted haplotype residuals based on allele indicator across all marker pairs. Deviations from this line suggest that allelic interactions affect haplotype residuals. The significance of the difference in deviation between parental and recombinant haplotypes is given above each panel (interaction contrasts, calculated using package *phia*). Whether differences indicate BDM interactions or heterosis is given in each panel. *Marker pair CD has only three values, because for the *II.I* haplotype expected values equalled 0, and no residual could be computed.

TABLE 4.2. Taxonomic assignment and proposed function of OTUs with more than 0.5% difference in relative abundance between Algarrobo-1 and Viçosa-1.

Phylum	Class	Order	Family	Genus	Species	GreenGenes OTU reference	GenBank accession number	Relative abundance (%) Algarrobo-1	Viçosa-1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*		646549	AM936014.1	31.6	34.3
Proposed function: Environmental sample form polluted industrial site									
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Psychrobacter*	*maritimus*	324143	EU000245.1	2.7	0.0
Proposed function: Bacterium sampled in South Korea, present in sea ice									
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Citrobacter*		559204	GU272271.1	13.1	10.6
Proposed function: Extracted from phyllosphere of dichlorvos-treated rape plants, found almost everywhere in soil and water									
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae			797229	HM186527.1	2.0	3.2
Proposed function: Uncultured bacterium found at 9-52 m below ground									
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Yersinia*	*ruckeri*	759061	FN668384.1	3.6	4.7
Proposed function: Fish pathogen of Atlantic salmon									
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae			637931	HM845874.1	0.0	1.0
Proposed function: Uncultured bacterium found in wounds of diabetic mice									
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	*Neisseria*		4305062	JQ453291.1	0.9	0.0
Proposed function: Uncultured bacterium found in human mouth									
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae			813457	HM461200.1	2.7	2.0
Proposed function: Enterobacter, cultured soil bacterium from Colombia									
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Paracoccus*		590586	GU061873.1	0.0	0.6
Proposed function: Uncultured marine bacterium from South Chinese Sea									
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	*lividum*	832674	HQ538673.1	0.6	0.0
Proposed function: Soil bacterium isolated from Chinese sewer system									
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Lactococcus*		716006	EU560799.1	0.5	0.0
Proposed function: Uncultured glucose fermenting bacterium found in intestinal tract of *Apriona germari* beetle larvae									

TABLE 4.3. Taxonomic assignment and proposed function of OTUs with more than 100 reads difference in absolute read abundance between Algarrobo-1 and Viçosa-1.

Phylum	Class	Order	Family	Genus	Species	GreenGenes OTU reference	GenBank accession number	Relative abundance (%) Algarrobo-1	Viçosa-1
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Psychrobacter*	*maritimus*	324143	EU000245.1	1300	0
Proposed function: Bacterium sampled in South Korea, present in sea ice									
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae			637931	HM845874.1	0	468
Proposed function: Uncultured bacterium found in wounds of diabetic mice									
Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	*Neisseria*		4305062	JQ453291.1	427	0
Proposed function: Uncultured bacterium found in human mouth									
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	*lividum*	832674	HQ538673.1	276	0
Proposed function: Soil bacterium isolated from Chinese sewer system									
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Paracoccus*		590586	GU061873.1	0	269
Proposed function: Uncultured marine bacterium from South Chinese Sea									
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Lactococcus*		716006	EU560799.1	265	0
Proposed function: Uncultured glucose fermenting bacterium found in intestinal tract of *Apriona germari* beetle larvae									
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rhodoferax*		719367	HQ008584.1	201	0
Proposed function: Phototrophic bacterium sampled in freshwater in Argentina									
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Kocuria*	*palustris*	1002005	JF064759.1	185	0
Proposed function: Aerobic actinobacterium found on *Typha angustifolia* leaves on the Danube river									
Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	*Chryseobacterium*		656229	GQ246712.1	161	0
Proposed function: Found in and frequently associated with dairy products									
Proteobacteria	Gammaproteobacteria	Legionellales				*de novo* cluster 43		0	160
Proposed function: Unknown Legionellales bacterium									
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Streptococcus*		1082539	JF146348.1	0	155
Proposed function: Uncultured bacterium found at human skin									
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*licheni-formis*	574051	FJ907196.1	0	147
Proposed function: Found in melon juice, but usually associated with soil and ground-dwelling birds									
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Achromobacter*		558264	GQ417006.1	0	145
Proposed function: Ubiquitous bacterium sampled on metal objects									

TABLE 4.3. Continued.

Phylum	Class	Order	Family	Genus	Species	GreenGenes OTU reference	GenBank accession number	Relative abundance (%) Algarrobo-1	Viçosa-1
Actinobacteria	Actinobacteria	Actinomycetales	Promicromono-sporaceae	*Cellulosimicrobium*		732609	AB489904.1	0	142
Proposed function: *Luteimicrobium subarcticum*, sampled in soil from the subarctic Rishiri Island (Japan)									
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Nevskia*	*ramosa*	104155	JF006394.1	0	129
Proposed function: Ubiquitous ammonia collecting freshwater bacterium									
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae			240468	EU234312.1	0	124
Proposed function: Uncultured bacterium found in penicillin-polluted wastewater									
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*		1083508	JF037881.1	0	112
Proposed function: Bacterium from a diverse group of soil bacteria / pathogens									
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae			866365	JF043115.1	111	0
Proposed function: Unknown Caulobacteraceae									
Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	*Aeromonas*		839235	HM779182.1	106	0
Proposed function: Dominant bacterium found in zebrafish gut									

ing bacterium identified in the intestinal tract of *Apriona germari* beetle larvae (GenBank accession number EU560799.1), that was previously found to be associated with arthropods. Since bacteria belonging to this genus ferment glucose into lactic acid, it is likely that this OTU has a role in digestion rather than serve as a reproductive parasite. None of the listed OTUs has previously been associated with reproduction of a secondary species.

Discussion

We studied hybrid incompatibility between populations of the haplodiploid spider mite *T. evansi*. Strong but incomplete hybrid breakdown was previously shown to occur between two genetic lineages of this species (Gotoh et al. 2009), and we confirmed these observations with interlineage crosses between the Algarrobo-1 and Viçosa-1 populations (FIGURE 4.3). We show that the observed hybrid breakdown has a genetic component, because allele frequency patterns and two-locus haplotype counts of viable and inviable hybrids are consistently different. More specifically, we were able to show that both nuclear and cytonuclear BDM interactions lie at the basis of hybrid incompatibility in this species (FIGURES 4.4 and S4.2, TABLE 4.1). This is in line with previous research on haplodiploid *Nasonia* wasps, where hybrid breakdown is also explained by the combined effects of nuclear and cytonuclear defects (Gibson et al. 2013). As an explanation of our results, we suggest that the two genetic lineages, over the time of their divergence, have accumulated genetic differences due to drift and adaptation, which reduce the viability of interlineage hybrids as a consequence of BDM incompatibilities.

Contrary to our expectations, we found that recombinant haplotypes at some marker pairs were overrepresented in viable genotypes compared to inviable genotypes (FIGURE 4.4, TABLE 4.1). This contrasts with the BDM model, and indicates that recombination between the two lineages also produced heterotic interactions with positive effects on viability. Heterosis is not an uncommon phenomenon, although it is usually observed in F1 progeny (Chen 2013). Heterosis in second-generation offspring, as a counterpart to hybrid breakdown, is much less studied. In one intriguing example, Kulmuni and Pamilo (2014) demonstrated sex-specific effects of recombination between parental genomes on fitness of *Formica* wood ants, where hybrid F2 females had increased fitness but hybrid F2 males were completely inviable. Future work on hybrid breakdown in other systems could validate the simultaneous occurrence of BDM incompatibilities and heterosis, as well as the occurrence of heterosis in second- and later generation offspring.

Neither of the two parental populations was infected with *Wolbachia*, *Cardinium* or *Spiroplasma* bacteria, as assessed using standard PCR techniques (FIGURE S4.3). In order to assess the possibility that other bacteria affected the reproductive compatibility of

our mite populations, we listed the OTUs with different relative (TABLE 4.2) or absolute (TABLE 4.3) abundance between the parental populations in a 16S sequencing procedure. This analysis yielded several OTUs with different abundances, but we found no indication that these specific OTUs were associated with arthropods or functioned as reproductive parasites in any species. However, due to the short sequence length several OTUs could only be identified to the family or genus level, making it difficult to assess their role in reproductive isolation. Therefore, we cannot exclude the possibility that the microbes represented by these OTUs affect reproductive isolation in spider mites through hitherto undescribed mechanisms.

In the *viable I – viable II* comparison, we found significantly different allele frequencies at two loci (FIGURE S4.2). Because all microsatellite loci used in this study are nuclear, this indicates that cytonuclear interactions affect viability. Theoretically, mitochondrial loci are expected to frequently engage in cytonuclear interactions, due to the translocation of genes for functioning of organelles to the nuclear genome (Burton et al. 2013). Indeed, cytonuclear effects on hybrid breakdown have previously been found in wasps (Koevoets et al. 2012). Another possibility is that epigenetically inherited differences, such as through genomic imprinting, methylation or histone modifications, cause changes in transposable element activation that are detrimental when active in a hybrid background (Lafon-Placette & Köhler 2015). Disentangling these alternative explanations requires identification and functional characterization of the incompatibility genes followed by a gene expression assay, to see if it is the allele per se that causes the incompatibility, as would be expected for interactions between nuclear and mitochondrial loci, or if it is a dosage-dependent effect caused by deregulation of dosage-sensitive interactions.

According to the BDM model of hybrid incompatibility interactions between alleles of heterospecific origin have detrimental effects on hybrid viability. Consistent with these predictions, at some marker pairs we found an excess of parental two-locus haplotypes and a lack of recombinant haplotypes in the viable – inviable comparisons, supporting the BDM model for explaining hybrid breakdown in *T. evansi*. An open question, however, is whether BDM incompatibility is caused by many allelic interactions with small effects, or by few interactions with large effects (Maheshwari & Barbash 2011). Although it has been shown that strong hybrid breakdown can be caused by the multiplicative effect of many loci (Dion-Côté et al. 2014), genomic rearrangements such as translocations, inversions and chromosome duplications form another potential cause of incompatibility (Brown & O'Neill 2010). Spider mites have holokinetic chromosomes, where spindle fibres attach to a diffuse kinetochore along the length of a chromosome during cell division, instead of connecting to a localized centromere. It has been suggested that due to this feature, organisms with holokinetic chromosomes exhibit aneuploidy more often, because segregation during cell division does not depend

on attachment to a centromere (d'Alençon et al. 2010). Thus, it is not unlikely that genome rearrangements could play a role in speciation in spider mites.

Under Mendelian segregation, we expected to find a 1:1 distribution of alleles at each locus. We observed, however, that pooled across all loci and hybrid groups only 46.0% (SE = 1.1%) of the observed alleles derived from lineage I, and across loci there was even more variation, ranging from 32% (SE = 2.9%) to 68% (SE = 2.8%). Correcting for sample size within each hybrid group does not affect this pattern. Since our analysis is based on comparisons between hybrid groups, we did not assume Mendelian segregation and these biased allele distributions do not harm the validity of our results. Nevertheless, it seems that alleles do not segregate randomly, which suggests that allele distributions were affected by segregation-distorting processes such as meiotic drive or by experimental artefacts such as PCR bias (Kanagawa 2003). Even though we tried to exclude PCR bias by excluding individuals with more than two missing alleles, it remains possible that certain inviable genotypes cease development earlier than others, and thus accumulate less DNA. If the markers of these individuals therefore have a lower chance of amplifying in PCR and also have non-random allele combinations, then that could explain the observed bias. Alternatively, since selfish genetic elements are hypothesized to play a role in BDM incompatibilities (Johnson 2010), meiotic drive of such loci can also disturb Mendelian segregation of alleles. However, the biased recovery of alleles observed here is mild in comparison to an overall 2:1 bias in hybrid F2 *Nasonia* wasps, where cytonuclear effects and complex incompatibilities with more than two loci were suggested as potential explanations (Gadau et al. 1999).

Our demonstration of BDM incompatibilities in *T. evansi* is based upon comparisons between genotypes of hybrid groups, distinguished by their phenotype, viable or inviable, and their cytotype. Genotypes consist of allele frequencies at eight microsatellite loci. We employed this method, because it does not require a priori knowledge of the location of these markers on the genome, of linkage between them, or of possible deviations from Mendelian segregation. In addition, because we genotyped haploid males only, dominance plays no role in the expression of BDM loci. These characteristics make this method suitable for investigation of hybrid incompatibilities in other non-model systems for which genetic markers are available. Although we used microsatellite markers, in principle any genetic marker can be used, including other genetic markers that are often used for non-model organisms without a sequenced genome, such as amplification fragment length polymorphisms (Vos et al. 1995). Arguably, the density of genetic markers on the genome in this study is low, prohibiting any conclusions about the number and location of BDM loci in *T. evansi*. Nonetheless, we see this study as a demonstration of our method to study hybrid incompatibility in non-model organisms, and as a first step to understanding hybrid incompatibility in *T. evansi*. Future work could increase the genetic marker density in order to investigate BDM incompatibilities in *T. evansi* in more detail.

Insight into the genetic mechanisms underlying speciation requires an understanding of broad patterns across a range of taxa. However, because few studies have investigated the genetic causes of hybrid incompatibility beyond the limited set of current model organisms, several important questions remain unanswered, such as: (1) which evolutionary force predominantly drives BDM incompatibilities (Maheshwari & Barbash 2011); (2) to what extent do hybrid inviability and sterility have different genetic causes (Xu & He 2011); (3) what happens to BDM incompatibilities after renewed contact between previously separated populations (Lindtke & Buerkle 2015); and (4) if it is common for populations to harbour polymorphisms for BDM interactions (Cutter 2012). We demonstrate that by studying haploid life stages in species with incomplete incompatibility, relatively simple methods are sufficient to detect allelic interactions. We expect that a similar approach works equally well in other taxa, provided that they have haploid life stages and that both affected and unaffected stages can be sampled. Since an estimated 20% of all animals show haplodiploid reproduction (Crozier & Pamilo 1996), there is ample opportunity for identification of other haplodiploid hybrid incompatibilities in the animal kingdom. More generally, hybrid breakdown has been reported in several taxa with haploid life stages, including ants (Kulmuni & Pamilo 2014), wasps (Gibson et al. 2013), algae (Niwa et al. 2010), mosses (McDaniel et al. 2008), and fungi (Turner et al. 2011), indicating potentially promising species for further study. An important next step in these taxa would be to assess the feasibility of sampling inviable hybrids, which would allow direct comparisons between viable and inviable genotypes. In this respect, the importance of finding haploid systems with incomplete hybrid inviability becomes key, as they will provide valuable insight into aspects of speciation that are otherwise hard to study in these taxa.

Data archiving

For both populations, all quality-checked and chimera-filtered 16S sequences are available at DDBJ/EMBL/GenBank under accession numbers KAHN00000000 (Algarrobo-1) and KAHO00000000 (Viçosa-1), and the versions described in this paper are the first versions KAHN01000000 and KAHO01000000. All other data sets and R scripts are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.0j4m5 (Knegt et al. 2016).

Acknowledgements

We thank the late prof. Maurice W. Sabelis for his support and guidance. This research was supported by grants from the Dutch Science Foundation (NWO): TOP-grants 854.11.005 (to BK) and 864.10.005 (to ETK), and ALW-TTI Green Genetics grant 828.08.001 (to BCJS); and from the European Research Council: ERC Grant Agreement 335542 (to ETK). YS was funded as a postdoc via the budget of prof. Maurice W.

Sabelis for his Royal Dutch Academy of Sciences (KNAW) professorship. We also thank Harold Lemereis, Ludek Tikovsky and R. Fernando Inchauste Callahuara for growing our plants, and the Population Biology research group as well as three anonymous reviewers for valuable comments on the manuscript.

References

Alba JM, Schimmel BCJ, Glas JJ, Ataide LMS, Pappas ML, Villarroel CA, et al. (2015). Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytol* **205**: 828-840.

Bates D, Maechler M, Bolker B, Walker S (2013). *lme4: Linear Mixed-effects Models using Eigen and S4*. R package version 1.0-4. URL http://CRAN.R-project.org/package=lme4

Bateson W (1909). Heredity and variation in modern lights. In: Seward AC (ed) *Darwin and Modern Science*, Cambridge University Press: Cambridge, UK, pp 85-101.

Bonato O (1999). The effect of temperature on life history parameters of *Tetranychus evansi* (Acari: Tetranychidae). *Exp Appl Acarol* **23**: 11-19.

Boubou A, Migeon A, Roderick GK, Auger P, Cornuet J, Magalhães S, et al. (2012). Test of colonization scenarios reveals complex invasion history of the red tomato spider mite *Tetranychus evansi*. *PLoS One* **7**: e35601.

Brown JD, O'Neill RJ (2010). Chromosomes, conflict, and epigenetics: chromosomal speciation revisited. *Annu Rev Genomics Hum Genet* **11**: 291-316.

Brucker RM, Bordenstein SR (2013). The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science* **341**: 667-669.

Burton RS, Pereira RJ, Barreto FS (2013). Cytonuclear genomic interactions and hybrid breakdown. *Annu Rev Ecol Evol Syst* **44**: 281-302.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. (2010a). QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335-336.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL and Knight R (2010b). PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**: 266-267.

Chae E, Bomblies K, Kim S, Karelina D, Zaidem M, Ossowski S, et al. (2014). Species-wide genetic incompatibility analysis identifies immune genes as hot spots of deleterious epistasis. *Cell* **159**: 1341-1351.

Charlesworth B, Coyne JA, Barton NH (1987). The relative rates of evolution of sex chromosomes and autosomes. *Am Nat* **130**: 113-146.

Chen ZJ (2013). Genomic and epigenetic insights into the molecular bases of heterosis. *Nat Rev Genet* **14**: 471-482.

Crawley MJ (2013). *The R Book*, 2nd edition. John Wiley and Sons: Chichester, UK.

Crozier RH, Pamilo P (1996). *Evolution of social insect colonies: sex allocation and kin selection*. Oxford University Press, Oxford, UK.

Cutter AD (2012). The polymorphic prelude to Bateson-Dobzhansky-Muller incompatibilities. *Trends Ecol Evol* **27**: 209-218.

d'Alençon E, Sezutsu H, Legeai F, Permal E, Bernard-Samain S, Giminez S, et al. (2010). Extensive synteny conservation of holokinetic chromosomes in Lepidoptera despite high rates of local genomic rearrangements. *Proc Natl Acad Sci U S A* **107**: 7680-7685.

De Rosario-Martinez H (2015). *phia: post-hoc interaction analysis*. R package version 0.2-0. URL http://CRAN.R-project.org/package=phia.

DeSantis TZ, Higenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* **72**: 5069-5072.

Dion-Côté A, Renaut S, Normandeau E, Bernatchez L (2014). RNA-seq reveals transcriptomic shock involving transposable elements reactivation in hybrids of young lake whitefish species. *Mol Biol Evol* **31**: 1188-1199.

Dobzhansky Th (1936). Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics* **21**: 113-135.

Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstädter J, et al. (2008). The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol* **6**. DOI**:** 10.1186/1741-7007-6-27.

Edgar RC (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460-2461.

Gadau J, Page RE jr, Werren JH (1999). Mapping of hybrid incompatibility loci in *Nasonia*. *Genetics* **153**: 1731-1741.

Gibson JD, Niehuis O, Peirson BRE, Cash EI, and Gadau J (2013). Genetic and developmental basis of F2 hybrid breakdown in *Nasonia* parasitoid wasps. *Evolution* **67**: 2124-2132.

Gotoh T, Araki R, Boubou A, Migeon A, Ferragut F, Navajas M (2009). Evidence of co-specificity between *Tetranychus evansi* and *Tetranychus takafujii* (Acari: Prostigmata, Tetranychidae): comments on taxonomic and agricultural aspects. *Int J Acarol* **35**: 485-501.

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, et al. (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res* **21**: 494-504.

Haberman SJ (1973). The analysis of residuals in cross-classified tables. *Biometrics* **29**: 205-220.

Hothorn T, Bretz F, Westfall P (2008). Simultaneous inference in general parametric models. *Biom J* **50**: 346-363.

Hou J, Friedrich A, Gounot J, Schacherer J (2015). Comprehensive survey of condition-specific reproductive isolation reveals genetic incompatibility in yeast. *Nat Commun* **6**: 7214.

Johnson NA (2010). Hybrid incompatibility genes: remnants of a genomic battlefield? *Trends Genet* **26**: 317-325.

Kanagawa T (2003). Bias and artifacts in multitemplate polymerase chain reactions (PCR). *J Biosci Bioeng* **96**: 317-323.

Koevoets T, van de Zande L, Beukeboom LW (2012). Temperature stress increases hybrid incompatibilities in the parasitic wasp genus *Nasonia*. *J Evol Biol* **25**: 304-316.

Kulmuni J, Pamilo P (2014). Introgression in hybrid ants is favored in females, but selected against in males. *Proc Natl Acad Sci U S A* **111**: 12805-12810.

Lafon-Placette C, Köhler C (2015). Epigenetic mechanisms of postzygotic reproductive isolation in plants. *Curr Opin Plant Biol* **23**: 39-44.

Lindtke D, Buerkle CA (2015). The genetic architecture of hybrid incompatibilities and their effect on barriers to introgression in secondary contact. *Evolution* **69**: 1978-2004.

Maheshwari S, Barbash DA (2011). The genetics of hybrid incompatibilities. *Annu Rev Genet* **45**: 331-355.

Matute DR, Butler IA, Turissini DA, Coyne JA (2010). A test of the snowball theory of the rate of evolution of hybrid incompatibilities. *Science* **329**: 1518-1521.

McDaniel SF, Willis JH, Shaw AJ (2008). The genetic basis of developmental abnormalities in interpopulation hybrids of the moss *Ceratodon purpureus*. *Genetics* **179**: 1425-1435.

Muller HJ (1942). Isolating mechanisms, evolution and temperature. *Biol Symp* **6**: 71-125.

Niwa K, Kobiyama A, Sakamoto T (2010). Interspecific hybridization in the haploid blade-forming marine crop *Porphyra* (Bangiales, Rhodophyta): occurrence of allodiploidy in surviving F1 gametophytic blades. *J Phycol* **46**: 693-702.

Orr HA, Presgraves DC (2000). Speciation by postzygotic isolation: forces, genes and molecules. *BioEssays* **12**: 1085-1094.

Presgraves DC (2010). The molecular evolutionary basis of species formation. *Nat Rev Genet* **11**: 175-180.

R Development Core Team (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Rieseberg LH, Blackman BK (2010). Speciation genes in plants. *Ann Bot* **106**: 439-455.

Stebbins GL (1958). The inviability, weakness, and sterility of interspecific hybrids. *Adv Genet* **9**: 147-215.

Tao Y, Hartl DL (2003). Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauretania*. III. Heterogeneous accumulation of hybrid incompatibilities, degree of dominance, and implications for Haldane's rule. *Evolution* **57**: 2580-2598.

Turner E, Jacobson DJ, Taylor JW (2011). Genetic architecture of a reinforced, postmating, reproductive isolation barrier between *Neurospora* species indicates evolution via natural selection. *PLoS Genet* **7**: e1002204.

Vala F, Breeuwer JAJ, Sabelis MW (2000). Wolbachia-induced 'hybrid breakdown' in the two-spotted spider mite *Tetranychus urticae* Koch. *Proc R Soc B* **267**: 1931-1937.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* **23**: 4407-4414.

Xu M, He X (2011). Genetic incompatibility dampens hybrid fertility more than hybrid viability: yeast as a case study. *PLoS One* **6**: e18341.

Supporting Information

TABLE S4.1. Specifications of microsatellite markers used in genetic analyses. Allele sizes were determined by genotyping 20 adult males of the parental populations. All eight markers were fixed for different alleles in the parental populations. Each primer pair had a fluorescent dye attached to either the forward or reverse primer indicated by colour codes *PET*, *VIC*, or *FAM*.

Locus name (this study)	Locus name (Boubou et al. 2012)	Allele size lineage I (bp)	Allele size lineage II (bp)	Primer sequence (5' to 3')
A	evaTG1-D9[SA]	253	247	Fw: *PET*-GCCAATTGAAGGGTTACAGG
				Rev: CATTCACAAGCAATGTTATTACCAG
B	evaTC2-A2	170	172	Fw: *PET*-CAATTGATGGTTTCTGTTTGG
				Rev: CATTATCGCTTCACTCATCGTC
C	evaATCT2-G11	121	124	Fw: GGTTGACCGTGAAGAGAG
				Rev: *VIC*-CAGAATGACAGTTACAATTGC
D	evaTC3-D3	121	124	Fw: *FAM*-GTCGTCCGGGATTTTTTCTG
				Rev: GTTGCTTGTTTAATTGTTGTCACTG
E	evaTC5-E6[1]	302	298	Fw: CCACGACCAGTCTTGATTG
				Rev: *PET*-GTGATGTCGAATGAGCAGG
F	evaTC1-H4	166	184	Fw: *PET*-CGATATAATTGTCAATGGTG
				Rev: GTATCAAGTATATTCCTATATGATG
G	evaATCT1-H4	166	184	Fw: GCACAGTTGAGAACGGGCTAAG
				Rev: *VIC*-CCTGTTTCTTTACTCATCCTGTCCC
H	evaTC1-A12	166	183	Fw: AGCATTTTAATGTTTCCTTTG
				Rev: *FAM*-GTTTCACTTGTAAATGGCTATG

TABLE S4.2. Specifications of primers used for the detection of symbionts.

Target	Primer	Sequence	Locus	Fragment size	Ref.
Wolbachia	76F	5'-TTGTAGCCTGCTATGGTATAACT-3'	*16S*	900 bp	O'Neill
	1012R	5'-GAATAGGTATGATTTTCATGT-3			et al. 1992
Cardinium	CLO-F	5'-GCGGTGTAAAATGAGCGTG-3'	*16S*	450 bp	Weeks
	CLO-R1	5'-ACCTMTTCTTAACTCAAGCCT-3'			et al. 2003
Spiroplasma	ApDnaAF1	5'-ATTCTTCAGTAAAAATGCTTGGA-3'	*dnaA*	385 bp	Fukatsu
	ApDnaAR1	5'-ACACATTTACTTCATGCTATTGA-3'			et al. 2001
Tetranychus evansi	β-actin-F	5'-CAGCCATGTATGTTGCCATC-3'	β-*actin*	166 bp	Feng
	β-actin-R	5'-AAATCACGACCAGCCAAATC-3'			et al. 2010
Bacterial DNA	341F	5'-TCCTACGGGNGGCWGCAG-3'	*16S*	444 bp	Modified
	785R	5'-TGACTACHVGGGTATCTAAKCC-3'			from
					Klindworth
					et al. 2013

TABLE S4.3. Means (standard error) and sample sizes for parental oviposition (eggs per day), F1 hatch rate (proportion hatched), F1 sex ratio (proportion male), F1 oviposition (eggs per day) and F2 hatch rate (proportion hatched) in all cross treatments (female × male). For parental oviposition, F1 hatch rate and F1 sex ratio we report a time series over four consecutive leaf discs from day 0 until day 9. Sample sizes decrease over time as data are excluded due to female mortality. Note that data from one individual female can be included in up to four time points. Hence, these data are not independent, and we included female identity as a random factor in our statistical analyses. In addition, because female mortality differed among treatments, we included the leaf disc sequence as a random factor as well.

Parental oviposition	**Day 0 – 2**	**n**	**Day 2 – 4**	**n**	**Day 4 – 7**	**n**	**Day 7 – 9**	**n**
I × I	4.58 (0.38)	33	5.11 (0.53)	28	4.63 (0.68)	18	3.18 (0.74)	11
II × II	4.93 (0.41)	37	4.68 (0.41)	31	2.27 (0.60)	17	3.17 (1.74)	3
I × II	5.62 (0.40)	38	5.23 (0.39)	28	2.79 (0.66)	16	5.50 (1.50)	2
II × I	4.36 (0.26)	39	4.93 (0.47)	27	2.83 (0.44)	23	4.67 (0.64)	6
F1 hatch rate								
I × I	0.85 (0.03)	32	0.80 (0.04)	28	0.87 (0.02)	24	0.74 (0.09)	12
II × II	0.92 (0.03)	35	0.89 (0.02)	32	0.85 (0.06)	22	0.71 (0.16)	7
I × II	0.92 (0.02)	37	0.79 (0.05)	34	0.79 (0.07)	24	0.81 (0.07)	8
II × I	0.76 (0.04)	37	0.83 (0.04)	33	0.85 (0.03)	22	0.80 (0.05)	9
F1 sex ratio								
I × I	0.15 (0.04)	32	0.13 (0.03)	25	0.21 (0.04)	24	0.18 (0.14)	7
II × II	0.28 (0.05)	34	0.17 (0.02)	30	0.24 (0.09)	11	0.39 (0.20)	3
I × II	0.44 (0.06)	32	0.32 (0.06)	28	0 (0)	6	0.83 (0.11)	5
II × I	0.24 (0.06)	29	0.19 (0.04)	29	0.13 (0.03)	18	0.28 (0.16)	6
F1 oviposition	**Unmated**	**n**	**Mated**	**n**				
I × I	3.62 (0.38)	39	3.31 (0.42)	27				
II × II	2.88 (0.26)	49	3.41 (0.37)	22				
I × II	3.11 (0.41)	27	3.34 (0.42)	25				
II × I	2.60 (0.29)	84	2.69 (0.37)	27				
F2 hatch rate								
I × I	0.89 (0.03)	36	0.92 (0.03)	27				
II × II	0.89 (0.03)	46	0.93 (0.05)	22				
I × II	0.02 (0.01)	28	0.06 (0.02)	23				
II × I	0.07 (0.02)	67	0.05 (0.02)	24				

TABLE S4.4. Haplotype counts for all hybrid groups and marker pairs. Per hybrid group, sample size is indicated in italics in a separate column.

	Viable hybrids					Viable hybrids					Inviable hybrids					Inviable hybrids				
Marker	cytotype I					cytotype II					cytotype I					cytotype II				
pair	I,I	II,II	I,II	II,I	*n*	I,I	II,II	I,II	II,I	*n*	I,I	II,II	I,II	II,I	*n*	I,I	II,II	I,II	II,I	*n*
AB	42	13	4	17	*76*	27	13	4	27	*71*	12	11	8	17	*48*	7	16	7	11	*41*
AC	16	21	23	6	*66*	11	28	19	10	*68*	7	22	16	12	*57*	8	14	8	14	*44*
AD	14	19	20	8	*61*	10	25	19	10	*64*	7	19	10	12	*48*	8	10	6	14	*38*
AE	13	21	32	11	*77*	6	19	17	13	*55*	5	16	17	9	*47*	3	19	11	10	*43*
AF	8	22	36	9	*75*	10	17	18	20	*65*	12	12	11	18	*53*	8	18	7	10	*43*
AG	28	15	18	17	*78*	5	23	24	12	*64*	6	24	16	8	*54*	8	18	8	9	*43*
AH	40	7	6	25	*78*	25	8	6	32	*71*	6	16	17	15	*54*	6	15	10	14	*45*
BC	21	16	35	1	*73*	13	10	41	7	*71*	9	10	28	12	*59*	13	17	9	12	*51*
BD	19	13	31	4	*67*	13	9	37	7	*66*	9	7	20	12	*48*	13	16	6	12	*47*
BE	20	12	46	5	*83*	15	10	26	6	*57*	8	12	26	6	*52*	6	17	15	11	*49*
BF	14	12	52	4	*82*	25	8	28	7	*68*	17	10	17	10	*54*	7	14	13	14	*48*
BG	44	10	24	7	*85*	12	9	40	6	*67*	9	15	27	5	*56*	8	17	12	13	*50*
BH	58	3	10	14	*85*	49	5	9	11	*74*	11	10	24	11	*56*	9	15	12	16	*52*
CD	20	44	2	4	*70*	21	47	1	0	*69*	22	32	0	0	*54*	27	25	0	0	*52*
CE	5	35	17	17	*74*	8	22	11	12	*53*	5	30	12	11	*58*	8	17	18	10	*53*
CF	4	35	19	15	*73*	11	23	10	21	*65*	14	23	8	18	*63*	11	19	12	11	*53*
CG	16	19	7	34	*76*	7	33	12	12	*64*	9	37	12	7	*65*	12	20	13	10	*55*
CH	18	6	5	47	*76*	18	9	4	40	*71*	12	30	10	13	*65*	13	17	14	13	*57*
DE	5	29	18	16	*68*	8	19	10	11	*48*	5	19	12	8	*44*	8	13	18	8	*47*
DF	5	32	19	11	*67*	10	21	10	19	*60*	14	16	8	12	*50*	11	14	12	10	*47*
DG	19	19	5	27	*70*	7	29	11	12	*59*	9	25	12	5	*51*	12	16	13	8	*49*
DH	21	7	3	39	*70*	17	9	4	36	*66*	12	21	10	8	*51*	13	13	14	11	*51*
EF	5	43	21	14	*83*	9	19	10	15	*53*	11	24	3	16	*54*	10	24	7	10	*51*
EG	18	23	10	35	*86*	8	28	10	7	*53*	10	36	6	5	*57*	11	26	6	9	*52*
EH	26	11	2	47	*86*	15	8	6	28	*57*	10	31	5	9	*55*	13	24	5	12	*54*
FG	14	28	6	37	*85*	13	29	17	4	*63*	16	30	14	0	*60*	20	29	2	2	*53*
FH	16	9	4	56	*85*	33	13	1	21	*68*	21	28	10	2	*61*	20	26	3	5	*54*
GH	54	13	0	21	*88*	20	14	0	33	*67*	15	38	1	8	*62*	21	28	2	6	*57*

TABLE S4.5. Number of sequencing reads after joining paired-end reads and quality filtering. As the number of forward and reverse unjoined reads is identical, this table gives the number of forward *or* reverse unjoined reads, but not their sum. For downstream analysis the Viçosa-1 samples were pooled, whereas the Algarrobo-1_2 sample was excluded.

Sample	Number of unjoined reads	Number of joined reads	Number of reads after quality filtering	Number of reads after OTU filtering
Algarrobo-1_1	77116	59367	53929	48955
Algarrobo-1_2	151	*excluded*		
Viçosa-1_1	54915	42462	38537	47598
Viçosa-1_2	14396	11420	10226	

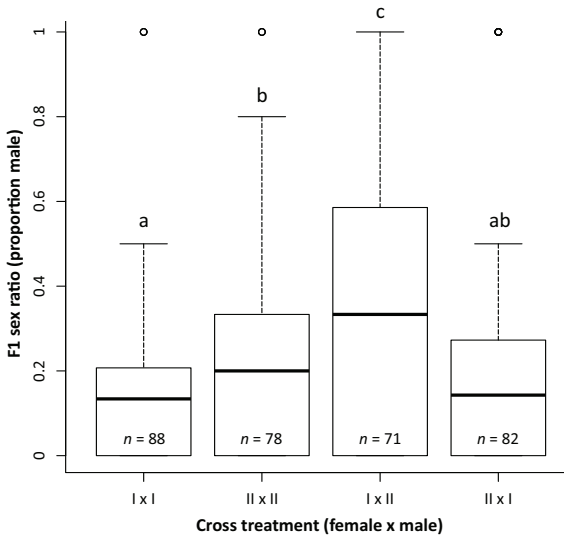


FIGURE S4.1. Effects of cross treatments on F1 sex ratio. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate upper fence (nearest observed value ≤ third quartile + 1.5 box height), circles indicate outliers and different letters indicate significant differences between treatments (post hoc contrasts assessed by pooling factor levels until only significant contrasts remain, with $p < 0.05$). Sample sizes are indicated within each box.

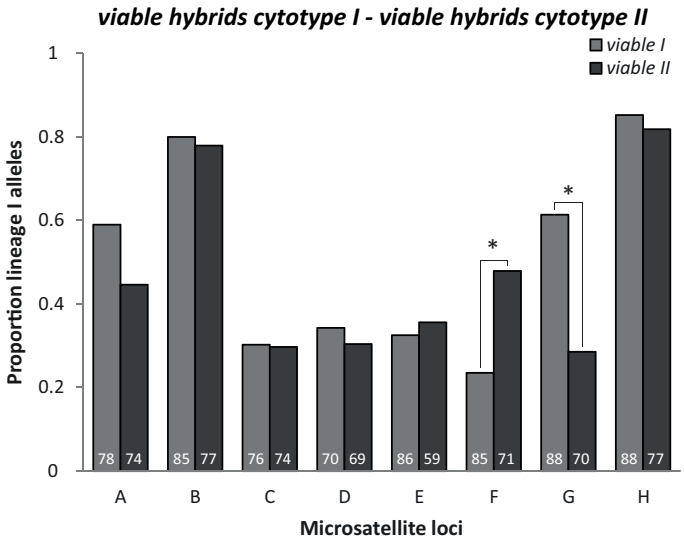
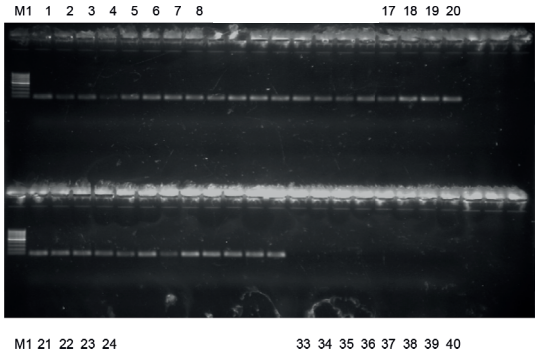


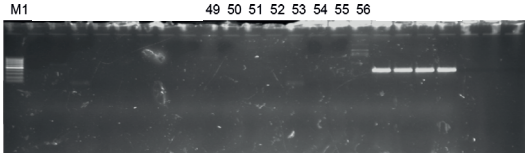
FIGURE S4.2. Microsatellite allele frequencies of the two viable groups. At each locus, the proportion of lineage I alleles is shown. Asterisks indicate significantly different allele frequencies between the two hybrid groups (Fisher's exact test of independence, Bonferroni corrected $p < 0.05$). Sample sizes are indicated at the bottom of each bar.

A



number	population	replicate	locus / species	band
M1	*100 bp ladder*			
1	Viçosa-1	1	*β*-actin	yes
2	Viçosa-1	2	*β*-actin	yes
3	Viçosa-1	3	*β*-actin	yes
4	Viçosa-1	4	*β*-actin	yes
5	Viçosa-1	5	*β*-actin	yes
6	Viçosa-1	6	*β*-actin	yes
7	Viçosa-1	7	*β*-actin	yes
8	Viçosa-1	8	*β*-actin	yes
17	Algarrobo-1	1	*β*-actin	yes
18	Algarrobo-1	2	*β*-actin	yes
19	Algarrobo-1	3	*β*-actin	yes
20	Algarrobo-1	4	*β*-actin	yes
21	Algarrobo-1	5	*β*-actin	yes
22	Algarrobo-1	6	*β*-actin	yes
23	Algarrobo-1	7	*β*-actin	yes
24	Algarrobo-1	8	*β*-actin	yes
33	Viçosa-1	1	*Cardinium*	no
34	Viçosa-1	2	*Cardinium*	no
35	Viçosa-1	3	*Cardinium*	no
36	Viçosa-1	4	*Cardinium*	no
37	Viçosa-1	5	*Cardinium*	no
38	Viçosa-1	6	*Cardinium*	no
39	Viçosa-1	7	*Cardinium*	no
40	Viçosa-1	8	*Cardinium*	no

B



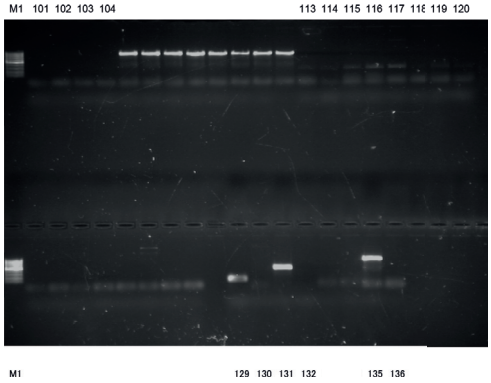
number	population	replicate	locus / species	band
M1	*100 bp ladder*			
49	Algarrobo-1	1	*Cardinium*	no
50	Algarrobo-1	2	*Cardinium*	no
51	Algarrobo-1	3	*Cardinium*	no
52	Algarrobo-1	4	*Cardinium*	no
53	Algarrobo-1	5	*Cardinium*	no
54	Algarrobo-1	6	*Cardinium*	no
55	Algarrobo-1	7	*Cardinium*	no
56	Algarrobo-1	8	*Cardinium*	no

C

number	population	replicate	locus / species	band
M1	*100 bp ladder*			
97	Viçosa-1	1	*Wolbachia*	no
98	Viçosa-1	2	*Wolbachia*	no
99	Viçosa-1	3	*Wolbachia*	no
100	Viçosa-1	4	*Wolbachia*	no

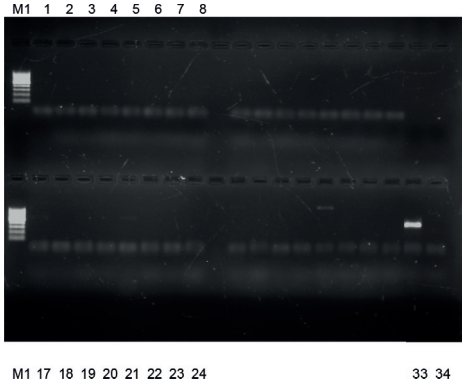
FIGURE S4.3. Endosymbiont screening results using standard PCR. PCR products were loaded on 1% agarose gels and stained using ethidium bromide. Each photo (**A**-**E**) is a separate gel. Sample specifications are given in the tables next to the gel photos, with corresponding numbers above and below the gel. Because we ran these samples within the context of another experiment, some samples are irrelevant for this research and hence excluded from the tables and not represented with a number above or below the gel.

D



number	population	replicate	locus / species	band
M1		*100 bp ladder*		
101	Viçosa-1	5	*Wolbachia*	no
102	Viçosa-1	6	*Wolbachia*	no
103	Viçosa-1	7	*Wolbachia*	no
104	Viçosa-1	8	*Wolbachia*	no
113	Algarrobo-1	1	*Wolbachia*	no
114	Algarrobo-1	2	*Wolbachia*	no
115	Algarrobo-1	3	*Wolbachia*	no
116	Algarrobo-1	4	*Wolbachia*	no
117	Algarrobo-1	5	*Wolbachia*	no
118	Algarrobo-1	6	*Wolbachia*	no
119	Algarrobo-1	7	*Wolbachia*	no
120	Algarrobo-1	8	*Wolbachia*	no
129	positive control		*β*-actin	yes
130	negative control		*β*-actin	no
131	positive control		*Cardinium*	yes
132	negative control		*Cardinium*	no
135	positive control		*Wolbachia*	yes
136	negative control		*Wolbachia*	no

E



number	population	replicate	locus / species	band
M1		*100 bp ladder*		
1	Viçosa-1	1	*Spiroplasma*	no
2	Viçosa-1	2	*Spiroplasma*	no
3	Viçosa-1	3	*Spiroplasma*	no
4	Viçosa-1	4	*Spiroplasma*	no
5	Viçosa-1	5	*Spiroplasma*	no
6	Viçosa-1	6	*Spiroplasma*	no
7	Viçosa-1	7	*Spiroplasma*	no
8	Viçosa-1	8	*Spiroplasma*	no
17	Algarrobo-1	1	*Spiroplasma*	no
18	Algarrobo-1	2	*Spiroplasma*	no
19	Algarrobo-1	3	*Spiroplasma*	no
20	Algarrobo-1	4	*Spiroplasma*	no
21	Algarrobo-1	5	*Spiroplasma*	no
22	Algarrobo-1	6	*Spiroplasma*	no
23	Algarrobo-1	7	*Spiroplasma*	no
24	Algarrobo-1	8	*Spiroplasma*	no
33	positive control		*Spiroplasma*	yes
34	negative control		*Spiroplasma*	no

FIGURE S4.3. Continued

Appendix S4.1

Adjusted residual allele frequency

This appendix illustrates the calculation of adjusted residual allele frequencies, using locus A as an example. At locus A, we observed the following alleles in *viable I* and *inviable I*:

	Viable hybrids cytotype I	Inviable hybrids cytotype I	*Sum*
Allele type I	46	23	***69***
Allele type II	32	34	***66***
Sum	***78***	***57***	***135***

Let $O(x_{i,j})$ be the number of alleles of type i observed in hybrid group j. Expected values are then obtained by:

$$E\left(x_{i,j}\right)=\frac{\sum_{j=1}^{2}x_{i,j}\cdot\sum_{i=1}^{2}x_{i,j}}{\sum x_{i,j}},$$

giving

$$E\left(x_{1,1}\right)=\frac{69\cdot 78}{135}=39.87,\ E\left(x_{1,2}\right)=\frac{69\cdot 57}{135}=29.13$$

$$E\left(x_{2,1}\right)=\frac{66\cdot 78}{135}=38.13,\ E\left(x_{2,2}\right)=\frac{66\cdot 57}{135}=27.87$$

Adjusted residuals are then given by

$$\textit{adjusted residual}\left(x_{i,j}\right)=\frac{O\left(x_{i,j}\right)-E\left(x_{i,j}\right)}{\sqrt{E\left(x_{i,j}\right)\cdot\left(1-\frac{\sum_{j=1}^{2}x_{i,j}}{\sum x_{i,j}}\right)\cdot\left(1-\frac{\sum_{i=1}^{2}x_{i,j}}{\sum x_{i,j}}\right)}},$$

rendering

$$\textit{adjusted residual}\left(x_{1,1}\right)=\frac{46-39.87}{\sqrt{39.87\cdot\left(1-\frac{69}{135}\right)\cdot\left(1-\frac{78}{135}\right)}}=2.14,\ \textit{adjusted residual}\left(x_{1,2}\right)=\frac{23-29.13}{\sqrt{29.13\cdot\left(1-\frac{69}{135}\right)\cdot\left(1-\frac{57}{135}\right)}}=-2.14$$

$$\textit{adjusted residual}\left(x_{2,1}\right)=\frac{32-38.13}{\sqrt{38.13\cdot\left(1-\frac{66}{135}\right)\cdot\left(1-\frac{78}{135}\right)}}=-2.14,\ \textit{adjusted residual}\left(x_{2,2}\right)=\frac{34-27.87}{\sqrt{27.87\cdot\left(1-\frac{66}{135}\right)\cdot\left(1-\frac{57}{135}\right)}}=2.14$$

Note that adjusted residuals $x_{i,1}$ and $x_{i,2}$ are each other's additive inverses, because comparing *viable I* to *inviable I* is equivalent to comparing *inviable I* to *viable I*. Similarly, adjusted residuals $x_{1,j}$ and $x_{2,j}$ are each other's additive inverses, because comparing allele type I to allele type II is equivalent to comparing allele type II to allele type I.

Appendix S4.2

Adjusted residual haplotype count

This appendix illustrates the calculation of adjusted residual haplotype counts, using marker pair AB as an example. At marker pair AB, we observed the following haplotypes in *viable I* and *inviable I*:

	Viable hybrids cytotype I	Inviable hybrids cytotype I	*Sum*
Haplotype *I.I*	42	12	***54***
Haplotype *II.II*	13	11	***24***
Haplotype *I.II*	4	8	***12***
Haplotype *II.I*	17	17	***34***
Sum	***76***	***48***	***124***

Let $O(x_{i,j})$ be the number of haplotypes of type i that we observed in hybrid group j. Expected values are then obtained by:

$$E\left(x_{i,j}\right)=\frac{\sum_{j=1}^{2}x_{i,j}\cdot\sum_{i=1}^{4}x_{i,j}}{\sum x_{i,j}},$$

giving

$$E\left(x_{1,1}\right)=\frac{54\cdot 76}{124}=33.10,\ E\left(x_{1,2}\right)=\frac{54\cdot 48}{124}=20.90$$

$$E\left(x_{2,1}\right)=\frac{24\cdot 76}{124}=14.71,\ E\left(x_{2,2}\right)=\frac{24\cdot 48}{124}=9.29$$

$$E\left(x_{3,1}\right)=\frac{12\cdot 76}{124}=7.35,\ E\left(x_{3,2}\right)=\frac{12\cdot 48}{124}=4.65$$

$$E\left(x_{4,1}\right)=\frac{34\cdot 76}{124}=20.84,\ E\left(x_{4,2}\right)=\frac{34\cdot 48}{124}=13.16$$

Adjusted residuals are then given by

$$\textit{adjusted residual}\left(x_{i,j}\right)=\frac{O\left(x_{i,j}\right)-E\left(x_{i,j}\right)}{\sqrt{E\left(x_{i,j}\right)\cdot\left(1-\frac{\sum_{j=1}^{2}x_{i,j}}{\sum x_{i,j}}\right)\cdot\left(1-\frac{\sum_{i=1}^{4}x_{i,j}}{\sum x_{i,j}}\right)}},$$

rendering

$$\textit{adjusted residual}\left(x_{1,1}\right)=\frac{42-33.10}{\sqrt{33.10\cdot\left(1-\frac{54}{124}\right)\cdot\left(1-\frac{76}{124}\right)}}=3.31,\ \textit{adjusted residual}\left(x_{1,2}\right)=\frac{12-20.90}{\sqrt{20.90\cdot\left(1-\frac{54}{124}\right)\cdot\left(1-\frac{48}{124}\right)}}=-3.31$$

$$\textit{adjusted residual}\left(x_{2,1}\right)=\frac{13-14.71}{\sqrt{14.71\cdot\left(1-\frac{24}{124}\right)\cdot\left(1-\frac{76}{124}\right)}}=-0.80,\ \textit{adjusted residual}\left(x_{2,2}\right)=\frac{11-9.29}{\sqrt{9.29\cdot\left(1-\frac{24}{124}\right)\cdot\left(1-\frac{48}{124}\right)}}=0.80$$

$$\textit{adjusted residual}\left(x_{3,1}\right)=\frac{4-7.35}{\sqrt{7.35\cdot\left(1-\frac{12}{124}\right)\cdot\left(1-\frac{76}{124}\right)}}=-2.09,\ \textit{adjusted residual}\left(x_{3,2}\right)=\frac{8-4.65}{\sqrt{4.65\cdot\left(1-\frac{12}{124}\right)\cdot\left(1-\frac{48}{124}\right)}}=2.09$$

$$\textit{adjusted residual}\left(x_{4,1}\right)=\frac{17-20.84}{\sqrt{20.84\cdot\left(1-\frac{34}{124}\right)\cdot\left(1-\frac{76}{124}\right)}}=-1.59,\ \textit{adjusted residual}\left(x_{4,2}\right)=\frac{17-13.16}{\sqrt{13.16\cdot\left(1-\frac{34}{124}\right)\cdot\left(1-\frac{48}{124}\right)}}=1.59$$

Note that adjusted residuals $x_{i,1}$ and $x_{i,2}$ are each other's additive inverses, because comparing *viable I* to *inviable I* is equivalent to comparing *inviable I* to *viable I*.

Appendix S4.3

Allele indicator statistic

In order to regress the adjusted haplotype residuals against some allele frequency metric, we defined an 'allele indicator' statistic which is the sum of two adjusted residual allele frequencies. As an example, we calculate the allele indicator of marker pair AB in the comparison of hybrid groups *viable I* to *inviable I*, and show how it is used in combination with the adjusted residual haplotype counts of marker pair AB.

At loci A and B, we obtained the following adjusted residual allele frequencies:

		Viable hybrids cytotype I	Inviable hybrids cytotype I
Marker A	**Allele type I**	2.14	-2.14
	Allele type II	-2.14	2.14
Marker B	**Allele type I**	2.30	-2.30
	Allele type II	-2.30	2.30

Since the allele indicator is defined as the sum of the two respective adjusted residual allele frequencies, we obtain the following allele indicators for marker pair AB:

	Formula	Calculation	Allele indicator	Adjusted residual haplotype count
Haplotype *I.I*	$res_A(x_{1,1}) + res_B(x_{1,1})$	2.14 + 2.30	4.43	3.31
Haplotype *II.II*	$res_A(x_{1,2}) + res_B(x_{1,2})$	-2.14 – 2.30	-4.43	-0.80
Haplotype *I.II*	$res_A(x_{1,1}) + res_B(x_{1,2})$	2.14 – 2.30	-0.16	-2.09
Haplotype *II.I*	$res_A(x_{1,2}) + res_B(x_{1,1})$	-2.14 + 2.30	0.16	-1.59
Haplotype *I.I*	$res_A(x_{2,1}) + res_B(x_{2,1})$	- 2.14 – 2.30	-4.43	-3.31
Haplotype *II.II*	$res_A(x_{2,2}) + res_B(x_{2,2})$	2.14 + 2.30	4.43	0.80
Haplotype *I.II*	$res_A(x_{2,1}) + res_B(x_{2,2})$	-2.14 + 2.30	0.16	2.09
Haplotype *II.I*	$res_A(x_{2,2}) + res_B(x_{2,1})$	2.14 – 2.30	-0.16	1.59

$res_Y(x_{i,j})$ indicates the adjusted residual allele frequency of allele i in hybrid group j at marker Y.

The shaded part is included in the table for completeness, but was excluded from the regression (see below). As a consequence of rounding to two digits, some allele indicators do not match their calculation. We used the unrounded values in the regression.

In the regression of adjusted residual haplotype counts against the allele indicator statistic, the goal was to investigate if at certain marker pairs the adjusted residual haplotype counts deviated from their expected values based on the allele indicator statistic. In that context, comparing hybrid groups *viable I* to *inviable I* is equivalent to comparing *inviable I* to *viable I*. This is reflected in the adjusted residual haplotype counts, as well as in the allele indicators, where half of the values are additive inverses of the other half. Consequently, for our purpose one of the two comparisons is redundant. To avoid pseudoreplication, we therefore included only (the non-shaded) half of the values in the regression.

References in supporting information

Boubou, A., Migeon, A., Roderick, G.K., Auger, P., Cornuet, J., Magalhães, S., and Navajas, M. (2012). Test of colonization scenarios reveals complex invasion history of the red tomato spider mite *Tetranychus evansi*. *PLoS One 7*: e35601.

Feng, H., Wang, L., Liu, Y., He, L., Li, M., Lu, W., and Xue, C. (2010). Molecular expression and characterization of a heat shock protein gene (HSP90) from the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval). *Journal of Insect Science 10*: 1-14.

Fukatsu, T., Tsuchida, T., Nikoh, N., and Koga, R. (2001). *Spiroplasma* symbiont of the pea aphid, *Acyrthosiphon pisum* (Insecta: Homoptera). *Applied and Environmental Microbiology 67*: 1284-1291.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glöckner, F.O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation based diversity studies. *Nucleic Acids Research 41*: e1.

O'Neill, S.L., Giordano, R., Colbert, A.M.E., Karr, T.L., and Robertson, H.M. (1992). 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proceedings of the National Academy of Sciences USA 89*: 2699-2702.

Weeks, A.R., Velten, R., and Stouthamer, R. (2003). Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proceedings of the Royal Society London B 270*: 1857-1865.

5

Spider mites maintain defence suppression after 60 generations of experimental evolution on tomato lines that lack inducible jasmonic acid defences

Bram Knegt, Robert C. Schuurink,
E. Toby Kiers & Martijn Egas

Abstract

Herbivores employ various offensive strategies to overcome the defences of their host plants. While it is recognized that offensive strategies can be costly, it is unknown how these costs influence the selection of herbivore offensive strategies across different environments. Here, we study the costs associated with suppression of inducible plant defence by herbivorous arthropods. This strategy can strongly promote herbivore performance, but may require metabolic investments. To investigate this possibility, we allowed defence-suppressing *Tetranychus evansi* spider mites to evolve in environments where we manipulated their tomato hosts for the expression of jasmonic-acid (JA) dependent defence signalling. We used *defenseless-1* tomato mutants to mimic plants with constitutive JA-dependent defences only, and *35S::prosystemin* plants to mimic plants with induced JA-dependent defences. We also included *Phaseolus vulgaris* bean as a distantly related host in which expression of induced defence is regulated via different pathways. We expected that when plant defence is artificially low or high, defence suppression is no longer necessary or possible, causing *T. evansi* to change their defence suppression phenotype. After approximately 60 generations, *T. evansi* strains evolved on tomato mutants and bean showed similar performance on wildtype tomato plants. We also found no differences in the accumulation of JA, JA-isoleucine and salicylic acid in wildtype tomato plants infested with the evolved strains, nor in the expression of marker genes for inducible plant defence. This indicates that metabolic costs associated with defence suppression by *T. evansi* are low, or that host plant manipulation provides more benefits than lowered defences only.

Introduction

Plants and herbivores are natural antagonists. Because plants can suffer serious damage from herbivores, they have evolved various mechanisms to prevent or reduce herbivory, such as dense trichome coverage to obstruct herbivore movement (Traw & Dawson 2002, Glas et al. 2012), or defensive compounds toxic to herbivores (Smith 1955, Heckel 2014). Many of these defensive mechanisms are costly to maintain (Cipollini et al. 2014), and plants therefore evolved inducible mechanisms of defences in response to perception of damage (Chester 1933, Karban & Baldwin 1997), such as induction of digestive inhibitors (Green & Ryan 1972, Ryan 1990), and production of volatiles attractive to predators and parasitoids (Baldwin & Schultz 1983, Heil 2014).

Herbivores respond to induced defences in different ways (Karban & Agrawal 2002). Some herbivores avoid heavily defended plants or tissues (de Moraes et al. 2001), others resist defensive compounds through detoxification (Smith 1955, Heckel 2014), and yet others dilute them by attacking plants in large numbers (Wood 1973, Raffa 2001). Recently, another offensive herbivore strategy was described: by manipulating a plant's physiological processes, herbivores can actually suppress expression of induced plant defence and thereby increase their performance (Musser et al. 2002, Kant et al. 2015, Stahl et al. 2018). For example, *Myzus persicae* aphids produce a salivary effector protein, Mp55, that reduces expression of defensive glucosinolates and hydrogen peroxides in *Arabidopsis thaliana* host plants (Elzinga et al. 2014). Some other herbivores employ symbiotic microorganisms to achieve a similar goal: *Macrosteles quadrilineatus* leafhoppers transfer a type of phytoplasma that secretes effector proteins to downregulate jasmonic acid (JA) synthesis, a hormone crucial for the regulation of induced plant defence against herbivores (Sugio et al. 2011).

Although suppression of plant defence promotes herbivore performance (Denno & Kaplan 2007, Kant et al. 2015), such suppression may at times be an unnecessary investment for two reasons. First, the production of defence-suppressing effectors or the maintenance of symbioses with microorganisms could ensue metabolic costs (Dupas & Boscaro 1999, Gwynn et al. 2005). The production of enzymes that detoxify plant defensive compounds, for example, can trade off with food conversion efficiency (Creswell et al. 1992), intrinsic rate of population increase (Castañeda et al. 2010), and fecundity (Agrawal et al. 2002). Defence suppression could require similar investments from herbivores. Second, costs and benefits of defence suppression may vary depending on the degree to which plant defence is induced in the first place (Heil 2010). Cross-talk between defence signalling pathways, for example, can preclude plants from defending effectively against different attackers (Glazebrook 2005, Thaler et al. 2012). As another example, plants may choose not to mount an induced defence response after herbivore attack when resources are low and competition is high (Voelckel et al. 2001, Kigathi et al. 2013, Orrock et al. 2015, Hahn & Maron 2016, Züst & Agrawal 2017). Under such conditions defence suppression by herbivores may not be necessary.

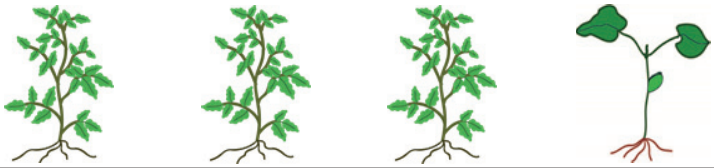
Metabolic costs are difficult to quantify or to manipulate directly in any herbivore species (Whittaker & Feeny 1971, Appel & Martin 1992). However, by manipulating host plants for their expression of plant defence, and studying the evolution of herbivore traits over generations on these hosts, we can ask how plant defence drives selection of herbivore defence suppression. We hypothesize that when plant defence is artificially low or high, defence suppression is no longer necessary or possible, causing a mediation of the level of defence suppression due to selection against a costly trait (e.g., antagonistic pleiotropy, Cooper & Lenski 2000) or due to genetic drift (Halligan & Keightley 2009). Here, we test this hypothesis by allowing *Tetranychus evansi* spider mites to evolve on host plants manipulated for their expression of inducible plant defence.

The tomato red spider mite *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae) is a neotropical herbivore predominantly infesting plants from the Solanaceae family, including tomato, pepper and tobacco (Navajas et al. 2013, Migeon and Dorkeld 2018). It is especially adapted to high temperatures, reaching generation times of < 10 days above 30 °C (Bonato 1999, Gotoh et al. 2010). In general, cultivated tomato (*Solanum lycopersicum*) responds to spider mite infestations with accumulation of the plant hormones JA and salicylic acid (SA), which lead to the expression of defence-associated compounds such as proteinase inhibitors (PIs), polyphenol oxidases (PPOs) and pathogenesis-related proteins (PRs) (Li et al. 2002a, Ament et al. 2004, Kant et al. 2004, Martel et al. 2015, Arnaiz et al. 2018). However, *T. evansi* suppresses tomato JA and SA-dependent plant defence downstream of hormone accumulation, and benefits through increased fecundity (Sarmento et al. 2011, Alba et al. 2015, Ataide et al. 2016). *Tetranychus evansi* likely achieves this suppression through injecting salivary effector proteins into the plant while feeding (Jonckheere et al. 2016, Villarroel et al. 2016, Schimmel et al. 2017a), independently of microorganisms (Staudacher et al. 2017).

To investigate the effects of tomato inducible defence responses on the evolution of defence suppression by *T. evansi* spider mites, we introduced a genetically diverse *T. evansi* population to four different experimental evolution environments. First, we used wild-type cultivated tomato as a control treatment for plants capable of showing an induced defence response. Because *T. evansi* fecundity correlates negatively with tomato JA levels (Ataide et al. 2016), *T. evansi* is likely susceptible to induced plant defences. Suppression of these induced responses is therefore beneficial for *T. evansi*, and we expected *T. evansi* to retain its defence suppression phenotype in this treatment (TABLE 5.1).

Second, we used tomato *defenseless1* (def-1) JA accumulation mutants (Howe et al. 1996) as a treatment without inducible JA-dependent plant defence. Def-1 plants are impaired in their JA biosynthesis, and do not express induced levels of JA and JA-dependent defence responses such as PIs upon spider mite infestations (Howe & Ryan 1999, Li et al. 2002a, Ament et al. 2004). Indeed, susceptible, defence-inducing *T. urticae* generally reach higher performance on def-1 than on wildtype tomato plants (Li et al.

TABLE 5.1. Past evidence of *Tetranychus evansi* performance and suppression of JA-dependent defences on wildtype, def-1 and PS tomato plants, and on bean plants. The expected change in suppression of JA-dependent plant defences after experimental evolution on each host is given in the last row.



	wildtype	**def-1**	**PS**	**bean**
JA defences	inducible	constitutively low	constitutively high	inducible
Past evidence of *T. evansi* performance	high (Sarmento et al. 2011, Alba et al. 2015, de Oliveira et al. 2016, Godinho et al. 2016, Schimmel et al. 2017a, b, Paulo et al. 2018)	high (Alba et al. 2015, Ataide et al. 2016)	low (Schimmel 2016)	high (Ataide 2013, Paulo et al. 2018)
Past evidence of suppression of JA-dependent plant defence by *T. evansi*	yes (Sarmento et al. 2011, Alba et al. 2015, de Oliveira et al. 2016, Godinho et al. 2016, Villarroel et al. 2016, Schimmel et al. 2017a, b)	unknown	unknown	no evidence found (Ataide 2013)
Expected change in defence suppression	no change	less suppression	no change or less suppression*	no expectation

*depends on whether *T. evansi* is able to suppress the constitutively-induced JA-dependent defences in PS plants, see main text.

2002a, Kant et al. 2008). Defence-suppressing *T. evansi* have similar performance on def-1 and wildtype plants (Alba et al. 2015, Ataide et al. 2016), showing that *T. evansi* suppress defences of wildtype plants to a level that is as low as in def-1 plants. Because inducible JA-dependent defences are absent in def-1 plants, selection for their suppression will also be absent, potentially driving *T. evansi* to accumulate mutations or dysfunctional gene combinations within the genes involved in defence manipulation. In addition, if defence suppression entails metabolic costs such as effector production (Jonckheere et al. 2016, Villarroel et al. 2016, Schimmel et al. 2017a), then selection will favour individuals not paying this cost. Consequently, we expected *T. evansi* to evolve reduced defence suppression in a def-1 environment (TABLE 5.1).

Third, we used *35S::prosystemin* (PS) tomato plants (McGurl et al. 1994), which constitutively overexpress the *prosystemin* gene, to mimic plants with induced JA-dependent defences. Prosystemin is a polypeptide that converts into systemin (Pearce et al. 1991),

which interacts with receptors in the plasma membranes of phloem parenchyma cells to initiate a cascade that leads to the release of linolenic acid (Ryan 2000, Narváez-Vásquez & Ryan 2004). Because linolenic acid is the cellular substrate of the JA biosynthesis pathway (Narváez-Vásquez et al. 1999, Wasternack & Hause 2013), systemin is thought to prime plants for damage-induced accumulation of JA (Li et al. 2003). Nevertheless, also in the absence of wounding, PS plants constitutively express induced levels of JA (Chen et al. 2006), PIs (McGurl et al. 1994, Jacinto et al. 1998, Degenhardt et al. 2010) and PPOs (Constabel et al. 1995), with negative consequences for spider mite performance (Li et al. 2002a, Kant et al. 2008, Glas et al. 2014, Alba et al. 2015). However, there is a possibility that *T. evansi* also suppresses defences in PS plants, because the constitutive overexpression of JA-dependent plant defence in PS plants is caused by processes upstream of hormone accumulation, whereas *T. evansi* suppresses plant defence downstream of hormone production (Alba et al. 2015). Whether this occurs is currently unknown. If *T. evansi* is able to suppress the constitutively-induced JA-dependent defences in PS plants, then it will likely maintain its defence suppression phenotype in PS environments. Conversely, if *T. evansi* is unable to suppress defences in PS plants, then selection will favour non-suppressors as in the def-1 environment (TABLE 5.1).

Lastly, *T. evansi* is found on several Fabaceae species including the common bean *Phaseolus vulgaris* (Migeon & Dorkeld 2018). Bean plants can induce defences to which spider mites are susceptible, because non-inducing *T. urticae* mites reached higher performance than defence-inducing *T. urticae* on *P. vulgaris* (Kant et al. 2008). Although the induced defence response of bean plants to spider mites is, like in tomato, regulated by JA and SA (Ozawa et al. 2000, Arimura et al. 2002), the defences produced by bean and tomato plants differ. *Tetranychus urticae* resistant to induced tomato defence, for instance, have equally low performance on *P. vulgaris* as *T. urticae* susceptible to tomato defence (Kant et al. 2008). Additionally, previous research indicates that adaptation of *T. urticae* to tomato (Fry 1990) or cucumber (Gould 1979, Agrawal 2000) is reversible by culturing populations for several generations on bean, underlining a difference in quality among these host plants. *Tetranychus evansi* induces lower levels of SA and SA-dependent plant defence in *P. vulgaris* than *T. urticae*, but not of JA and JA-dependent defence marker genes (Ataide 2013). When coinfesting a *P. vulgaris* leaf with *T. evansi*, *T. urticae* reaches higher performance than in single infestations (Ataide 2013). This suggests that *T. evansi* is able to suppress plant defence also in *P. vulgaris*, although it is unknown if this occurs through the same mechanism as in tomato. Consequently, we expected *T. evansi* cultured on *P. vulgaris* to maintain its suppression of bean defence, but we did not have an expectation for its repercussions for defence suppression in tomato (TABLE 5.1).

After approximately 60 generations (~30 months) of culturing in each of the four experimental evolution environments, we assessed the fecundity of the evolved *T. evan-*

si strains on wildtype, def-1 and PS tomato, and on *P. vulgaris* bean plants. We first investigated if there was a response to selection, i.e., a difference in performance among strains from different experimental environments. Then, to disentangle defence suppression (manipulation of plant defence expression) and resistance (traits that allow mites to maintain high fecundity in the presence of induced plant defences), we measured phytohormone accumulation and expression of several defence marker genes in wildtype tomato plants after infestation with each of the experimental strains.

Materials and Methods

Spider mite populations

We used two populations of *T. evansi* spider mites. Population Viçosa-1 was collected on *S. lycopersicum* cv. Santa Clara in a glasshouse of the Federal University of Viçosa, Brazil, in 2002 (Sarmento et al. 2007), and transported to Amsterdam in 2010 under export permit 03/2010/UTRA-VIÇ/DT-MG (National Plant Protection Organization of Brazil; register number BR-334) and import declaration number 2010/016 (Plant Protection Service of the Netherlands). Population Algarrobo-1 was collected on *S. nigrum* near Malaga, Spain, in 2011 (Alba et al. 2015), and transported to Amsterdam the same year. We maintained base cultures of these populations on detached leaves of *S. lycopersicum* cv. Castlemart (hereafter 'tomato'), which were grown in a greenhouse (25: 18 °C; 16: 8 h light: dark photoperiod; 50–60% relative humidity) for 4-6 weeks, after which we placed detached leaves on wet cotton wool in open, plastic trays in a controlled environment (25 °C; 16: 8 h light: dark photoperiod; 60% relative humidity). In addition, we used a *T. urticae* Santpoort-2 spider mite population, which was collected on *Euonymus europea* near Santpoort, Netherlands, in 2001 (previously called 'KMB' in Kant et al. 2008, renamed to 'Santpoort-2' by Alba et al., 2015), and cultured on detached *P. vulgaris* cv. Speedy leaves under the same conditions.

Production of initial population

To create an initial population that is genetically diverse with respect to tomato defence suppression, we performed crosses between the Viçosa-1 and Algarrobo-1 *T. evansi* populations. These populations are genetically differentiated at mitochondrial and nuclear loci, because they belong to different, previously described *T. evansi* lineages (Gotoh et al. 2009, Alba et al. 2015). Crosses between the two genetic *T. evansi* lineages are partially incompatible due to genetic Bateson-Dobzhansky-Muller incompatibilities, such that only ~5% of interlineage F2 offspring is viable (Gotoh et al. 2009, Knegt et al. 2017). Viable offspring nevertheless have recombined genotypes, indicating that interactions among alleles from different lineages are not necessarily lethal, and because some interlineage allelic combinations are overrepresented among viable offspring, genetic recombination may increase viability through heterosis (Knegt et al. 2017). Potentially, such

recombination also affects the defence suppression phenotype of individuals in the population resulting from the cross.

In addition, the Viçosa-1 and Algarrobo-1 populations suppress tomato defence to different degrees, because plants infested with either population showed differential expression of defence-associated marker loci, which correlated with significant differences in performance of defence-inducing *T. urticae* spider mites that coinfest the same leaflet (Alba et al. 2015). Although no heritability estimates for defence suppression by *T. evansi* are available, we assumed that these differences are at least partly heritable, because population culturing and experiments were conducted under identical conditions, minimizing the potential influence of phenotypic plasticity. More generally, defence suppression varies among *T. evansi* populations (Knegt et al. submitted), suggesting the presence of genetic variation for this trait. Therefore, we assumed that crossing these two populations would produce, through combination and genetic recombination, a population phenotypically diverse with respect to defence suppression, and susceptible to selection in different environments.

To prevent genetic bottlenecks due to low viability in the F2 generation, we set up an initial population by performing large scale reciprocal crosses between the Viçosa-1 and Algarrobo-1 *T. evansi* populations. First, we allowed adult females from our base cultures to oviposit on detached tomato leaflets for two days. Eight days later, we collected as many females as possible from the resulting offspring generation that were in their pre-adult molting stage, and transferred them to fresh, detached leaflets with a maximum of 30 females per leaflet. On these leaflets we also introduced an equal number of adult males from the other population, taken from the base cultures, and we allowed them to mate with the emerging, virgin females for three days. Then, we removed all males and females, and allowed the offspring of the cross to develop into adults. F1 females are diploid and carry one set of chromosomes from each genetic lineage, but because spider mites have a haplodiploid mode of reproduction (Schrader 1923), F1 males are haploid and thus carry only one, maternally derived chromosome set. Upon emergence as adults, F1 females likely mated with males from their own cohort, thus establishing a backcross with their maternal genetic lineage.

We used males and females from this F1 generation to start the initial population for our experiments. The initial population consisted of 326 F1 females from a lineage I (♀) x lineage II (♂) cross and 325 F1 females from a lineage II (♀) x lineage I (♂) cross, as well as 170 lineage I and 145 lineage II adult males (lineage names as in Boubou et al. [2012]). We allowed this population to experience and overcome the viability minimum in the F2 generation, and cultured it on detached tomato leaves for 70 days (~5 generations). We used adult females from this population to start the experimental evolution experiments. In parallel, we also maintained this population as a base population under culture conditions similar to the Viçosa-1 and Algarrobo-1 base populations (hereafter 'initial population').

Experimental evolution treatments

We introduced 20 adult females from the initial population to one of four different treatments: *S. lycopersicum* cv. Castlemart leaflets ('wildtype'), *S. lycopersicum def-1* leaflets ('def-1'), *S. lycopersicum 35S::prosystemin* leaflets ('PS'), and *P. vulgaris* cv. Speedy leaves ('bean'). Plants for all three *S. lycopersicum* treatments were grown in a greenhouse for four weeks before leaflets were cut, and *P. vulgaris* plants were three weeks old when we cut their leaves for use in the treatments. We used detached leaves and leaflets rather than intact plants for three reasons. First, *T. evansi* is able to suppress tomato defences locally, even when challenged with competing, defence-inducing *T. urticae* on the same leaflet (Schimmel et al. 2017a, b). This shows that *T. evansi* can manipulate plant defence signalling within leaflets, even when a source of defence induction is present. Second, local and systemic JA-dependent plant defence is triggered by signals upstream of JA induction (Campos et al. 2014), whereas *T. evansi* suppresses plant defences downstream of hormone production (Alba et al. 2015). Therefore, although leaf abscission may cause a production of damage-associated signals by the wounded tissues that lead to a temporal JA burst (McCloud & Baldwin 1997), *T. evansi* likely locally suppresses any resulting JA-dependent defence induction. Lastly, using detached leaf material is consistent with previous experimental evolution studies with spider mites (Magalhães et al. 2007, 2009, 2011, 2014).

We placed these detached leaves and leaflets with their abaxial side facing upwards on wet cotton wool in open, plastic trays in a controlled environment (25 °C; 16: 8 h light: dark photoperiod; 60% relative humidity). We replicated each treatment five times, such that there were in total twenty subsets of the initial population (hereafter 'strains').

In the first generations after transferring the mites to the experimental arenas, the strains in the bean treatment suffered substantial mortality, which drove one replicate to extinction. The remaining nineteen strains established a growing population. Each strain received two fresh leaves or leaflets each week, which remained in the arena for two weeks such that four leaves or leaflets were present in the arenas at all times. Strains generally overexploited fresh material within one week, after which they migrated to fresh material. When we removed overexploited leaf material that had been present in the arenas for two weeks, we removed all mites inhabiting those leaves or leaflets with them for practical reasons. We changed the location of strains in the climate room each week.

Performance assays of evolved strains on detached leaf material

Thirty months (~60 generations) after transferring mites to their experimental evolution arenas, we started experiments to measure if the evolved strains had changed their defence suppression phenotype. Because previous experimental evolution studies with spider mites showed adaptive change of gene expression and performance on novel hosts within 15-30 generations (Magalhães et al. 2009, Wybouw et al. 2015), we assumed

that this time was sufficient for the defence suppression phenotype of our strains to evolve, even if selection were weaker than in these previous experiments.

To investigate if the evolved strains had adapted to their experimental evolution environments, we assayed their performance in set-ups that resemble their own as well as the other three environments. We grew *S. lycopersicum* wildtype, def-1 and PS plants for four and bean plants for three weeks in a greenhouse, and then placed detached leaflets (*S. lycopersicum* genotypes) and leaves (*P. vulgaris*) on wet cotton wool in open, plastic trays in a climate room under the same conditions as the experimental evolution environments. We infested each leaflet or leaf with five adult females of synchronized age (14 days after oviposition, i.e., ~2 days after emergence as adults) from one evolved strain, measured their fecundity by counting the number of eggs present in the arena after four days, and counted the number of alive females one day and four days after their introduction. We assessed the performance of each strain on each plant genotype with three replicates, i.e., twelve replicates per strain. We also included a control with mites from the initial population, which we cultured as a base population in parallel to the experimental evolution treatments. This control had nine replicates per plant genotype.

We placed eight leaves or leaflets in the same tray, and performed experiments in three blocks in time, with treatment combinations (strain x plant genotype) randomly distributed across trays and blocks. We excluded 27 cases in which the total number of females, dead or alive, after four days was lower than five (i.e., with 'missing' females). To correct for differences in the number of surviving females, we calculated daily oviposition rates per female, assuming that dead females had stopped ovipositing halfway between the time it was observed to be dead and the last time it was observed to be alive.

Infestation experiments and sampling of leaf material

To investigate if the evolved strains induced different responses in wildtype tomato plants, we grew *S. lycopersicum* cv. Castlemart plants in a greenhouse (25: 18 °C; 16: 8 h light: dark photoperiod; 50–60% relative humidity) for 10 days, after which we transferred them to a climate room (25 °C; 16: 8 h light: dark photoperiod; 60% relative humidity) to acclimatize for 11 days. When plants were 21 days old, we infested one leaflet from the second, third and fourth true leaf, counting from the base of the stem, with 15 adult females of synchronized age (14 days after oviposition) each, i.e., 45 in total per plant, from one evolved strain for 7 days. We confined mites to the infested leaflets by applying a lanolin barrier at the base of the leaflet. We performed these experiments in five blocks in time, and included six replicates, i.e., six plants, per evolved strain. We chose to prevent variation among blocks from contributing noise to measurements of individual strains, and therefore included all replicates for each evolved strain within one block. This introduces collinearity between block and experimental treatments, but has the advantage of measuring defence expression phenotypes of individ-

ual strains relative to control treatments more precisely. Each block included six clean, uninfested plants as a negative control for mite infestation, six plants infested with *T. evansi* Viçosa-1 mites as a benchmark for defence suppression, as well as six plants infested with *T. urticae* Santpoort-2 mites as a benchmark for defence induction. Clean plants also received lanolin.

We distributed treatments evenly across the climate room. The first four blocks each included all replicates of four experimental strains and the three control or benchmark treatments. In the fifth block we tested the last three experimental strains as well as the controls and benchmarks, but also included a treatment in which we infested six plants with mites from the initial population, as a control for adaptation to the conditions of the experimental arenas. After 7 days of mite infestation we cut the infested leaflets, placed them in 15 mL tubes with the three leaflets pooled per plant, and immediately froze them in liquid nitrogen. We stored leaf samples at -80 °C until further processing.

Plant hormone extraction and measurements

We ground the frozen leaf samples by vortexing for 45 s together with two slim, sterile metal rods, and manual grinding with a sterile pestle for 30 s. We kept the samples frozen at all times, and afterwards transferred them to 2 mL Eppendorf tubes. We used the methods of Wu et al. (2007) for extraction and analysis of phytohormones, with slight modifications explained in Alba et al. (2015). Briefly, we dissolved 140 (s.d. 17) g leaf material per sample in 1 mL ethyl acetate containing D_5-JA (100 ng mL^{-1}) and D_6-SA (100 ng mL^{-1}) isotopes (C/D/N Isotopes, Pointe-claire, Canada), using a Precellys 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6500 rpm twice for 25 s. We then centrifuged tubes for 20 min at 15,000 rpm at 4 °C, transferred the supernatant to new tubes, and repeated the washing step with the pellet and 0.5 mL ethyl acetate without isotopes for higher yield. We combined both supernatants, evaporated the ethyl acetate using a CentriVap Centrifugal Concentrator (Labconco, Kansas City, MO, USA), resuspended the residue in 250 µL of 70% (v/v) LC-MS grade methanol, centrifuged for 5 min at 15,000 rpm at 4 °C, and transferred 180 µL of the supernatant into glass vials. We determined the amount of phytohormones in each sample using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on 20 µL injections onto Pursuit XRs 5 columns (C18; 50 × 2.0 mm, Agilent Technologies, Santa Clara, CA, USA) in a Varian 320-MS LC/MS (Agilent Technologies) system. We used the D_5-JA and D_6-SA internal standards to calculate the phytohormone recovery rate (D_5-JA: 5.52 [s.d. 1.35] %, D_6-SA: 5.60 [s.d. 1.54] %) and the amount of JA and SA in ng g^{-1} fresh weight (FW) per sample. In addition, we quantified the amount of JA-isoleucine (JA-Ile), the receptor-binding form of JA (Thines et al. 2007), in each sample by comparison to a dilution series of external JA-Ile standard.

RNA extraction and cDNA synthesis

We extracted RNA from the ground, frozen leaf samples using the hot phenol method of Verwoerd et al. (1989), yielding at least 3000 ng RNA / μL per sample. We diluted samples with higher RNA yield to this concentration, and subsequently removed DNA from our samples using an Ambion TURBO DNAse kit (Thermo Fisher Scientific, Waltham, MA, USA). To this end, we added 4.0 μL DNAse mastermix (2.0 μL 10x DNAse buffer, 0.5 μL DNAse, 1.5 μL H_2O) to 16 μL of each RNA solution, digested DNA at 37 °C for 40 min, then added 2 μL DNAse inactivation reagent, inactivated DNAses at room temperature for 5 min, centrifuged the samples at 13,000 rpm for 5 min, and finally transferred 10 μL of the DNA-free RNA-rich supernatant to new tubes. Then, we synthesized cDNA from RNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) by adding 1 μL oligo $(dT)_{18}$ primer to target mRNA, allowing the primer to anneal at 70 °C for 5 min, then adding 9 μL reverse transcriptase (RT) mastermix (4.0 μL 5x RT buffer, 2.0 μL dNTPs, 0.5 μL RT, 2.5 μL H_2O), allowing RT to synthesize cDNA at 42 °C for 60 min, and subsequently inactivated the RT enzyme at 70 °C for 10 min. We diluted the resulting cDNA solutions 10 times.

Gene expression assays

To investigate the responses induced by the evolved strains and control populations in wildtype tomato plants, we performed quantitative reverse transcription polymerase chain reactions (qRT-PCR) of three genes associated with tomato defence: *proteinase inhibitor-IIf* (PI-IIf), *polyphenol oxidase-D* (PPO-D) and *pathogenesis-related protein 1a* (PR-1a). We used the tomato *actin* gene as a constantly expressed reference locus (Løvdal & Lillo 2009). We chose these genes because PI-IIf and PPO-D show JA-dependent expression (Li et al. 2004), PR-1a is SA-dependent (van Kan et al. 1995), and all three loci encode compounds presumably involved in defence against herbivores.

We used an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, USA) to perform qRT-PCR in 20 μL reactions (1 μL cDNA solution, 4 μL EvaGreen [Solis BioDyne, Tartu, Estonia], 13 μL H_2O, 1 μL forward primer [5 μM], and 1 μL reverse primer [5 μM] [specifications in TABLE S5.1]). Per locus we performed all reactions of samples within one experimental block on the same plate, in duplo, thus running two technical replicates of six biological replicates of seven treatments per plate. On each plate we also included a fourfold serial dilution, in duplo, of cDNA from the control treatment for defence induction to produce standard curves for the calculation of primer efficiency, and two negative controls with H_2O instead of cDNA. The PCR program consisted of heating at 50 °C for 2 min and 95 °C for 15 min, 40 cycles of denaturation at 95 °C for 15 s and annealing / extension at 60 °C for 1 min, and concluded with a melt curve program. We excluded reactions with a non-sigmoidal increase in flu-

orescence or with drops in fluorescence during melting curve analysis more than 2 °C different from the expected melting temperature of the target amplicon, in total 564 of 1888 reactions (28%). We calculated primer efficiency with the slope of a linear regression of dilution series cycle threshold values (C_t-values), and averaged these efficiencies across plates to obtain one efficiency estimate per locus. We determined the C_t-values of each reaction by specifying a threshold fluorescence per locus. Subsequently, we averaged the two technical replicates per sample into one C_t-value, and used this C_t-value to calculate the expression at each locus relative to the expression of the *actin* housekeeping gene, as [$efficiency\ locus^{-C_t(locus)}$ / $efficiency\ actin^{-C_t(actin)}$].

Statistics

We used R v.3.2.4 (R Core Team 2016) for all statistical analyses. First, we investigated if the culture conditions of the experimental environments affected the performance of wildtype strains compared to the initial population that was cultured as a base population. To do so, we defined a statistical model with oviposition rate (number of eggs per female per day) as response variable, an interaction between culture method (categorical, 2 levels) and host plant (categorical, 4 levels) as fixed terms, and experimental strain (categorical, 5 levels), as well as tray (categorical, 40 levels) nested in week (categorical, 3 levels), as random factors using package *lme4* (Bates et al. 2015). We assumed a Gaussian error distribution, and confirmed assumptions of normality, homogeneity of variance, independence and absence of negative fitted values. We calculated the significance of the culture method x host plant interaction using approximate F tests with a Kenward-Roger approximation as implemented in the package *pbkrtest* (Halekoh and Højsgaard 2014), and performed backward model simplification by omitting non-significant terms until only significant terms remained. Subsequently, we assessed post hoc contrasts among host plants using package *multcomp* (Hothorn et al. 2008) while applying Holm's correction for multiple comparisons.

Second, we investigated if a response to selection was measurable in the performance of the experimental strains. We defined a statistical model with oviposition rate as response variable, an interaction between experimental treatment (categorical, 4 levels) and host plant (categorical, 4 levels) as fixed terms, and experimental strain (categorical, 19 levels), as well as tray (categorical, 46 levels) nested in week (categorical, 3 levels), as random factors. We square root-transformed the response variable to meet assumptions of normality, homogeneity of variance, independence and absence of negative fitted values.

Third, to investigate if mite strains from the def-1, PS and bean environments had changed their performance relative to the wildtype strains, we compared their fecundity across all host plants in pairwise settings. We defined three statistical models with oviposition rate as response variable, an interaction between experimental treatment

(categorical, wildtype vs. one of the other three treatments = 2 levels) and host plant (categorical, 4 levels) as fixed terms, and experimental strain (categorical, 9 or 10 levels), as well as tray (categorical, 38 or 42 levels) nested in week (categorical, 3 levels), as random factors. We square root-transformed the response variable and performed backward model simplification until only significant terms remained. Subsequently, we assessed post hoc contrasts while applying Holm's correction for multiple comparisons.

Last, to compare phytohormone accumulation and expression of the PI-IIf, PPO-D and PR-1a genes among treatments and controls, we defined models with amount of phytohormone (continuous) or relative gene expression (continuous) as response variable, treatment (categorical, 4 treatments plus 4 controls = 8 levels) as fixed factor, and experimental strain (categorical, 19 strains plus 4 controls = 23 levels) nested in experimental block (categorical, 5 levels) as random terms. We log-transformed response variables to meet all model assumptions, calculated the significance of the treatment term using approximate F tests with a Kenward-Roger approximation, and assessed post hoc contrasts among treatments while applying Holm's correction.

Results

Performance assays

Approximately 60 generations after introducing the initial population to the experimental evolution environments, we measured the fecundity (eggs per female per day) of the experimental strains across detached leaflets of the three tomato genotypes and bean. First, we assessed if the wildtype strains showed evidence of adaptation to culture conditions, by comparing their performance to that of the initial population that was kept as a base culture. We found no indication of adaptation to the culture conditions per se, as oviposition rates were similar among wildtype strains and the initial population ($p = 0.799$, FIGURE 5.1, TABLE S5.2). The host plant during the performance assay did affect the performance of these strains ($p = 0.005$), due to low performance on bean compared to the def-1 tomato genotype (FIGURE 5.1).

Next, we asked if the experimental strains performed differently across the four host plants depending on their experimental environment. We found evidence of a response to selection, as the performance of experimental strains was significantly affected by an interaction between experimental treatment and host plant ($p < 0.001$, TABLE 5.2). This means that significant differences evolved in the fecundity of the experimental strains on the different host plants.

Then, we compared the performance of def-1, PS and bean strains to wildtype strains in three pairwise comparisons (FIGURE 5.2A-C, TABLE S5.3). We expected *T. evansi* to evolve reduced defence suppression in a def-1 environment, which would lead to reduced performance on wildtype tomato (TABLE 5.1). However, we found no evidence of such a response, as wildtype and def-1 strains reached similar performance on each

TABLE 5.2. Statistical test details of a mixed-effect model specifying the effects of experimental evolution treatments (wildtype, def-1, PS or bean) and host plant (wildtype, def-1, PS or bean) on *Tetranychus evansi* oviposition rate (eggs per female per day). Denominator degrees of freedom are estimated using a Kenward-Roger approximation.

Random terms	**Variance**	**Std. dev**	**% variance**			
tray:week	0.029	0.171	12			
experim. strain	0.000	0.000	0			
residual	0.216	0.464	88			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment × host plant	9.0973	1.0108	9	176.754	4.481	< 0.001

host plant (FIGURE 5.2A). In a PS environment, we expected *T. evansi* to maintain or reduce its degree of defence suppression, depending on whether it was able to suppress the constitutively overexpressed defences of PS plants (TABLE 5.1). In line with a maintained degree of defence suppression, we found that performance was not affected by a treatment x host plant interaction ($F_{3,73.25} = 0.90$, $p = 0.447$). However, PS strains performed worse than wildtype strains across all host plants (FIGURE 5.2B). Last, we found similar performance of bean strains and wildtype strains on the three tomato genotypes (FIGURE 5.2C).

Phytohormone accumulation and gene expression assays

To disentangle defence suppression (manipulation of plant defence expression) and resistance (traits that allow mites to maintain high fecundity in the presence of induced

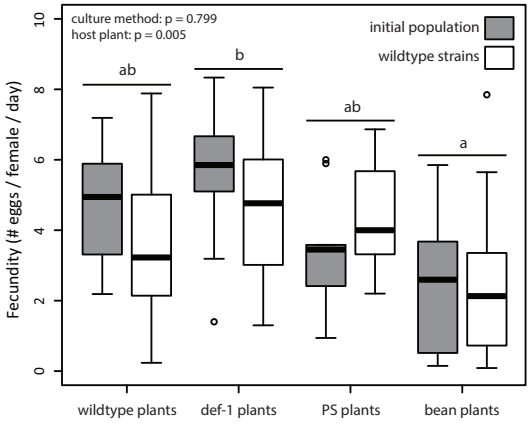


FIGURE 5.1. Performance of the initial population and wildtype strains across all host plants. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences among host plants as assessed through Holm-adjusted post hoc contrasts. See TABLE S5.2 for statistical details.

plant defences), we measured accumulation of phytohormones and expression of three defence marker genes in wildtype tomato plants after 7 days of infestation. If evolved strains had changed their level of defence suppression, then we expected wildtype tomato plants infested with these strains to accumulate more JA, JA-Ile, or SA, and to express

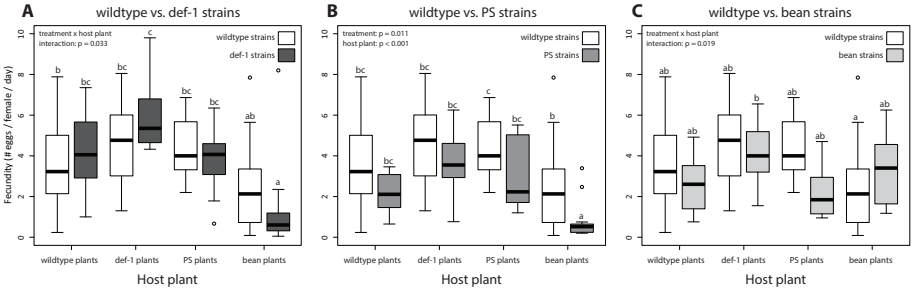


FIGURE 5.2. Performance of wildtype strains compared to (A) def-1 strains, (B) PS strains, and (C) bean strains. The significance of the experimental evolution treatment, the host plant during the performance assay, or their interaction, is given in the upper left corner of each graph. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences between treatments as assessed through Holm-adjusted post hoc contrasts. See TABLE S5.3 for statistical details.

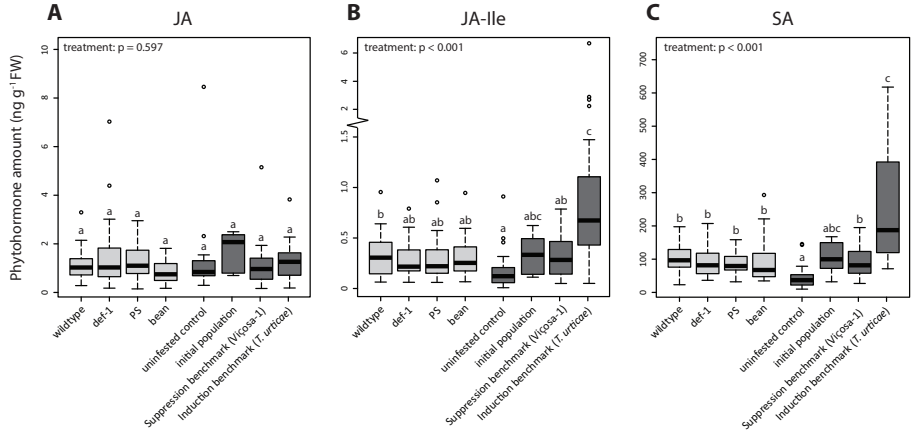


FIGURE 5.3. Amount of phytohormones JA (A), JA-Ile (B) and SA (C) in wildtype tomato plants after infestation with experimental strains or control populations for 7 days. Phytohormones were measured using LC-MS/MS, corrected for recovery of internal standards, and are expressed in ng per gram fresh leaf tissue. The significance of the experimental evolution treatment is given in the upper left corner of each graph. Results of experimental evolution strains are shown in light grey, results of control and benchmark treatments are shown in dark grey. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences between treatments as assessed through Holm-adjusted post hoc contrasts. See TABLE S5.4 for statistical details.

defence marker genes more strongly than plants infested with the benchmark population for defence suppression, Viçosa-1. Alternatively, if they had retained their suppression phenotype, then we expected similar accumulation of phytohormones and gene expression among treatments.

We found that JA, JA-Ile and SA accumulated to similar levels in plants infested with the evolved strains, Viçosa-1, and the initial population (FIGURE 5.3, TABLE S5.4). In plants infested with the benchmark population for defence induction, *T. urticae*, JA-Ile (FIGURE 5.3B) and SA (FIGURE 5.3C) levels were significantly higher than in plants infested with the evolved strains or Viçosa-1. We observed no significant differences in JA levels among treatments (FIGURE 5.3A). Similarly, expression of all three assayed genes was significantly affected by treatment (FIGURE 5.4, TABLE S5.5), but none of the evolved strains suppressed expression to levels that were different among each other, nor from the suppression benchmark or the initial population. Expression of *PI-IIf* (FIGURE 5.4A) and *PPO-D* (FIGURE 5.4B) was variable, such that we observed no significant difference between the suppression and induction benchmarks (Viçosa-1 and *T. urticae*), nor with most of the experimental evolution treatments. Expression of *PR-1a* was higher in plants infested with the induction benchmark *T. urticae*, but similar among all other treatments (FIGURE 5.4C).

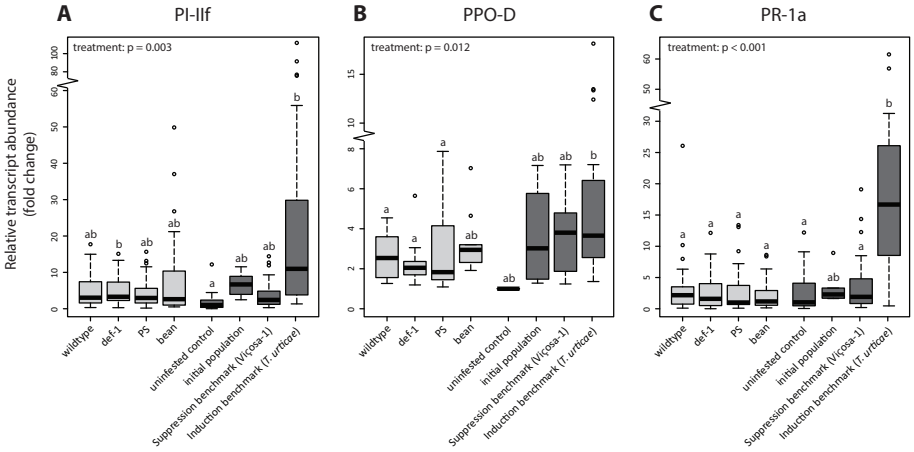


FIGURE 5.4. Expression of the plant defence-associated genes *PI-IIf* (A), *PPO-D* (B), and *PR-1a* (C) in wildtype tomato plants after infestation with experimental strains, control or benchmark populations for 7 days. Gene expression was measured using qRT-PCR, and expression is relative to that of *actin* and normalized to the treatment with the lowest median relative expression. The significance of the experimental evolution treatment is given in the upper left corner of each graph. Results of experimental evolution strains are shown in light grey, results of benchmark treatments are shown in dark grey. Thick lines indicate treatment median, boxes encompass data from first to third quartile, whiskers indicate fences (nearest observed value ≥ first or ≤ third quartile ± 1.5 box height), circles indicate outliers, and different letters indicate significant differences between treatments as assessed through Holm-adjusted post hoc contrasts. See TABLE S5.5 for statistical details.

Discussion

Our aim was to investigate if suppression of inducible plant defence by herbivores is selected against when induced plant defence is either absent or constitutive, as would be expected when expression of this trait involves costs. We allowed a genetically diverse population of *T. evansi* spider mites to evolve on tomato host plants with manipulated levels of JA-dependent inducible plant defences and on bean, and found that strains adapted to wildtype, def-1 or PS plants did not differ in performance on wildtype tomato (FIGURE 5.2). We also found no difference in accumulation of the phytohormones JA, JA-Ile or SA in wildtype tomato plants infested with the evolved strains (FIGURE 5.3), nor in the expression of plant genes associated with defence (FIGURE 5.4). This shows that all evolved strains have retained their level of tomato defence suppression.

Although we found no differences in the level of defence suppression, we did find a general response to selection (significant interaction between treatment and host plant on mite fecundity; TABLE 5.2). This response shows that the experimental strains have evolved differences that affect their fecundity across the four host plants, and is likely caused by traits that affect fecundity other than defence suppression, such as an altered ability to resist induced plant defences. For example, PS strains perform significantly less well on bean than wildtype strains (FIGURE 5.2AB), which could potentially be explained by a reduced ability to cope with bean defences.

There are three possible explanations for the lack of a change in the level of defence suppression among *T. evansi* strains adapted to different host plant environments. First, defence suppression by *T. evansi* may not entail large metabolic costs. This is because if costs were high, defence suppression would have been selected against through antagonistic pleiotropy (Cooper & Lenski 2000) in environments where defence suppression is ineffective. Previous research into trade-offs between metabolic costs of herbivore offense has found mixed results. Metabolic costs of detoxification established a trade-off with food conversion efficiency in *Spodoptera eridania* caterpillars (Creswell et al. 1992), and with intrinsic rate of population increase in *Sitobion avenae* aphids (Castañeda et al. 2010). In a parasite-host system, defence suppression by *Leptopilina boulardi* parasitoids was selected against when host diversity was high, probably due to ineffective suppression in some host species (Dupas & Boscaro 1999). In contrast, no trade-offs were found between the production of detoxification enzymes and growth rate in *Depressaria pastinacella* caterpillars (Berenbaum & Zangerl 1994), nor with growth parameters in *Heliothis zea* caterpillars (Neal 1987). In *T. urticae* spider mites, no trade-off was observed between production of large amounts of proteinaceous web and fecundity (Tien et al. 2009), but production of detoxification enzymes contributed to reduced performance when they were not necessary (Agrawal et al. 2002). Here, we found no difference in performance among *T. evansi* strains from environments with artificially low (def-1) or high (PS) levels of inducible plant defence, suggesting that metabolic costs

associated with the production of defence suppressing effectors do not trade off with life-history traits in *T. evansi*.

Second, it could be adaptive for herbivores to only suppress induced plant defence when it is actually expressed by the plant. Herbivores often react to induced plant defences with responses that are phenotypically plastic (Després et al. 2007), such as dose-dependent production of detoxification enzymes (Broadway 1997, Li et al. 2002b). Hence, if defence suppression by *T. evansi* is a plastic trait, then not suppressing plant defence when it is not necessary or possible can save metabolic costs. This hypothesis can potentially explain the absence of a difference in performance among strains evolved in environments with artificially low or high levels of inducible plant defence. Recently, Schimmel et al. (2017a, b) observed that when previously established *T. evansi* on tomato were challenged with competing, defence-inducing *T. urticae* on the same leaflets, they increased their fecundity and their expression of genes encoding salivary effectors possibly involved in defence suppression (Villarroel et al. 2016). Simultaneously, plants decreased their phytohormone concentrations and defence marker gene expression at the local *T. evansi* feeding sites. This observation suggests that defence suppression by *T. evansi* is phenotypically plastic depending on the presence of competitors or their effects on plant defence.

Third, there could be no response to selection due to a lack of heritable variation (Lynch & Walsh 1998). *Tetranychus evansi* populations harbour heritable variation for defence suppression (Knegt et al. submitted), and the population that we used here resulted from a cross between two genetically differentiated *T. evansi* populations. This hybrid population likely had more genetic variation than its parental populations, because the parental populations have significantly different defence suppression phenotypes (Alba et al. 2015), and F2 offspring of a cross between these populations have recombinant genotypes (Knegt et al. 2017). Although recombination may indeed increase variation in defence suppression, recombination between genetic material of closely-related parents is predicted to generate more phenotypic variation than between distantly-related lineages (Hosseini et al. 2016). In addition, F2 hybrids suffered 95% mortality due to incompatible genetic interactions (Knegt et al. 2017). Previous research has shown that genetic incompatibilities are non-randomly distributed across genomes, and may concentrate in areas of genomic coadaptation (Hohenlohe et al. 2012). If the genes underlying defence suppression in *T. evansi* are located in such a coadapted genomic region, then recombination in this region likely decreased hybrid fitness and was selected against in our cross. Genetic variation for defence suppression in the hybrid population then may not have exceeded variation in the parental populations, and could even have been lower due to the genetic bottleneck caused by mortality in the F2 generation. Mapping of linkage disequilibrium along the *T. evansi* genome, as well as localization of the genes underlying defence suppression will provide more insight into the effects of recombination on *T. evansi* defence suppression.

Even in the absence of costs, traits that are not utilized are expected to degrade over time due to accumulation of mutations in the underlying genes (Halligan & Keightley 2009). However, we found no evidence of a loss of functionality of defence suppression in any of our treatments. Sixty generations may be too short for mutations to erode the genes underlying defence suppression, but an alternative explanation is that *T. evansi* not only suppresses inducible tomato defences (Sarmento et al. 2011a, Alba et al. 2015), but simultaneously also manipulates other aspects of plant quality. In general, a plant's response to herbivore attack is not restricted to defence only, but also involves modification of its primary metabolism on a large scale (Schultz et al. 2013, Zhou et al. 2015). Plants can channel resources away from the infested tissues, but herbivores may try to change their feeding sites into nutrient sinks (e.g., Kaiser et al. 2010). There are indications that such processes are involved in interactions between tomato plants and spider mites. For example, when tomato plants are challenged with a severe *T. urticae* infestation, their infested leaves senesce and shed, potentially as a defence mechanism to prevent further infestation of their tissues. In contrast, tomato leaves infested with *T. evansi* remain green and appear healthy until they are overexploited, suggesting that plants continue to supply the infested leaves with nutrients (Liu et al. 2017).

There are more indications that *T. evansi* manipulates the nutrient flows of their host plants. For example, tomato plants infested with *T. evansi* contain higher soluble sugar concentrations than uninfested plants (Ximénez-Embún et al. 2016) or plants infested with *T. urticae* (Schimmel 2016). Additionally, *T. evansi* potentially alters the distribution of nutrients throughout leaflets, such that adjacent spider mites may feed from less nutritious tissues (Schimmel et al. 2017b). Whether *T. evansi* manipulates plants to supply their feeding sites with additional nutrients or sugars, and if it benefits from access to these resources remains to be demonstrated. However, if physiological manipulation of the plant by *T. evansi* is not restricted to defence suppression only, then the benefits conferred by these other mechanisms may have been present in all our experimental evolution environments. Selection may then have sustained defence suppression and its correlated other effects on plant physiology, even in the absence of effective defence suppression.

Bean (*P. vulgaris*) is a host plant distantly related to tomato, and represents an environment in our experiments in which defence is regulated differently than in tomato. Defensive differences (e.g., secondary metabolites) among plant species can prohibit herbivores from maintaining high performance across all plant species, because antagonistic pleiotropy (e.g., metabolic costs) between resistance traits generates trade-offs between performance on different hosts (Jaenike 1990, Grosman et al. 2015). Among spider mites, host plant specialization and trade-offs in performance have predominantly been studied in *T. urticae*. In this species, exposure to a novel host plant generally increases its performance after a few generations (Gould 1979, Fry 1990, Agrawal et al.

2002, Magalhães et al. 2007), due to increased detoxification of plant compounds and attenuation of the plant's defence response (Dermauw et al. 2013, Wybouw et al. 2015). In line with metabolic costs associated with such detoxification pathways, *T. urticae* performance on novels host trades off with performance on the ancestral host (Gould 1979, Agrawal et al. 2002). However, trade-offs are not ubiquitous for *T. urticae*, because some studies report no trade-off among novel and ancestral hosts (Fry 1990, Magalhães et al. 2009). Here, we found that *T. evansi* did not significantly increase its fecundity on bean after exposure to this host for sixty generations, nor did bean-adapted strains reduce their fecundity on tomato (FIGURE 5.2C). Def-1 and PS strains, however, performed poorly on bean (FIGURE 5.2AB). Thus, in line with independently evolving resistance traits (Kawecki 1994), these results indicate that bean can be a challenging host for *T. evansi*, but also that there is no trade-off between performance on bean and tomato for *T. evansi*.

Two potentially confounding factors complicate the interpretation of the fecundity assays conducted in this study. First, life-history traits such as fecundity can not only be affected by genetic adaptation, but also by the environment that individuals or their mothers experienced previously, i.e., juvenile and maternal effects (Mousseau & Fox 1998, Magalhães et al. 2011). The influence of juvenile and maternal effects can be minimized by allowing control and treated populations to spend one or a few generations in a common environment before assessing their performance (Kawecki & Ebert 2004, Magalhães et al. 2011), but in this study we transferred individuals from the host they evolved on directly to the host on which we measured their fecundity. Consequently, the results of our performance assays should be interpreted as the net outcome of genetic adaptation and juvenile and maternal effects. Nevertheless, because we found no differences among the evolved strains in the responses they induced in wildtype tomato hosts (FIGURES 5.3 and 5.4), it is unlikely that maternal effects obscured significant differences in their performance on wildtype tomato.

Second, spider mites are generally cultured on detached leaf material. Detaching a leaf from its plant causes a stress response, leading to the accumulation of defence compounds also in the absence of herbivore infestation (Heil 2009). Thus, this culture method can expose mites to plants with induced defences. Consistent with previous experimental evolution studies on host change in spider mites (Magalhães et al. 2007, 2009, 2011, 2014), the environments in which our strains evolved also consisted of detached leaves or leaflets. These detached leaflets may therefore have had their defences induced, potentially rendering the wildtype environment similar to the PS environment. However, *T. evansi* suppress tomato defences locally, also when plant defence is induced within the same leaflet (Schimmel et al. 2017a, b). This may be possible because local and systemic JA-dependent plant defence is triggered by signals upstream of JA induction (Campos et al. 2014), whereas *T. evansi* suppresses plant defences down-

stream of hormone production (Alba et al. 2015). We therefore discard the suggestion that induced defences in detached leaflets precluded *T. evansi* to suppress defences in wildtype treatments. For the same reasons, it seems likely that *T. evansi* was also able to suppress tomato defences in PS plants. This interpretation is supported by our result that PS and wildtype strains performed equally well in wildtype and PS environments (FIGURE 5.2B), and suppressed defences to levels similar to a defence-suppression benchmark (FIGURES 5.3 and 5.4).

Suppression of induced plant defence by herbivorous arthropods can provide substantial benefits in terms of increased performance (Kant et al. 2015), but its metabolic costs have previously not been investigated. Substantial metabolic costs have been measured for production of other compounds involved in plant-herbivore interactions, such as induced expression of defence compounds for plants (Cipollini et al. 2014) or detoxification enzymes in herbivores (Creswell et al. 1992, Agrawal et al. 2002, Castañeda et al. 2010). Here, we found no difference in fecundity among *T. evansi* strains evolved on hosts where induced plant defence was either absent or constitutively over-expressed, nor in the responses they induced in their wildtype tomato hosts. Therefore, metabolic costs associated with defence suppression in *T. evansi* are likely small. Energetic costs of offensive traits can play an important role in host range evolution of herbivorous arthropods (Karban & Agrawal 2002), and low costs may facilitate the development of polyphagous diets (Berenbaum & Zangerl 1994, Després et al. 2007, Castañeda et al. 2009). Additionally, phenotypic plasticity in defence suppression may allow herbivores to prevent unnecessary costs in the absence of defence suppression, and host plant manipulation may also confer other benefits than suppression of induced plant defences only. Future research into the expression of *T. evansi* salivary effectors could measure their expression in *T. evansi* when feeding on def-1 and PS host plants, to investigate the possibility of phenotypic plasticity in defence suppression.

Acknowledgements

We thank dr. JM Alba for help with lab analyses, and M. de Vries for help with LC-MS/MS analyses. This work was supported by the Dutch Science Foundation (NWO): TOP-grant 854.11.005, and by the European Research Council (ERC): Grant Agreement 335542.

References

Agrawal AA (2000). Host-range evolution: adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology*, **81**, 500-508.

Agrawal AA, Vala F, Sabelis MW (2002). Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? *The American Naturalist*, **159**, 553-565.

Alba JM, Schimmel BCJ, Glas JJ, Ataide LMS, Pappas ML, Villarroel CA, Schuurink RC, Sabelis MW, Kant MR (2015). Spider mites suppress tomato defences downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist*, **205,** 828-840.

Ament K, Kant MR, Sabelis MW, Haring MA, Schuurink RC (2004). Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiology*, **135**, 2025-2037.

Appel HM, Martin MM (1992). Significance of metabolic load in the evolution of host specificity of *Manduca sexta*. *Ecology*, **73**, 216-228.

Arimura G-I, Ozawa R, Nishioka T, Boland W, Koch T, Kühnemann F, Takabayashi J (2002). Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *The Plant Journal*, **29**, 87-98.

Arnaiz A, Talavera-Mateo L, Gonzalez-Melendi P, Martinez M, Diaz I, Santamaria ME (2018). *Arabidopsis* Kunitz trypsin inhibitors in defense against spider mites. *Frontiers in Plant Sciences*, **9**, 1-16.

Ataide LMS (2013). Induction and suppression of plant defences by herbivorous mites: two different strategies with advantages and disadvantages. PhD thesis, Federal University of Viçosa, Viçosa. Available online: http://locus.ufv.br/handle/123456789/916.

Ataide LMS, Pappas ML, Schimmel BCJ, Lopez-Orenes A, Alba JM, Duarte MVA, Pallini A, Schuurink RC, Kant MR (2016). Induced plant-defenses suppress herbivore reproduction but also constrain predation of their offspring. *Plant Science*, **252,** 300-310.

Baldwin IT, Schultz JC (1983). Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science*, **221**, 277-279.

Bates D, Mächler M, Bolker BM, Walker SC (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**. doi: 10.18637/jss.v067.i01.

Berenbaum MR, Zangerl AR (1994). Costs of inducible defense: protein limitation, growth, and detoxification in parsnip webworms. *Ecology*, **75**, 2311-2317.

Bonato O (1999). The effect of temperature on life history parameters of *Tetranychus evansi* (Acari: Tetranychidae). *Experimental and Applied Acarology*, **23,** 11-19.

Boubou A, Migeon A, Roderick GK, Auger P, Cornuet J-M, Magalhães S, Navajas M (2012). Test of colonisation scenarios reveals complex invasion history of the red tomato spider mite *Tetranychus evansi*. *PLoS One*, **7**, e35601.

Broadway RM (1997). Dietary regulation of serine proteinases that are resistant to serine proteinase inhibitors. *Journal of Insect Physiology*, **43**, 855-874.

Castañeda LE, Figueroa CC, Fuentes-Contreras E, Niemeyer HM, Nespolo RF (2009). Energetic costs of detoxification systems in herbivores feeding on chemically defended host plants: a correlational study in the grain aphid, *Sitobion avenae*. *Journal of Experimental Biology*, **212**, 1185-1190.

Castañeda LE, Figueroa CC, Nespolo RF (2010). Do insect pests perform better on highly defended plants? Costs and benefits of induced detoxification defences in the aphid *Sitobion avenae*. *Journal of Evolutionary Biology*, **23**, 2474-2483.

Chen H, Jones AD, Howe GA (2006). Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. *FEBS Letters*, **580**, 2540-2546.

Chester KS (1933). The problem of acquired physiological immunity in plants. *The Quarterly Review of Biology*, **8**, 275-324.

Cipollini D, Walters D, Voelckel C (2014). Costs of resistance in plants: from theory to evidence. *Annual Plant Reviews*, **47**, 263-308.

Constabel CP, Bergey DR, Ryan CA (1995). Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 407-411.

Cooper VS, Lenski RE (2000). The population genetics of ecological specialization in evolving *Escherichia coli* populations. *Nature*, **407**, 736-739.

Creswell JE, Merritt SZ, Martin MM (1992). The effect of dietary nicotine on the allocation of assimilated food to energy metabolism and growth in fourth-instar larvae of the southern armyworm, *Spodoptera eridania* (Lepidoptera: Noctuidae). *Oecologia*, **89**, 449-453.

Degenhardt DC, Refi-Hind S, Stratmann JW, Lincoln DE (2010). Systemin and jasmonic acid regulate constitutive and herbivore-induced systemic volatile emissions in tomato, *Solanum lycopersicum*. *Phytochemistry*, **71**, 2024-2037.

De Moraes CM, Mescher MC, Tumlinson JH (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature*, **410**, 577-580.

Denno RF, Kaplan I (2007). Plant-mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. In: *Ecological communities: plant mediation in indirect interaction webs* (eds Ohgushi T, Craig TP, Price PW). Cambridge University Press, London.

De Oliveira EF, Pallini A, Janssen A (2016). Herbivores with similar feeding modes interact through the induction of different plant responses. *Oecologia*, **180,** 1-10.

Dermauw W, Wybouw N, Rombauts S, Menten B, Vontas J, Grbić M, Clark RM, Feyereisen R, Van Leeuwen T (2013). A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. *Proceedings of the National Academy of Sciences of the United States of America*, **110,** E113-22.

Després L, David J-P, Gallet C (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology and Evolution*, **22**, 298-307.

Dupas S, Boscaro M (1999). Geographic variation and evolution of immunosuppressive genes in a *Drosophila* parasitoid. *Ecography*, **22**, 284-291.

Elzinga DA, de Vos M, Jander G (2014). Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. *Molecular Plant-Microbe Interactions*, **27**, 747-756.

Fry JD (1990). Trade-offs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. *The American Naturalist*, **136**, 569-580.

Glas JJ, Alba JM, Simoni S, Villarroel CA, Stoops M, Schimmel BCJ, Schuurink RC, Sabelis MW, Kant MR (2014). Defense suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. *BMC Biology*, **12,** 98.

Glas JJ, Schimmel BCJ, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012). Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. *International Journal of Molecular Sciences*, **13**, 17077-17103.

Glazebrook J (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, **43**, 205-227.

Godinho DP, Janssen A, Dias T, Cruz C, Magalhães S (2016). Down-regulation of plant defence in a resident spider mite species and its effect upon con- and heterospecifics. *Oecologia*, **180,** 161-167.

Gotoh T, Araki R, Boubou A, Migeon A, Ferragut F, Navajas M (2009). Evidence of co-specificity between *Tetranychus evansi* and *Tetranychus takafujii* (Acari: Prostigmata, Tetranychidae): comments on taxonomic and agricultural aspects. *International Journal of Acarology*, **35,** 485-501.

Gotoh T, Sugimoto N, Pallini A, Knapp M, Hernandez-Suarez E, Ferragut F, Ho C, Migeon A, Navajas M, Nachman G (2010). Reproductive performance of seven strains of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae) at five temperatures. *Experimental and Applied Acarology*, **52,** 239-259.

Gould F (1979). Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. *Evolution*, **33**, 791-802.

Green TR, Ryan CA (1972). Wound induced proteinase inhibitor in plant leaves: a possible defence mechanism against insects. Science, 175, 776-777.

Grosman AH, Molina-Rugama AJ, Mendes-Dias R, Sabelis MW, Menken SBJ, Pallini A, Breeuwer JAJ, Janssen A (2015). No adaptation of a herbivore to a novel host but loss of adaptation to its native host. *Scientific Reports*, **5**, 16211.

Gwynn DM, Callaghan A, Gorham J, Walters KFA, Fellowes MDE (2005). Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. *Proceedings of the Royal Society B*, **272**, 1803-1808.

Hahn PG, Maron JL (2016). A framework for predicting intraspecific variation in plant defense. *Trends in Ecology and Evolution*, **31**, 646-656.

Halekoh U, Højsgaard S (2014). A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models – the R package pbkrtest. *Journal of Statistical Software*, **59**. doi: 10.18637/jss.v059.i09.

Halligan DL, Keightley PD (2009). Spontaneous mutation accumulation studies in evolutionary genetics. *Annual Review of Ecology, Evolution and Systematics*, **40**, 151-172.

Heckel DG (2014). Insect detoxification and sequestration strategies. *Annual Plant Reviews*, **47**, 77-114.

Heil M (2009). Damaged-self recognition in plant herbivore defence. *Trends in Plant Science*, **14**, 356-363.

Heil M (2010). Plastic defence expression in plants. *Evolutionary Ecology*, **24**, 555-569.

Heil M (2014). Herbivore-induced plant volatiles: targets, perception and unanswered questions. *New Phytologist*, **204**, 297-306.

Hohenlohe PA, Bassham S, Currey M, Cresko WA (2012). Extensive linkage disequilibrium and parallel adaptive divergence across threespine stickleback genomes. *Philosophical Transactions of the Royal Society of London B*, **367**, 395-408.

Hosseini S-R, Martin OC, Wagner A (2016). Phenotypic innovation through recombination in genome-scale metabolic networks. *Proceedings of the Royal Society of London B*, **283**, 20161536.

Hothorn T, Bretz F, Westfall P (2008). Simultaneous inference in general parametric models. *Biomedical Journal*, **50,** 346-363.

Howe GA, Lightner J, Browse J, Ryan CA (1996). An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *The Plant Cell*, **8**, 2067-2077.

Howe GA, Ryan CA (1999). Suppressors of systemin signaling identify genes in the tomato wound response pathway. *Genetics*, **153**, 1411-1421.

Jacinto T, Fernandes KVS, Machado OLT, Siqueira-Júnior CL (1998). Leaves of transgenic tomato plants overexpressing prosystemin accumulate high levels of cystatin. *Plant Science*, **138**, 35-42.

Jaenike J (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, **21**, 243-273.

Jonckheere W, Dermauw W, Zhurov V, Wybouw N, Van den Bulcke J, Villarroel CA, Greenhalgh R, Grbić M, Schuurink RC, Tirry L, Baggerman G, Clark RM, Kant MR, Vanholme B, Menschaert G, Van Leeuwen T (2016). The salivary protein repertoire of the polyphagous spider mite *Tetranychus urticae*: a quest for effectors. *Molecular and Cellular Proteomics*, **15,** 3594-3613.

Kaiser W, Huguet E, Casas J, Commin C, Giron D (2010). Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proceedings of the Royal Society of London B*, **277**, 2311-2319.

Kant MR, Ament K, Sabelis MW, Haring MA, Schuurink RC (2004). Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiology*, **135,** 483-495.

Kant MR, Jonckheere W, Knegt B, Lemos F, Liu J, Schimmel BCJ, Villarroel CA, Ataide LMS, Dermauw W, Glas JJ, Egas M, Janssen A, Van Leeuwen T, Schuurink RC, Sabelis MW, Alba JM (2015). Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany*, **115**, 1015-1051.

Kant MR, Sabelis MW, Haring MA, Schuurink RC (2008). Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proceedings of the Royal Society B*, **275,** 443-452.

Karban R, Agrawal AA (2002). Herbivore offense. *Annual Review of Ecology and Systematics*, **33**, 641-664.

Karban R, Baldwin IT (1997). *Induced Responses to Herbivory*. Chicago University Press, Chicago, USA.

Kawecki TJ (1994). Accumulation of deleterious mutations and the evolutionary cost of being a generalist. *The American Naturalist*, **144**, 833-838.

Kawecki TJ, Ebert D (2004). Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225-1241.

Kigathi RN, Weisser WW, Veit D, Gershenzon J, Unsicker SB (2013). Plants suppress their emission of volatiles when growing with conspecifics. *Journal of Chemical Ecology*, **39**, 537-545.

Knegt B, Meijer TT, Kant MR, Kiers ET, Egas M. *Tetranychus evansi* spider mite populations suppress tomato defences to varying degrees. *Ecology and Evolution*, in review.

Knegt B, Potter T, Pearson NA, Sato Y, Staudacher H, Schimmel BCJ, Kiers ET, Egas M (2017). Detection of genetic incompatibilities in non-model systems using simple genetic markers: hybrid breakdown in the haplodiploid spider mite *Tetranychus evansi*. *Heredity*, **118**, 311-321.

Li G, Liu G, Xu C, In Lee G, Bauer P, Ling H-Q, Ganal MW, Howe GA (2003). The tomato suppressor of *prosystemin-mediated responses2* gene encodes a fatty acid desaturase required for the biosynthesis of jasmonic acid and the production of a systemic wound signal for defense gene expression. *The Plant Cell*, **15**, 1646-1661.

Li X, Schuler MA, Berenbaum MR (2002b). Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature*, **419**, 712–715.

Li C, Williams MM, Loh Y, Lee GI, Howe GA (2002a). Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. *Plant Physiology*, **130,** 494-503.

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004). The tomato homolog of *CORONATINE-INSENSITIVE1* is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell*, **16**, 126-143.

Lynch M, Walsh B (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, USA.

Løvdal T, Lillo C (2009). Reference gene selection for quantitative real-time PCR normalization in tomato subjected to nitrogen, cold, and light stress. *Analytical Biochemistry*, **387,** 238-242.

Magalhães S, Blanchet E, Egas M, Olivieri I (2009). Are adaptation costs necessary to build up a local adaptation pattern? *BMC Evolutionary Biology*, **9**, 182.

Magalhães S, Blanchet E, Egas M, Olivieri I (2011). Environmental effects on the detection of adaptation. *Journal of Evolutionary Biology*, **24**, 2653-2662.

Magalhães S, Cailleau A, Blanchet E, Olivieri I (2014). Do mites evolving in alternating host plants adapt to host switch? *Journal of Evolutionary Biology*, **27**, 1956-1964.

Magalhães S, Fayard J, Janssen A, Carbonell D, Olivieri I (2007). Adaptation in a spider mite population after long-term evolution on a single host plant. *Journal of Evolutionary Biology*, **20**, 2016-2027.

Martel C, Zhurov V, Navarro M, Martinez M, Cazaux M, Auger P, Migeon A, Santamaria ME, Wybouw N, Diaz I, Van Leeuwen T, Navajas M, Grbić M, Grbić V (2015). Tomato whole genome transcriptional response to *Tetranychus urticae* identifies divergence of spider mite-induced responses between Tomato and Arabidopsis. *Molecular Plant-Microbe Interactions*, **28**, 343-361.

McCloud ES, Baldwin IT (1997). Herbivory and caterpillar regurgitants amplify the wound-induced

increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. *Planta*, **203**, 430-435.

McGurl B, Orozco-Cardenas M, Pearce G, Ryan CA (1994). Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9799-9802.

Migeon A, Dorkeld F (2018). Spider Mites Web: a comprehensive database for the Tetranychidae. http://www.montpellier.inra.fr/CBGP/spmweb.

Mousseau TA, Fox CW (1998). The adaptive significance of maternal effects. *Trends in Ecology and Evolution*, **13**, 403-407.

Musser RO, Hum-Musser SM, Eichenseer H, Pfeiffer M, Ervin G, Murphy JB, Felton GW (2002). Caterpillar saliva beats plant defences. *Nature*, **416**, 599-600.

Narváez-Vásquez J, Florin-Christensen J, Ryan CA (1999). Positional specificity of a phospholipase A activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. *The Plant Cell*, **11**, 2249-2260.

Narváez-Vásquez J, Ryan CA (2004). The cellular localization of prosystemin: a functional role for phloem parenchyma in systemic wound signaling. *Planta*, **218**, 360-369.

Navajas M, de Moraes GJ, Auger P, Migeon A (2013). Review of the invasion of *Tetranychus evansi*: biology, colonization pathways, potential expansion and prospects for biological control. *Experimental and Applied Acarology*, **59,** 43-65.

Neal JJ (1987). Metabolic costs of mixed-function oxidase induction in *Heliothis zea*. *Entomologia Experimentalis et Applicata*, **43**, 175-179.

Orrock JL, Sih A, Ferrari MCO, Karban R, Preisser EL, Sheriff MJ, Thaler JS (2015). Error management in plant allocation to herbivore defence. *Trends in Ecology and Evolution*, **30**, 441-445.

Ozawa R, Arimura G-I, Takabayashi J, Shimoda T, Takaaki N (2000). Involvement of jasmonate- and salicylate-related signaling pathways for the production of specific herbivore-induced volatiles in plants. *Plant and Cell Physiology*, **41**, 391-398.

Paulo JT, Godinho DP, Silva A, Branquinho C, Magalhães S (2018). Suppression of plant defences by herbivorous mites is not associated to adaptation to host plants. *International Journal of Molecular Sciences*, **19**, 1783.

Pearce G, Strydom D, Johnson S, Ryan CA (1991). A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science*, **253**, 895-897.

R Core Team (2016) R: A language and environment for statistical computing. Foundation for statistical computing, Vienna. https://www.R-project.org/

Raffa KF (2001). Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. *Chemoecology*, **11**, 49-65.

Ryan CA (1990). Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual Review of Phytopathology*, **28**, 425-449.

Ryan CA (2000). The systemin signaling pathway: differential activation of plant defensive genes. *Biochimica et Biophysica Acta*, **1477**, 112-121.

Sarmento RA, Lemos F, Bleeker PM, Schuurink RC, Pallini A, Oliveira MGA, Lima ER, Kant MR, Sabelis MW, Janssen A (2011). A herbivore that manipulates plant defence. *Ecology Letters*, **14,** 229-236.

Sarmento RA, Venzon M, Pallini A, Oliveira EE, Janssen A (2007). Use of odours by *Cycloneda sanguinea* to assess patch quality. *Entomologia Experimentalis et Applicata*, **124**, 313-318.

Schimmel BCJ (2016). From induction to suppression: how to manipulate plant defences. PhD thesis, University of Amsterdam, Amsterdam.

Schimmel BCJ, Ataide LMS, Chafi R, Villarroel CA, Schuurink RC, Kant MR (2017a). Overcompensation of defense suppression by herbivores in response to competition. *New Phytologist*, **214,** 1688-1701.

Schimmel BCJ, Ataide LMS, Kant MR (2017b). Spatiotemporal heterogeneity of tomato induced defense responses affects spider mite performance and behavior. *Plant Signaling & Behavior*, **12**, e1370526.

Schrader F (1923). Haplodiploidie bei einer Spinnmilbe. *Archiv für Mikroskopische Anatomie*, **97**, 610-622.

Schultz JC, Appel HM, Ferrieri AP, Arnold TM (2013). Flexible resource allocation during plant defense responses. *Frontiers in Plant Science*, **4**, 324.

Smith J (1955). Detoxification mechanisms in insects. *Biological Reviews*, **30**, 455-475.

Stahl E, Hilfiker O, Reymond P (2018). Plant–arthropod interactions: who is the winner? *The Plant Journal*, **93**, 703-728.

Staudacher H, Schimmel BCJ, Lamers MM, Wybouw N, Groot AT, Kant MR (2017). Independent effects of a herbivore's bacterial symbionts on its performance and induced plant defences. *International Journal of Molecular Sciences*, **18,** 182.

Sugio A, Kingdom HN, MacLean AM, Grieve VM, Hogenhout SA (2011). Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, E1254-E1263.

Thaler JS, Humphrey PT, Whiteman NK (2012). Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, **17**, 260-270.

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007). JAZ repressor proteins are targets of the SCF complex during jasmonate signaling. *Nature*, **448**, 661-666.

Tien NSH, Sabelis MW, Egas M (2009). Heritability of defence and life-history traits in the two-spotted spider mite. *Evolutionary Ecology Research*, **11**, 1271-1281.

Traw MB, Dawson TE (2002). Differential induction of trichomes by three herbivores of black mustard. *Oecologia*, **131**, 526-532.

Van Kan JAL, Cozijnsen T, Danhash N, de Wit PJGM (1995). Induction of tomato stress protein mRNAs by ethephon, 2,6-dichloroisonicotinic acid and salicylate. *Plant Molecular Biology*, **27,** 1205-1213.

Verwoerd TC, Dekker BMM, Hoekema A (1989). A small-scale procedure for the rapid isolation of plant RNAs. *Nucleic Acids Research*, **17,** 2362.

Villarroel CA, Jonckheere W, Alba JM, Glas JJ, Dermauw W, Haring MA, Van Leeuwen T, Schuurink RC, Kant MR (2016). Salivary proteins of spider mites suppress defences in *Nicotiana benthamiana* and promote mite reproduction. *The Plant Journal*, **86,** 119-131.

Voelckel C, Schittko U, Baldwin IT (2001). Herbivore-induced ethylene burst reduces fitness costs of jasmonate and oral secretion-induced defenses in *Nicotiana attenuata*. *Oecologia*, **127**, 274-280.

Wasternack C, Hause B (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of Botany*, **111**, 1021-1058.

Whittaker RH, Feeny PP (1971). Allelochemics: chemical interactions between species. *Science*, **171**, 757-770.

Wood DL (1973). Selection and colonization of ponderosa pine by bark beetles. In: *Symposia of the Royal Entomological Society of London, number 6, Insect/Plant Relationships* (ed. van Emden HF), pp 101-117.

Wu J, Hettenhausen C, Meldau S, Baldwin IT (2007). Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. *The Plant Cell*, **19**, 1096-1122.

Wybouw N, Zhurov V, Martel C, Bruinsma KA, Hendrickx F, Grbić V, Van Leeuwen T (2015). Adaptation of a polyphagous herbivore to a novel host plant extensively shapes the transcriptome of herbivore and host. *Molecular Ecology*, **24,** 4647-4663.

Ximénez-Embún MG, Ertego F, Castañera P (2016). Drought-stressed tomato plants trigger bottom–up effects on the invasive *Tetranychus evansi*. *PLoS One*, **11**, e0145275.

Zhou S, Lou Y-R, Tzin V, Jander G (2015). Alternation of plant primary metabolism in response to insect herbivory. *Plant Physiology*, **169**, 1488-1498.

Züst T, Agrawal AA (2017). Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. *Annual Review of Plant Biology*, **68**, 513-534.

Supplementary information

TABLE S5.1. Specifications of primers used for qRT-PCR.

Target locus	Primer	Sequence (5' → 3')	Reference
actin	forward	TTAGCACCTTCCAGCAGATGT	Tomato Genome
	reverse	AACAGACAGGACACTCGCACT	Consortium (2012)
PI-IIf	forward	GACAAGGTACTAGTAATCAATTATCC	Graham et al. (1985)
	reverse	GGGCATATCCCGAACCCAAGA	
PPO-D	forward	GCCCAATGGAGCCATATC	Newman et al. (1993)
	reverse	ACATTCGATCCACATTGCTG	
PR-1a	forward	TGGTGGTTCATTTCTTGCAACTAC	van Kan et al. (1992)
	reverse	ATCAATCCGATCCACTTATCATTTTA	

TABLE S5.2. Statistical test details of mixed-effect models specifying the effects of culture method (initial population vs. wildtype strains) and host plant (wildtype, def-1, PS or bean) on oviposition rate (eggs per female per day). Results for models with and without a culture method x host plant interaction are shown. Denominator degrees of freedom are estimated using a Kenward-Roger approximation.

Model *with* an interaction between treatment and plant

Random terms	Variance	Std. dev	% variance			
tray:week	0.184	0.428	4.29%			
week	0.000	0.000	0.00%			
experim. strain	0.196	0.443	4.59%			
residual	3.893	1.973	91.11%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
culture method x host plant	14.246	4.749	3	64.573	1.096	0.357

Model *without* an interaction between treatment and plant

Random terms	Variance	Std. dev	% variance			
tray:week	0.108	0.329	2.51%			
week	0.000	0.000	0.00%			
experim. strain	0.186	0.431	4.32%			
residual	4.004	2.001	93.17%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
culture method	1.026	1.026	1	1.264	0.097	0.799
host plant	61.662	20.554	3	67.753	4.472	0.005

TABLE S5.3. Statistical test details of three mixed-effect models specifying the effects of experimental evolution treatment (wildtype vs. def-1, PS or bean) and host plant (wildtype, def-1, PS or bean) on oviposition rate (eggs per female per day). Denominator degrees of freedom are estimated using a Kenward-Roger approximation.

A. Wildtype vs. def-1 strains

Random terms	**Variance**	**Std. dev**	**% variance**			
tray:week	0.062	0.249	20.65%			
week	0.000	0.000	0.00%			
experim. strain	0.000	0.000	0.00%			
residual	0.239	0.489	79.35%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment × host plant	2.283	0.761	3	89.96	3.032	0.033

B. Wildtype vs. PS strains

Random terms	**Variance**	**Std. dev**	**% variance**			
tray:week	0.027	0.165	10.01%			
week	0.000	0.000	0.00%			
experim. strain	0.000	0.011	0.05%			
residual	0.245	0.495	89.94%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment	2.893	2.893	1	7.859	10.923	0.011
host plant	11.093	3.698	3	82.607	14.308	<0.001

C. Wildtype vs. bean strains

Random terms	**Variance**	**Std. dev**	**% variance**			
tray:week	0.013	0.115	4.94%			
week	0.008	0.091	3.07%			
experim. strain	0.000	0.000	0.00%			
residual	0.247	0.497	91.99%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment × host plant	2.779	0.926	3	78.066	3.507	0.019

TABLE S5.4. Statistical test details of three mixed-effect models specifying the effects of treatment (experimental evolution strains, uninfested control, suppression and induction benchmarks and initial population) on phytohormone amount (ng g^{-1} FW). Denominator degrees of freedom are estimated using a Kenward-Roger approximation.

A. JA

Random terms	**Variance**	**Std. dev**	**% variance**			
strain:round	0.016	0.127	3.54%			
round	0.040	0.200	8.83%			
residual	0.397	0.630	87.63%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment	2.251	0.322	7	23.226	0.797	0.597

B. JA-Ile

Random terms	**Variance**	**Std. dev**	**% variance**			
strain:round	0.085	0.291	13.01%			
round	0.038	0.195	5.83%			
residual	0.529	0.727	81.15%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment	24.147	3.450	7	23.206	6.522	<0.001

C. SA

Random terms	**Variance**	**Std. dev**	**% variance**			
strain:round	0.074	0.272	22.20%			
round	0.137	0.371	41.17%			
residual	0.122	0.350	36.63%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment	10.325	1.475	7	23.094	12.060	<0.001

TABLE S5.5. Statistical test details of three mixed-effect models specifying the effects of treatment (experimental evolution strains, uninfested control, suppression and induction benchmarks and initial population) on expression of genes associated with induced plant defence (expression relative to *actin*). Denominator degrees of freedom are estimated using a Kenward-Roger approximation.

A. PI-IIf

Random terms	**Variance**	**Std. dev**	**% variance**			
strain:round	0.414	0.644	28.47%			
round	0.208	0.456	14.24%			
residual	0.842	0.917	57.29%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment	25.497	3.643	7	23.457	4.321	0.003

B. PPO-D

Random terms	**Variance**	**Std. dev**	**% variance**			
strain:round	0.000	0.00	0.00%			
round	0.120	0.346	31.75%			
residual	0.257	0.507	68.25%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment	7.615	1.088	7	14.383	4.058	0.012

C. PR-1a

Random terms	**Variance**	**Std. dev**	**% variance**			
strain:round	0.222	0.471	12.04%			
round	0.622	0.789	33.77%			
residual	1.000	1.000	54.20%			
Fixed terms	**Sum of squares**	**Mean sum of squares**	**Df1**	**Df2**	**F**	**p**
treatment	51.756	7.394	7	23.280	7.393	<0.001

References in supplementary information

Graham, J.S., Pearce, G., Merryweather, J., Titani, K., Ericsson, L.H. & Ryan, C.A. (1985) Wound-induced proteinase inhibitors from tomato leaves. II. The cDNA-deduced primary structure of pre-inhibitor II. *Journal of Biological Chemistry*, **260**, 6561-6564.

van Kan, J.A.L., Joosten, M.H.A.J., Wagemakers, C.A.M., van den Berg-Velthuis, G.C.M. & de Wit, P.J.G.M. (1992) Differential accumulation of messenger-RNAs encoding extracellular and intracellular PR proteins in tomato induced by virulent and avirulent races of *Cladosporium fulvum*. *Plant Molecular Biology*, **20**, 513-527.

Newman, S.M., Eannetta, N.T., Yu, H., Prince, J.P., de Vicente, M.C., Tanksley, S.D. & Steffens, J.C. (1993) Organization of the tomato polyphenol oxidase gene family. *Plant Molecular Biology*, **21**, 1035-1051.

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, **485**, 635-641.

6

General discussion

Main aim of the thesis

Herbivorous arthropods employ a wide variety of offensive adaptations to attack their host plants (Karban & Agrawal 2002, Walling 2008). Why has this diversity evolved? What factors determine the success of an offensive strategy? Under which circumstances is it beneficial to employ a particular offensive strategy, and not another? Although offensive behaviour is ubiquitous among herbivores, the advantages and disadvantages of herbivore offensive strategies have received little attention compared to defensive strategies of plants. This is surprising, because offensive strategies can predispose herbivores to engage in behaviour that is damaging to natural environments and agriculture. Herbivores that detoxify plant defensive compounds, for example, are inclined to develop resistance against pesticides (Krieger et al. 1971, Dermauw et al. 2013, 2018). Likewise, herbivores that manipulate evolutionarily conserved plant signalling pathways across plant species are more apt to invade and disturb non-native environments than herbivores that intricately manipulate the physiology of a single host plant species (Pearse & Altermatt 2013).

Understanding the evolution of herbivore offensive strategies requires insight into its evolutionary costs and benefits (Blaazer et al. 2018). In this thesis, I studied the costs and benefits of a particular offensive strategy: suppression of inducible plant defences. Defence suppression benefits herbivores by preventing exposure to otherwise damaging plant defences, such as toxic or digestive inhibitory compounds, but also by reducing recruitment of natural enemies through plant volatiles (Musser et al. 2002, Zhang et al. 2009, Kant et al. 2015). The success of this strategy is illustrated by its occurrence in various major arthropod lineages, such as butterflies, whiteflies, aphids, thrips and mites, many of which are agricultural pests (Kant et al. 2015). However, defence suppression may also entail costs. Producing defence-suppressing enzymes, for example, or maintaining symbioses with defence-suppressing microorganisms, could ensue metabolic costs (Dupas & Boscaro 1999, Gwynn et al. 2005). Suppressed plants also constitute a suitable resource for competing herbivores, and may increase predator efficiency (Bruessow et al. 2010, Ataide et al. 2016). Defence-suppressing herbivores may therefore have to balance these costs against the benefits of lowered plant defences, especial-

Considering the potential costs associated with defence suppression, why do herbivores employ this offensive strategy, when other strategies are also possible? To study why defence suppression constitutes such a successful herbivore offensive strategy, I investigated its costs and benefits for the defence-suppressing spider mite *Tetranychus evansi*.

Overview of the contributions of this thesis

Previous studies found that *T. evansi* benefits from defence suppression through increased fecundity, but the absolute and relative size of the benefit varied substantially among these studies (Sarmento et al. 2011a, b, Godinho et al. 2016, de Oliveira et al. 2016, Schimmel et al. 2017a, b). Therefore, in CHAPTER 2, I reviewed past evidence, published and unpublished, of the benefits of defence suppression conferred to *T. evansi* in terms of increased fecundity. I found that, on average, *T. evansi* increase their oviposition rate with approximately one egg per day on tissues previously infested by conspecifics, corresponding to an increase in fecundity of 9-12%, a substantial increase for species with exponential growth (Sabelis 1991). This benefit varied considerably among studies, ranging from strongly positive to none at all or even slightly negative. Some of this variation could be explained by the time that spider mite populations had been cultured in laboratories before being used in experiments, such that weaker benefits were found when populations had been maintained in the laboratory for longer time. These results indicate that *T. evansi* adapted to culture conditions, and that the net effect of suppression of plant defence on the performance of spider mites in subsequent infestations is subject to phenotypic change over generations. Consequently, *T. evansi* populations harbour heritable variation for their plant-mediated interactions with other herbivores, and thus have the potential to adapt to plant-mediated ecological interactions.

The meta-analysis in CHAPTER 2 not only quantified the average benefit of defence suppression for *T. evansi*, but also confirmed previously published assertions regarding potential ecological costs. Specifically, I found that induction of tomato defences by defence-inducing genotypes of the spider mite species *Tetranychus urticae* decreased the fecundity of spider mites, *T. evansi* and *T. urticae*, in subsequent infestations with approximately one egg per day. This is in line with my assumption that *T. evansi* is susceptible to induced defences, and confirms that the presence of *T. urticae* can pose an ecological cost to *T. evansi* through its effects on plant defence (Kant et al. 2008, Sarmento et al. 2011a, b, Alba et al. 2015, Godinho et al. 2016, de Oliveira et al. 2016). Moreover, I found that *T. urticae* also attained an increase in fecundity of one egg per day from defence suppression by *T. evansi*. This shows that lowered tomato defences are profitable for spider mites in general, and that, on average, defence suppression increases not only the performance of *T. evansi* itself, but also that of its competitors. I also found, however, that these effects may depend on the timing and density of infestation, and on the

scale within the plant at which these interactions occur (Alba et al. 2015, de Oliveira et al. 2016, Schimmel et al. 2017a, b).

The phenotypic change in plant-mediated interactions with other spider mite populations observed in CHAPTER 2 provides indirect evidence for intraspecific variation in defence suppression within *T. evansi* populations. To measure intraspecific variation in defence suppression more directly, in CHAPTER 3 I investigated *T. evansi* populations from several locations around the world. Because the costs of defence suppression likely depend on biotic interactions with competitors and natural enemies, I expected *T. evansi* from different environments to have evolved to suppress plant defences to varying levels. By measuring the expression of several marker genes for tomato defence induction, I found significant variation among *T. evansi* populations in the degree to which they suppressed jasmonic acid (JA)-dependent and salicylic-acid (SA)-dependent defence pathways. This complements the findings of CHAPTER 2, by showing that variation for defence suppression is not only present within, but also among *T. evansi* populations.

Additionally, in CHAPTER 3 I found a trend that invasive *T. evansi* populations suppress plant defence less strongly than populations from the native South-American range. Because specialist natural enemies, such as *Phytoseiulus longipes* predatory mites (da Silva et al. 2010), are absent outside the native range of *T. evansi* (Ferragut et al. 2013), a possible explanation is that reduced biotic interactions with natural enemies allowed invasive *T. evansi* populations to relax their suppression of inducible plant defence. However, geographical range and genetic lineage were completely collinear variables in this dataset: all invasive populations belonged to the *T. evansi* genetic 'lineage I', while native populations belonged to genetic 'lineage II'. Differentiation between these lineages likely preceded migration outside South America (Boubou et al. 2012). Therefore, an alternative explanation is that in South America differences among the habitats of the two *T. evansi* lineages selected for different levels of defence suppression.

CHAPTERS 2 and 3 demonstrate that *T. evansi* populations harbour intraspecific variation for defence suppression within and among populations. To investigate this further, I hypothesized that manipulating the benefits of defence suppression through experimental evolution could expose its costs. Specifically, in environments where inducible plant defences are either absent or constitutive, I expected *T. evansi* to lower its level of defence suppression through drift or through selection against metabolic costs. Experimental evolution, however, requires a source population with sufficient genetic variation. I hypothesized that a cross between *T. evansi* mites from the two genetically differentiated lineages (Gotoh et al. 2009, Boubou et al. 2012) would produce such a genetically diverse population through combination and recombination of their genetic material. Therefore, in CHAPTER 4, I performed reciprocal crosses between *T. evansi* from lineages I and II. First, I confirmed that offspring suffered substantial post-zygotic

hybrid breakdown, by showing that only ~5% of hybrid F2 offspring hatched. Then, by genotyping viable and inviable hybrid offspring at eight microsatellite loci, I showed that Bateson-Dobzhansky-Muller incompatibilities underlie hybrid breakdown among these lineages. Moreover, I also found that viable hybrids nevertheless contained recombined genetic material, and that heterosis contributes to their viability.

After establishing a hybrid *T. evansi* population that contained recombined genetic material of lineages I and II, in CHAPTER 5 I exposed this population to experimental evolution on host plants with an altered inducible JA-dependent defence response. I used *defenseless-1* (def-1) tomato mutants in which JA-dependent inducible defences are absent (Howe et al. 1996), the *35S::prosystemin* (PS) tomato genotype in which these defences are constitutively overexpressed (McGurl et al. 1994), and *Phaseolus vulgaris* bean as a distantly related host with different defences. I expected that in the absence of inducible defences (def-1), when suppression of plant defences is not possible because they are constitutively overexpressed (PS), or because they are regulated differently than in tomato (bean), suppression is not necessary and would erode through drift or be selected against because of metabolic costs.

I maintained the hybrid *T. evansi* population for approximately 60 generations in these three environments and on wildtype controls, and then measured the performance of these mites on wildtype tomato, def-1, PS and bean. I found that *T. evansi* fecundity showed a general response to selection, but that fecundity on wildtype tomato remained high and was similar among all evolved strains. In line with these results, the degree to which wildtype tomato plants infested with these mites accumulated phytohormones and expressed defence gene markers was also similar among these strains. This shows that evolved strains retained their level of tomato defence suppression, and that metabolic costs of defence suppression are likely low. Alternative explanations could be that *T. evansi* prevents metabolic costs through only suppressing plant defences when it would be effective (i.e., phenotypic plasticity), or that host plant manipulation by *T. evansi* provides more benefits than defence suppression only, such as an altered nutrient distribution in the host plant (Kaiser et al. 2010). These benefits could have outweighed metabolic costs even when defence suppression was not necessary or possible.

Main conclusions of this thesis

Taken together, the results presented in this thesis help to better understand the evolution of defence suppression by *T. evansi*, and its associated costs and benefits. First, defence suppression is a trait for which sufficient heritable intraspecific variation exists in *T. evansi* to allow evolutionary change. This is substantiated by my finding that *T. evansi* populations suppress both JA- and SA-dependent defence marker genes to significantly different levels (CHAPTER 3). Additionally, I found that the effect size of increased fecundity through defence suppression decreases with the time that *T. evansi* populations

have been maintained in laboratories, representing heritable phenotypic change over generations (CHAPTER 2).

Second, a benefit of defence suppression conferred to *T. evansi* is an increase in fecundity of ~9-12% on average, across multiple laboratory experiments (CHAPTER 2). This benefit varied substantially among experiments, and a large part (~75%) of this variation could not be explained by differences in the experimental setups among these studies. Likewise, competing spider mites can impose a substantial cost on *T. evansi*, because they are able to benefit from defence suppression equally well as *T. evansi*, and can decrease the quality of a shared host through induction of plant defences. These costs are, however, as variable among studies as the benefits of defence suppression, and a large part of this variation remains unexplained. Therefore, factors that are currently unknown may substantially affect the size of costs and benefits of defence suppression for *T. evansi*.

Third, metabolic costs of defence suppression, for example to produce the effector proteins that interfere with host plant physiology, are likely low. They are in any case small enough to allow *T. evansi* populations to maintain their level of suppression in environments where defence suppression is not necessary (CHAPTER 5). This is, however, not evidence of the absence of metabolic costs, because *T. evansi* may also reduce metabolic costs through plasticity in the production of effector proteins. Alternatively, the benefits of host plant manipulation may outweigh the costs of effector production, if manipulation pertains more benefits than through suppression of JA-dependent inducible defences only.

Evolution of defence suppression by herbivores – suggestions for further research

Although defence suppression by *T. evansi* spider mites is an interesting phenomenon by itself, in this thesis it serves as model to study the evolutionary consequences of defence suppression as a herbivore offensive strategy. Therefore, in this section I will discuss the general relevance of my conclusions for understanding the evolution of defence suppression by herbivores. I will also list current gaps in our knowledge about costs and benefits of defence suppression, and suggest three directions for further research into the evolution of defence suppression as an offensive strategy.

General relevance of the conclusions of this thesis

Suppression of plant defences by herbivorous arthropods has mostly been investigated with an ecological or mechanistic focus. Ecological studies first demonstrated that plant responses induced by one herbivore species could actually benefit another (Denno & Kaplan 2007). For example, *Eulachnus agilis* aphids have higher survival and develop faster when they feed from pine needles that are also occupied by *Schizolachnus pineti*

aphids, presumably through induced changes in nutrient distribution within their host plant (Kidd et al. 1985). Similarly, *Spodoptera ornithogalli* caterpillars are only able to feed from *Lactuca serriola* plants after their latex canals have been severed by *Trichoplusia ni* caterpillars (Dussourd & Denno 1994).

In a next step, mechanistic studies demonstrated that herbivores produce compounds, or harbour symbiotic microorganisms, to actively manipulate physiological processes in plants (Kant et al. 2015, Stahl et al. 2018). For example, oral secretions of *Manduca sexta* caterpillars suppress expression of genes that are activated after wounding in *Nicotiana attenuata* plants (Schittko et al. 2001), and the first specific compound to be identified as a herbivore salivary constituent that suppresses plant defence was glucose oxidase in *Helicoverpa zea* caterpillars (Musser et al. 2002). Likewise, Yang et al. (2008) discovered that *Bemisia tabaci* whiteflies can transmit *Tomato yellow leaf curl China virus*, which induces the production of a protein, βC1, that suppresses JA-dependent genes and biosynthesis of defensive terpenoids (Li et al. 2014).

Now that the ecological consequences of defence suppression have been characterized in a number of systems (Kant et al. 2015), and its mechanisms have been revealed in some (Stahl et al. 2018), evolutionary questions can be asked, such as whether variation exists for the level of suppression. Other than in *T. urticae* (Kant et al. 2008), intraspecific variation in defence suppression by herbivores has only been investigated in *T. evansi* (Alba et al. 2015, this thesis). Variation among herbivore populations in their offensive strategies, and specifically in the level to which they suppress plant defences, is a necessary characteristic for defence suppression as a trait to evolve, and can have important consequences for community dynamics (Bolnick et al. 2011). Although directional selection may favour suppressors over non-suppressors and thus reduce genetic variation, this variation can theoretically be maintained as the costs and benefits of suppression vary across different host plant species, due to trade-offs with life-history traits, or change with the density of competitors and natural enemies (Kant et al. 2015, Gloss et al. 2016). Previously, several spider mite species were found to harbour intraspecific variation for their offensive strategy, where some populations suppressed and others induced plant defences (Takabayashi et al. 2000, Matsushima et al. 2006, Kant et al. 2008). Additionally, Alba et al. (2015) described two *T. evansi* populations that differed in the level to which they suppressed plant defences. In this thesis, I demonstrate significant intraspecific variation in defence suppression among herbivore populations from a wide geographic range (CHAPTER 3) and heritable variation within herbivore populations in lab cultures (CHAPTER 2). Together, these properties allow defence suppression to evolve in response to varying costs and benefits imposed by the herbivores' environment.

Herbivore offensive strategies such as detoxification of defensive plant compounds and suppression of inducible plant defences require the production of specific enzymes

(Heckel 2014, Stahl et al. 2018). However, the production of such enzymes can impose metabolic costs, as was demonstrated for the production of detoxification enzymes in caterpillars, aphids and spider mites (Creswell et al. 1992, Agrawal et al. 2002, Castañeda et al. 2010). Such costs can be relevant for the evolution of defence suppression, because they may impose a trade-off with life-history traits through competition for resources, and they can allow potential conspecific 'free-riders' that do not invest into defence suppression to attain a performance advantage over suppressors without paying a cost (Kant et al. 2015). In CHAPTER 5, I used the heritable, genetic variation in defence suppression among *T. evansi* populations demonstrated in CHAPTERS 2-4 as a starting point for experimental evolution. This allowed me to investigate the existence of metabolic costs for herbivores that suppress plant defences. I found that suppression of plant defence was retained in populations exposed to environments where suppression was either redundant or ineffective. My contribution thus is, that the metabolic costs of defence suppression are likely low, and that phenotypic plasticity in the production of defence-suppressing effectors, or attaining benefits other than suppression of plant defences only, are worthwhile subjects for further investigation.

A related, and an equally important question from an evolutionary perspective, is to what degree herbivores actually benefit from suppressed plant defences. Costs and benefits of defence suppression are typically quantified in terms of herbivore performance, through measuring changes in weight gain (Musser et al. 2002), survival (Mutti et al. 2008), oviposition rate (Kant et al. 2008), development time (Zarate et al. 2007), or population growth (Sarmento et al. 2011a). However, these costs and benefits can vary widely across studies, even when the same performance read-out is used. This is substantiated by the results of CHAPTER 2, where I quantify significant variation in the fecundity benefit conferred to spider mites through defence suppression by *T. evansi*, such that some studies even report significant negative effects on fecundity. Even though these studies represent carefully controlled lab experiments, ~75% of this variation could not be attributed to differences in their experimental set-ups. Therefore, my work (CHAPTER 2) has highlighted that unknown factors introduce major variability in performance measurements, asking for further investigation of these factors, or alternatively, to assess costs and benefits of defence suppression in more realistic natural settings instead.

Knowledge gaps of costs and benefits of defence suppression

Even though defence suppression and other herbivore offensive strategies have recently caught the attention of evolutionary biologists (Ali & Agrawal 2012, Blaazer et al. 2018), several potential costs and benefits of defence suppression await further study. In this section, I will bring up a number of gaps in our understanding of the evolutionary payoffs of defence suppression.

First, benefits of defence suppression have predominantly been characterised through measuring increases in herbivore performance (Kant et al. 2015, CHAPTER 2). However, benefits can also arise through suppression of indirect plant defences, e.g., by suppressing the production of plant volatiles that serve as cues for natural enemies to locate their prey. *Gossypium hirsutum* cotton plants, for example, produce significantly fewer volatiles when infested by both *Bemisia tabaci* whiteflies and *Spodoptera exigua* caterpillars, than when infested by *S. exigua* alone, presumably through suppression by *B. tabaci* (Rodriguez-Saona et al. 2003). Likewise, in other systems, plant defence suppression resulted in altered volatile emissions (Tooker et al. 2008, Kant et al. 2008, Zhang et al. 2009, Peñaflor et al. 2011, Schwartzberg et al. 2011, Sarmento et al. 2011a, Takai et al. 2018). In many systems, however, it remains unclear to what extent herbivores benefit from these altered volatile blends, although in some cases fitness benefits have been observed (Frago et al. 2017). This is an important gap in our knowledge, because top-down processes are on average more important than bottom-up processes in controlling arthropod herbivore populations (Vidal & Murphy 2018). Field experiments could provide more insight into the role of a suppressed production of plant volatiles in the evolution of defence suppression, e.g., by comparing the performance of defence suppressing herbivores on plants shielded off from natural enemies (through, for example, a cage) with the performance of herbivores on unprotected control plants.

Second, besides defence, herbivores can also manipulate other aspects of plant quality, such as nutrient content and plant morphology (Giron et al. 2016). For example, the galling wasp *Neuroterus quercusbaccarum* induces leaf galls in its *Quercus robur* oak hosts, in which it tightly manipulates nitrogen levels to optimize its survival (Hartley & Lawton 1992). There are indications that defence suppressing herbivores also manipulate other aspects of plant quality, not defence levels only. For example, spider mites feeding from leaf tissue close to a *T. evansi* feeding site on tomato experienced reduced fecundity, potentially because *T. evansi* recruited nutrients away from distant tissues (Schimmel et al. 2017b). Similarly, tomato plants infested with *T. evansi* contain higher soluble sugar content than plants infested with *T. urticae* (Schimmel 2016) or uninfested plants (Ximénez-Embún et al. 2016). If the manipulation of plant defence and nutrient content is linked in herbivores, e.g., because they are exerted by the same salivary effectors or because of linkage disequilibrium in the underlying genes, then these simultaneous manipulations have the potential to explain why defence suppression was maintained in my experimental evolution experiment (CHAPTER 5). Clearly, characterization of the different aspects of host plant manipulation by defence suppressing herbivores remains an important subject for further study.

Third, we lack information about the range of hosts in which herbivores can effectively suppress defences. Insight into the host range of defence suppressing herbivores is relevant, because it has been hypothesized that defence suppressors have broader host

ranges than herbivores that detoxify defensive plant compounds (Alba et al. 2011, Ali & Agrawal 2012, Blaazer et al. 2018). The idea behind this hypothesis is that plant defences are more diverse than their underlying molecular pathways. From the perspective of generalist herbivores, suppressing these pathways is then more efficient compared to detoxifying their more diverse end products. Although several studies have investigated possible differential plant responses to infestation with herbivores with different host breadths (Agrawal et al. 2000, Bidart-Bouzat & Kliebenstein 2011), no clear link was found. It is also unknown if specialist and generalist herbivores consistently differ in their salivary effectors and elicitors (Ali & Agrawal 2012).

A direct comparison of the host range of defence suppressors and herbivores that detoxify plant compounds could be another way to investigate the hypothesis that the former have broader host ranges than the latter, but we lack information about the range of hosts in which herbivores can effectively suppress defences. Previously, Ataide (2013) demonstrated that *T. urticae* can reach higher performance on *Phaseolus vulgaris* bean plants when these plants are co-infested with *T. evansi*. This suggests that the defences of *P. vulgaris* are compromised by *T. evansi*, but it is unknown if this occurs through the same mechanism as in tomato. Recently, this work was complemented by Paolo et al. (2018), who found that defence levels were similar among several plant species infested with *T. evansi* and uninfested controls. Further research is necessary to confirm whether the lack of increased defences is caused by suppression of the JA- or SA-inducible defence pathways, or by other mechanisms such as plant phenotypic plasticity (Hahn & Maron 2016).

A fourth gap in our knowledge is that we lack insight into the dynamics and plasticity of plant defence suppression by herbivores in time and space. Timing, for example, plays an important role, because the fecundity benefits of defence suppression by *T. evansi* are strongest after 1-2 days of feeding and decrease thereafter (de Oliveira et al. 2016). Likewise, the plant's defence response is differentially affected when defence suppressors occur in different densities (Alba et al. 2015), or when the sequence of arriving herbivores species differs (Schimmel et al. 2017a, b). Additionally, *T. evansi* potentially suppresses plant defences in a phenotypically plastic manner, because it may at times not be necessary to suppress defences (Chapter 5), or because altered levels of suppression may help in competition with other herbivore species (Schimmel et al. 2017a, b). Given that timing, density, order of infestation, and plasticity are all likely causes of variation in the costs and benefits of defence suppression, insight into these factors can potentially help to clarify part of the variation in fecundity benefit of defence suppression among laboratory experiments that I observed in Chapter 2. Further research is necessary to provide a more robust understanding of these dynamics of defence suppression.

Three suggestions for further research

To follow up on knowledge gaps identified in the previous section, here I provide three detailed suggestions for further research into the evolution of defence suppression by herbivores.

Phenotypic plasticity of offensive strategies in a related group of spider mites

To further explore the evolution of defence suppression, my first suggestion combines two open questions. The first question is if the phenotypically plastic response of herbivores to competitors differs between defence inducers and defence suppressors. In nature, plants are often attacked by multiple herbivores simultaneously. Each herbivore species induces its own response in the shared host plant, which will subsequently also affect coinfesting other herbivore species. Because such plant-mediated species interactions have strong consequences for herbivore performance (Poelman et al. 2008, Stam et al. 2014, CHAPTER 2), herbivores can be expected to adjust their offensive behaviour accordingly. My hypothesis is that phenotypic plasticity in offensive strategy upon the introduction of a competitor differs between defence inducers and defence suppressors. For example, defence inducers that are themselves resistant to the defences they induce, may induce even stronger defences when they receive cues that their host plant is also attacked by a potentially more susceptible competitor. Conversely, because competitors can pose a cost to defence suppressors (Sarmento et al. 2011a, Glas et al. 2014, CHAPTER 2), defence suppressors may try to monopolise their feeding site (Sarmento et al. 2011b), or may try to restrict its benefits to a confined area (Schimmel et al. 2017a, b).

The second question that I would address is whether defence suppression is an evolutionarily conserved trait among spider mites. Spider mites are thus far the only group of related herbivores (Matsuda et al. 2014) in which several species have been found to suppress (*T. evansi*, *T. urticae*, *T. ludeni*) or to induce (*T. urticae*, *T. kanzawai*) defences of one host plant (*Solanum lycopersicum* cultivated tomato) (Matsushima et al. 2006, Kant et al. 2008, Sarmento et al. 2011a, Godinho et al. 2016). If defence suppression is a conserved trait, then closely related species should employ similar molecular mechanisms (e.g. salivary effectors) to suppress the defence of their host plants. Alternatively, if defence suppression occurs through different mechanisms in each species, this would suggest a remarkable parallel trait evolution on a short evolutionary timescale.

The variation in offensive strategy among closely-related spider mites allows the comparison of their offensive strategies without being confounded with differences in phylogenetic relatedness. Additionally, plasticity in these species can be studied on a genomic scale using the sequenced genome of *T. urticae* (Grbić et al. 2011). Therefore, I propose to first determine gene expression in each of these five spider mite species in single infestations on tomato, through sequencing of their transcriptomes. Subsequently, these mites can be exposed to competition with another, defence-induc-

ing herbivore, and may respond by adjusting their offensive strategy. This phenotypic plasticity will stem from altered gene expression, and should thus be possible to measure through a second round of transcriptome sequencing. Finally, the observed changes in gene expression can be compared among species, and between defence inducers and suppressors. This will give information on whether defence inducers and defence suppressors use different genes in their response to a competitor, and if defence suppressors share an evolutionarily conserved response.

Herbivore offense syndromes

My second suggestion is to study defence suppression as an offensive syndrome. A 'syndrome' is a correlated set of traits whose associations are produced by selection. Different syndromes are mutually exclusive, because they are formed through evolutionary trade-offs rather than plasticity. A well-known example of syndromes is herbivore feeding guilds (Root 1967), which categorise herbivore species by the plant tissues they consume, such as leaf chewing herbivores (e.g., grasshoppers) or sap-sucking herbivores (e.g., aphids). Species from the same guild share a set of morphological and physiological traits that enable them to efficiently consume their preferred tissues. Another example is plant defence syndromes (Agrawal & Fishbein 2006), which were introduced to illustrate how plant defence trade-offs occur at a multivariable scale rather than at the level of single traits. This means that, for example, some plants may adopt a syndrome of tolerance to herbivory through expressing fast growth, high N levels, low chemical defence and low leaf toughness, whereas others employ a syndrome of resistance through low N levels, water content and specific leaf area, but also strong physical and chemical defences (Agrawal & Fishbein 2006). Because herbivores often employ multiple offensive traits simultaneously, such as avoidance and detoxification of host plant defences (Després et al. 2007), the possibility of multivariate trade-offs in herbivores seems warranted as well.

The reason that I advocate syndromes as a useful paradigm to study herbivore offensive strategies, is twofold. First, offensive syndromes allow the categorisation of herbivore offensive strategies in an evolutionarily meaningful way. The key is that herbivores that employ the same offense syndrome are expected to converge on expressing a similar set of correlated traits, even though they may be evolutionarily distantly related. Demonstrating that several groups of unrelated herbivores converge on employing a similar set of correlated offensive traits then supports the hypothesis that these traits provide an evolutionary solution to a given set of environmental conditions. As an example of convergent evolution in herbivore offense, larvae of the monarch butterfly *Danaus plexippus* sequester toxic cardenolides of their milkweed host plants as defence against natural enemies, and are themselves insensitive to cardenolides due to an amino acid change in their Na^+/K^+-ATPase enzymes (Holzinger & Wink 1996, Opitz & Müller 2009).

Surprisingly, *Chrysochus auratus* and *C. cobaltinus* beetles that also feed from milkweeds and sequester its chemicals, have evolved insensitivity to cardenolides through the exact same amino acid change, despite their evolutionary distance from butterflies (Labeyrie & Dobler 2004). Such convergent evolution indicates that target site insensitivity is an adequate evolutionary solution to the simultaneous challenge of feeding from plants with toxic compounds while obtaining protection against natural enemies. To further confirm the evolutionary association between target site insensitivity and sequestration, future research could investigate if other mechanisms of resistance, such as enzymatic detoxification (Heckel 2014) or producing decoy receptors (van der Hoorn & Kamoun 2008), are more often employed by herbivores that do not sequester host chemicals.

Second, studying herbivore offensive strategies as sets of covarying traits gives rise to new hypotheses. For example, Blaazer et al. (2018) recently hypothesized that defence suppressing spider mites such as *T. evansi* require traits that 'buffer' (Frank 2007) them against negative selection due to ecologically costly suppression of defences. The idea is that defence suppressing herbivores simultaneously evolve traits that mitigate the negative consequences of competition, because otherwise defence suppression would be too costly to maintain. Defence suppression is costly, because competing herbivores can benefit from suppressed defences, and can also reduce the quality of a shared host through induction of defences (Kant et al. 2008, Sarmento et al. 2011a, Glas et al. 2014, Kant et al. 2015, CHAPTER 2). Examples of traits that reduce these costs in *T. evansi* are the production of dense webbing that excludes competitors from feeding sites (Sarmento et al. 2011b), interference with the reproduction of competitors (Sato et al. 2014, 2016, Clemente et al. 2016, 2018), and localised hypersuppression of plant defences upon exposure to competition with *T. urticae* (Schimmel et al. 2017a, b). If other, unrelated, herbivore species that suppress plant defences also have traits that reduce competition, and if they possess these traits more often than non-defence suppressing relatives, then defence suppression and traits that reduce competition could form an offense syndrome. A hypothesis that could arise from this syndrome is that defence suppression is more likely to evolve in species-poor communities, or in species that already have strong 'buffers' against competitors in place.

Many syndromes can potentially be described among herbivore species, but when can a syndrome be considered as offensive? This is not a trivial question, because any trait that enhances herbivore performance will likely increase herbivore damage to their host plants and can thus be broadly seen as 'offensive'. However, such a broad definition of offense would render it too similar to herbivore fitness in general, and herbivore life history strategies are already actively studied (Nylin & Gotthard 1998, Blanckenhorn 2000, Awmack & Leather 2002, Moreau et al. 2017). Therefore, Karban & Agrawal (2002) have defined herbivore offensive traits as traits that increase the rate or efficiency of host use, such as feeding, ovipositing, or sequestering host chemicals, and that increase

herbivore performance. This explicitly excludes the amount of damage inflicted upon host plants, or its consequences for plant fitness. Examples of traits that can be recognised as offensive under this definition are host choice, suppression of inducible plant defences, detoxification of plant defensive compounds, and gregarious feeding. General life history traits that affect herbivore performance but that do not increase host use efficiency, such as body size or development time, are excluded.

Although the definition of Karban & Agrawal (2002) is useful in defining herbivore offense from the herbivore perspective, offense can also be directed to other organisms than plants, such as competing herbivores. Herbivores have a suite of traits that can help them in conflicts with competitors, such as territorial fighting in wood-eating termites (Florane et al. 2004), vibratory signalling display against intruders in shelter-building caterpillars (Yack et al. 2014) that may escalate into punching and hitting (Sigmon 2015), or reproductive interference (Sato et al. 2014, Gröning & Hochkirch 2008). Because herbivores may not always fight over resources related to their host plant such as food or territory, but also over other resources such as mates (Kemp & Wiklund 2001), herbivore offensive behaviour need not necessarily be limited to traits that increase the efficiency of host plant use. The definition of Karban & Agrawal (2002) is therefore only useful in the context of plant-herbivore interactions.

Studying herbivore offensive syndromes with a macroevolutionary perspective may be novel, but the notion that distantly related herbivore species can converge on similar offensive traits in similar environments has been explored by other authors. Rhoades (1985) distinguished 'opportunistic' herbivores that try to eat from many different plant species but are quickly repelled when a plant is well-defended, and 'stealthy' herbivores that overcome plant defences through detoxification, manipulation, or sequestration. These different strategies then are expected to produce different population dynamics, with opportunistic herbivores occurring in aggregations and having highly variable 'outbreak' dynamics, and stealthy herbivores living solitarily and maintaining low and relatively invariant population densities. An alternative model was suggested by Price et al. (1990, 1992), in which ovipositing females can be choosy or less choosy for a suitable host for her offspring. Species with choosy females have a restricted host range, occur in steady densities and have 'latent' population dynamics, whereas species with less-choosy females consume a large variety of lower-quality hosts and have variable ('eruptive') population dynamics. Last, Karban & Agrawal (2002) identified two axes along which herbivore offensive adaptations can be classified: whether the offensive trait works before or after ingestion of the plant tissue, and whether the trait is fixed or subject to phenotypic plasticity. Following this framework, manipulation of host plants should mostly occur before ingestion. Additionally, generalist herbivores feed from many different plants, and are thus expected to exert plasticity in their offensive traits, whereas specialist traits should more often be fixed.

The models of Rhoades (1985), Price et al. (1990, 1992) and Karban & Agrawal (2002) have in common that they contrast sets of correlated traits in herbivores, and formulate predictions for their population dynamics or host breadth. Because the sets of traits that they describe are not restricted to any particular herbivore clade, they can occur throughout distantly related herbivores, and thus constitute potential herbivore offense syndromes. Despite their relevance for the evolutionary ecology of plant herbivore interactions, and for understanding the occurrence of pest outbreaks, the predictions of these models remain largely untested (but see Pires et al. 2000). This is surprising, because the methods to compare offensive adaptations among herbivore species, while taking phylogenetic relatedness into account, are available (Agrawal & Fishbein 2006, Agrawal 2007, Agrawal et al. 2010). Future research could take up this challenge, and study associations between herbivores with similar sets of offensive traits and the ecological conditions in which they thrive.

Theoretical insight into defence suppression by herbivores

In the introduction of this thesis, I shortly reviewed research into defence suppression by parasites (referred to as 'immune evasion' in parasite-host literature). Because immune evasion is a well-recognised offensive strategy of many parasitic groups (Schmid-Hempel 2008), its mechanisms and evolutionary consequences have been described in more detail than in plant-herbivore research (Guiget et al. 2016). Most notably, several theoretical studies provide insight into costs and benefits of immune evasion. For example, Kamiya et al. (2018) demonstrate that immune evasion may spur the evolution of parasite virulence. This is because hosts with suppressed defences constitute favourable hosts for competing parasites, and within-host competition leads to the evolution of more competitive, and thus more virulent parasites. A major cost of virulence, however, is death of the host, and thereby reduced chances for successful transmission (Anderson & May 1979, Alizon et al. 2009). Consequently, when virulence increases too much, the costs of earlier host death may render investment into immune evasion unprofitable (Kamiya et al. 2018). Costs and benefits of immune evasion therefore strongly depend on the presence of competing parasites.

It is attractive to extrapolate these conclusions to plant-herbivore systems, and for example hypothesise that defence suppressing herbivores also evolve to overexploit their hosts faster to prevent the likelihood of coinfestation. Some evidence suggests that this might be the case. For instance, competition with herbivores that coinfest the same host plants imposes substantial costs to defence suppressors (Sarmento et al. 2011a, Glas et al. 2014, CHAPTER 2). In response, defence suppressors may try to monopolise their feeding site (Sarmento et al. 2011b) and interfere with the reproduction of competitors (Sato et al. 2014, 2016, Clemente et al. 2016, 2018). Additionally, they may also try to increase their competitive population growth through hypersuppression and

increased fecundity (Schimmel et al. 2017a, b). This latter response likely increases damage to the host plant, and may thereby reduce the time until the host is overexploited. This suggests that defence suppressing herbivores indeed overexploit their hosts faster when they sense the presence of competitors.

Although it is tempting to directly apply insights from parasite-host systems to plant-herbivore systems, caution is warranted for two reasons. First, herbivores have more possibilities for active dispersal than many parasites. Where parasites such as viruses and bacteria almost entirely depend on movement of their hosts or of vector organisms (e.g., mosquitos) to disperse and infect new hosts, herbivores are often capable of dispersal themselves. This independence likely reduces the evolutionary cost of host death, i.e., reduced chances for successful transmission (Anderson & May 1979, Alizon et al. 2009), because when their host dies, herbivores can disperse and search for a new host. Consequently, although host death may pose an upper limit to virulence evolution in defence suppressing parasites (Kamiya et al. 2018), this limit is likely relaxed or absent in defence suppressing herbivores. Because herbivore species (and also parasites) differ in their dispersal abilities, future theoretical studies could investigate the influence of dispersal possibilities on the evolution of virulence and defence suppression.

Second, theoretical insight into how natural enemies may affect costs and benefits of defence suppression is lacking. This is relevant, because parasites are less prone to predation or parasitism due to their physically often more intimate interaction with their hosts than herbivores (Marquis & Alexander 1992, Raffel et al. 2008). If natural enemies affect the evolution of defence suppression, they may do so more strongly in herbivores than in parasites. Obviously, these differences are not universally true: some herbivores also live within plant tissues (e.g., leaf miners, gall wasps, endophytic nematodes), and some natural enemies can attack endophytic herbivores within their host plants (e.g., entomopathogenic fungi). Nevertheless, how organisms in the third trophic level (predators, parasitoids, omnivores, pathogens) affect interactions among the first and second trophic level has more often been investigated in plant-herbivore systems than in parasite-host systems (e.g., Ode 2006, Poelman et al. 2008, Gols & Harvey 2009). Consequently, hypotheses on how natural enemies may affect the costs and benefits of defence suppression by herbivores exclusively come from plant-herbivore research.

Natural enemies have been suggested to affect the costs and benefits of defence suppression through multiple mechanisms, and intuition fails to provide clear predictions on their net effects. For example, suppression of plant defences can reduce the amount of volatiles released by plants (Tooker et al. 2008, Kant et al. 2008, Zhang et al. 2009, Peñaflor et al. 2011, Schwartzberg et al. 2011, Sarmento et al. 2011a, Frago et al. 2017, Takai et al. 2018), and may thus reduce the recruitment of natural enemies to such plants. At the same time, herbivores can sequester defensive compounds from their host plants as defence against natural enemies (Duffey 1980, Heckel 2014). However, when

herbivores suppress plant defences, they can potentially also sequester fewer defensive compounds, and may suffer increased predation (Ataide et al. 2016). In addition, defence suppression may inhibit formation of plant structures, such as trichomes (Traw & Bergelson 2003), that otherwise would have impeded predator movement (Riddick & Simmons 2014). Consequently, formal models that jointly consider these effects can provide important insight into the role of natural enemies in the evolution of defence suppression.

Defence suppression and its implications for pest management

Many herbivore species that have been found to suppress plant defences are agricultural pests, such as corn earworm (*Helicoverpa zea*) (Musser et al. 2002), Colorado potato beetle (*Leptinotarsa decemlineata*) (Lawrence et al. 2007), two-spotted spider mite (*Tetranychus urticae*) (Kant et al. 2008), silverleaf whitefly (*Bemisia tabaci*) (Zarate et al. 2007), beet armyworm (*Spodoptera exigua*) (Weech et al. 2008), western flower thrips (*Frankliniella occidentalis*) (Abe et al. 2012), and green peach aphid (*Myzus persicae*) (Bos et al. 2010). However, the implications of plant defence suppression for pest management have not been a central focus in this thesis. Nevertheless, insight into the evolutionary costs and benefits of herbivore offensive strategies can help to design more effective, and more durable pest management programs. Pesticide resistance, for example, can be a costly trait (Gassman et al. 2009, Cao et al. 2014). Fitness trade-offs therefore slow the evolution of pesticide resistance when pesticide-exposed pests are allowed to mate with individuals from 'refuges' where no pesticides are applied (Carrière et al. 2012, Farkas 2015). Resistance to multiple toxic plant compounds also evolves more slowly than resistance to single compounds (Zhao et al. 2003).

One reason why defence-suppressing herbivores could be apt to develop into pests, is that they can be expected to thrive in species-poor communities. This is because an important cost of defence suppression is competition with other herbivore species (Sarmento et al. 2011a, Glas et al. 2014, CHAPTER 2), and these occur less frequently in monocultures or pesticide-sprayed fields (Hendrickx et al. 2007). If low biodiversity indeed spurs defence-suppressing herbivores to develop into pests, then measures that increase biodiversity in agricultural fields, such as flower strips (Tschumi et al. 2015), might be especially effective against defence-suppressing crop pests. However, many defence-suppressing herbivores also have other offensive traits that contribute to their capacity to attack crops, such as pesticide resistance in the *Leptinotarsa decemlineata* Colorado potato beetle (Alyokhin et al. 2008) and in the two-spotted spider mite *T. urticae* (Van Leeuwen et al. 2010, Van Leeuwen & Dermauw 2016). Therefore, more research is needed to confirm if there is a functional relationship between defence sup-

pression and the propensity to become a crop pest, or if the reason that crop pests are overrepresented among defence-suppressing arthropods is simply the result of research bias towards species of agricultural importance.

Final consideration

In this thesis I have investigated costs and benefits in the evolution of suppression of inducible plant defences by *Tetranychus evansi* spider mites. As outlined in this discussion, my conclusions provide insight into why defence suppression is such a successful herbivore offensive strategy, and they also give rise to a multitude of new questions regarding its costs and benefits. Understanding the evolution of plant defence suppression by herbivores is work in progress, and I provided three suggestions for further research that I find particularly promising.

Importantly, inducible plant defences are not the only obstacle for herbivores to obtain high fitness. Although this thesis is disproportionately focused on why spider mites suppress these inducible defences, other factors are equally, if not more relevant for herbivore fitness, such as climatic conditions, food availability, and attack by natural enemies (Lill 2001, Bale et al. 2002, Lardies et al. 2004, Singer et al. 2004, Vidal & Murphy 2018). Herbivores have to balance their life-histories to attain optimal performance across all these hazards simultaneously, and overcoming plant defences may not always be the most pressing challenge. Therefore, just as wolves may choose not to attempt an exhausting chase of their prey in order to save resources during a cold winter, the benefits of suppressing plant defences may or may not outweigh its costs, depending on the stresses and opportunities in the environment of the herbivore.

References

Abe H, Tomitaka Y, Shimoda T, Seo S, Sakurai T, Kugimiya S, Tsuda S, Kobayashi M (2012). Antagonistic plant defense system regulated by phytohormones assists interactions among vector insect, thrips and a tospovirus. *Plant and Cell Physiology*, **53**, 204-212.

Agrawal AA (2000). Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos*, **89**, 493-500.

Agrawal AA (2007). Macroevolution of plant defense strategies. *Trends in Ecology and Evolution*, **22**, 103-109.

Agrawal AA, Fishbein M (2006). Plant defense syndromes. *Ecology*, **87**, S132-S149.

Agrawal AA, Conner JK, Rasmann S (2010). Tradeoffs and negative correlations in evolutionary ecology. In: *Evolution after Darwin: The First 150 Years* (eds: Bell MA, Futuyma DJ, Eanes WF, Levinton JS). Sinauer, Sunderland, USA.

Agrawal AA, Vala F, Sabelis MW (2002). Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? *The American Naturalist*, **159**, 553-565.

Alba JM, Glas JJ, Schimmel BCJ, Kant MR (2011). Avoidance and suppression of plant defenses by herbivores and pathogens. *Journal of Plant Interactions*, **6**, 221-227.

Alba JM, Schimmel BCJ, Glas JJ, Ataide LMS, Pappas ML, Villarroel CA, Schuurink RC, Sabelis MW, Kant MR (2015). Spider mites suppress tomato defences downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist*, **205,** 828-840.

Ali JG, Agrawal AA (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, **17**, 293-302.

Alizon S, Hurford A, Mideo N, van Baalen M (2009). Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology*, **22**, 245-259.

Alyokhin A, Baker M, Mota-Sanchez D, Dively G, Grafius A (2008). Colorado potato beetle resistance to insecticides. *American Journal of Potato Research*, **85**, 395-413.

Anderson RM, May RM (1979). Population biology of infectious diseases: part I. *Nature*, **280**, 361-367.

Ataide LMS (2013). Induction and suppression of plant defences by herbivorous mites: two different strategies with advantages and disadvantages. PhD thesis, Federal University of Viçosa, Viçosa. Available online: http://locus.ufv.br/handle/123456789/916.

Ataide LMS, Pappas ML, Schimmel BCJ, Lopez-Orenes A, Alba JM, Duarte MVA, Pallini A, Schuurink RC, Kant MR (2016). Induced plant-defenses suppress herbivore reproduction but also constrain predation of their offspring. *Plant Science*, **252,** 300-310.

Awmack CS, Leather SR (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817-844.

Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK, Butterfield J, Buse A, Coulson JC, Farrar J, Good JEG, Harrington R, Hartley S, Jones TH, Lindroth RL, Press MC, Symrnioudis I, Watt AD, Whittaker JB (2002). Herbivory in global climate change research: direct effects of rising temperatures on insect herbivores. *Global Change Biology*, **8**, 1-16.

Bidart-Bouzat MG, Kliebenstein D (2011). An ecological genomic approach challenging the paradigm of differential plant responses to specialist versus generalist insect herbivores. *Oecologia*, **167**, 677-689.

Blaazer CJH, Villacis-Perez EA, Chafi R, Van Leeuwen T, Kant MR, Schimmel BCJ (2018). Why do herbivorous spider mites suppress plant defenses? *Frontiers in Plant Science*, **9**, 1-16.

Blanckenhorn WU (2000). The evolution of body size: what keeps organisms small? *Quarterly Review of Biology*, **75**, 385-407.

Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur DA (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution*, **26**, 183-192.

Bos JIB, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA (2010). A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS Genetics*, **6**, e1001216.

Boubou A, Migeon A, Roderick GK, Auger P, Cornuet J-M, Magalhães S, Navajas M (2012). Test of colonisation scenarios reveals complex invasion history of the red tomato spider mite *Tetranychus evansi*. *PLoS One*, **7**, e35601.

Bruessow F, Gouhier-Darimont C, Buchala A, Metraux J-P, Reymond P (2010). Insect eggs suppress plant defence against chewing herbivores. *The Plant Journal*, **62**, 876-885.

Cao G, Fenh H, Guo F, Wu K, Li X, Liang G, Desneux N (2014). Quantitative analysis of fitness costs associated with the development of resistance to the Bt toxin Cry1Ac in *Helicoverpa armigera*. *Scientific Reports*, **4**, 5629.

Castañeda LE, Figueroa CC, Nespolo RF (2010). Do insect pests perform better on highly defended plants? Costs and benefits of induced detoxification defences in the aphid *Sitobion avenae*. *Journal of Evolutionary Biology*, **23**, 2474-2483.

Clemente SH, Rodrigues LR, Ponce R, Varela SAM, Magalhães S (2016). Incomplete species recognition entails few costs in spider mites, despite first-male precedence. *Behavioral Ecology and Sociobiology*, **70**, 1161-1170.

Clemente SH, Santos I, Ponce R, Rodrigues L, Varela SAM, Magalhães S (2018). Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly. *Behavioral Ecology*, **29**, 321-327.

Creswell JE, Merritt SZ, Martin MM (1992). The effect of dietary nicotine on the allocation of assimilated food to energy metabolism and growth in fourth-instar larvae of the southern armyworm, *Spodoptera eridania* (Lepidoptera: Noctuidae). *Oecologia*, **89**, 449-453.

da Silva FR, de Moraes GJ, Gondim jr MGC, Knapp M, Rouam SL, Paes JLA, de Oliveira GM (2010) Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screenhouse tomatoes. *Neotropical Entomology*, **39**, 991-995.

Denno RF, Kaplan I (2007). Plant-mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. In: *Ecological communities: plant mediation in indirect interaction webs* (eds Ohgushi T, Craig TP, Price PW). Cambridge University Press, London.

de Oliveira EF, Pallini A, Janssen A (2016). Herbivores with similar feeding modes interact through the induction of different plant responses. *Oecologia*, **180,** 1-10.

Dermauw W, Pym A, Bass C, Van Leeuwen T, Feyereisen R (2018). Does host plant adaptation lead to pesticide resistance in generalist herbivores? *Current Opinion in Insect Science*, **26**, 25-33.

Dermauw W, Wybouw N, Rombauts S, Menten B, Vontas J, Grbić M, Clark RM, Feyereisen R, Van Leeuwen T (2013). A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. *Proceedings of the National Academy of the Sciences USA*, **110**, E113-122.

Després L, David J-P, Gallet C (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology and Evolution*, **22**, 298-307.

Duffey SS (1980). Sequestration of plant natural products by insects. *Annual Review of Entomology*, **25**, 447-477.

Dupas S, Boscaro M (1999). Geographic variation and evolution of immunosuppressive genes in a *Drosophila* parasitoid. *Ecography*, **22,** 284-291.

Farkas TE (2015). Fitness trade-offs in pest management and intercropping with colour: an evolutionary framework and potential application. *Evolutionary Applications*, **8**, 847-853.

Ferragut, F., Garzón-Luque, E. & Pekas, A. (2013) The invasive spider mite *Tetranychus evansi* (Acari: Tetranychidae) alters community composition and host-plant use of native relatives. *Experimental and Applied Acarology*, **60**, 321-341.

Florane CB, Bland JM, Husseneder C, Raina AK (2004). Diet-mediated inter-colonial aggression in the formosan subterranean termite *Coptotermes formosanus*. *Journal of Chemical Ecology*, **30**, 2559-2574.

Frago E, Mala M, Weldegergis BT, Yang C, McLean A, Godfray HCJ, Gols R, Dicke M (2017). *Nature Communications*, **8**, 1-9.

Frank SA (2007). Maladaptation and the paradox of robustness in evolution. *PLoS One*, **2**, e1021.

Gassman AJ, Carrière Y, Tabashnik BE (2009). Fitness costs of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology*, **54**, 147-163.

Giron D, Huguet E, Stone GN, Body M (2016). Insect-induced effects on plants and possible effectors used by galling and leaf-mining insects to manipulate their host-plant. *Journal of Insect Physiology*, **84**, 70-89.

Glas JJ, Alba JM, Simoni S, Villarroel CA, Stoops M, Schimmel BCJ, Schuurink RC, Sabelis MW, Kant MR (2014). Defense suppression benefits herbivores that have a monopoly on their feeding site but can backfire within natural communities. *BMC Biology*, **12,** 98.

Gloss, A.D., Groen, S.C. & Whiteman, N.K. (2016) A genomic perspective on the generation and maintenance of genetic diversity in herbivorous insects. *Annual Review of Ecology, Evolution and Systematics*, **47**, 165-187.

Godinho DP, Janssen A, Dias T, Cruz C, Magalhães S (2016). Down-regulation of plant defence in a resident spider mite species and its effect upon con- and heterospecifics. *Oecologia*, **180,** 161-167.

Gols R, Harvey JA (2009). Plant-mediated effects in the Brassicaceae on the performance and behaviour of parasitoids. *Phytochemistry Reviews*, **8**, 187-206.

Gotoh T, Araki R, Boubou A, Migeon A, Ferragut F, Navajas M (2009). Evidence of co-specificity between *Tetranychus evansi* and *Tetranychus takafujii* (Acari: Prostigmata, Tetranychidae): comments on taxonomic and agricultural aspects. *International Journal of Acarology*, **35**, 485-501.

Gröning J, Hochkirch A (2008). Reproductive interference between animal species. *The Quarterly Review of Biology*, **83**, 257-282.

Grbić M, Van Leeuwen T, Clark RM, Rombauts S, Rouzé P, Grbić V, Osborne EJ, Dermauw W, et al.. (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature, 479, 487-492.

Guiget A, Dubreuil G, Harris MO, Appel HM, Schultz JC, Pereira MH, Giron D (2016). Shared weapons of blood- and plant-feeding insects: surprising commonalities for manipulating hosts. *Journal of Insect Physiology*, **84**, 4-21.

Gwynn DM, Callaghan A, Gorham J, Walters KFA, Fellowes MDE (2005). Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. *Proceedings of the Royal Society B*, **272**, 1803-1808.

Hahn PG, Maron JL (2016). A framework for predicting intraspecific variation in plant defense. *Trends in Ecology and Evolution*, **31**, 646-656.

Hartley SE, Lawton JH (1992). Host-plant manipulation by gall-insects: a test of the nutrition hypothesis. *Journal of Animal Ecology*, **61**, 113-119.

Heckel DG (2014). Insect detoxification and sequestration strategies. *Annual Plant Reviews*, **47**, 77-114.

Heil M (2010). Plastic defence expression in plants. *Evolutionary Ecology*, **24**, 555-569.

Hendrickx F, Maelfait J-P, Van WIngerden W, Schweiger O, Speelmans M, Aviron S, Augenstein I, Billeter R, Bailey D, Bukacek R, Burel F, Diekötter T, Dirksen J, Herzog F, Liira J, Roubalova M, Vandomme V, Bugter R (2007). How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. *Journal of Applied Ecology*, **44**, 340-351.

Holzinger F, Wink M (1996). Mediation of cardiac glycoside insensitivity in the monarch butterfly (*Danaus plexippus*): role of an amino acid substitution in the ouabain binding site of NA+,K+-ATPase. *Journal of Chemical Ecology*, **22**, 1921-1937.

Howe GA, Lightner J, Browse J, Ryan CA (1996). An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *The Plant Cell*, **8**, 2067-2077.

Kaiser W, Huguet E, Casas J, Commin C, Giron D (2010). Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proceedings of the Royal Society of London B*, **277**, 2311-2319.

Kamiya T, Mideo N, Alizon S (2018). Coevolution of virulence and immunosuppression in multiple infections. *Journal of Evolutionary Biology*, **31**, 995-1005.

Kant MR, Jonckheere W, Knegt B, Lemos F, Liu J, Schimmel BCJ, Villarroel CA, Ataide LMS, Dermauw W, Glas JJ, Egas M, Janssen A, Van Leeuwen T, Schuurink RC, Sabelis MW, Alba JM (2015). Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany*, **115**, 1015-1051.

Kant MR, Sabelis MW, Haring MA, Schuurink RC (2008). Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proceedings of the Royal Society B*, **275,** 443-452.

Karban R, Agrawal AA (2002). Herbivore offense. *Annual Review of Ecology and Systematics*, **33**, 641-664.

Kemp DJ, Wiklund C (2001). Fighting without weaponry: a review of male-male contest competition in butterflies. *Behavioral Ecology and Sociobiology*, **49**, 429-442.

Kidd NAC, Lewis GB, Howell CA (1985). An association between two species of pine aphid, *Schizolachnus pineti* and *Eulachnus agilis*. *Ecological Entomology*, **10**, 427-432.

Krieger RI, Feeny PP, Wilkinson CF (1971). Detoxication enzymes in the guts of caterpillars: an evolutionary answer to plant defenses? *Science*, **172**, 579-581.

Labeyrie E, Dobler S (2004). Molecular adaptation of *Chrysochus* leaf beetles to toxic compounds in their food plants. *Molecular Biology and Evolution*, **21**, 218-221.

Lardies MA, Carter MC, Bozinovic F (2004). Dietary effects on life history traits in a terrestrial isopod: the importance of evaluating maternal effects and trade-offs. *Oecologia*, **138**, 387-395.

Lawrence SD, Novak NG, Blackburn MB (2007). Inhibition of proteinase inhibitor transcripts by *Leptinotarsa decemlineata* regurgitant in *Solanum lycopersicum*. *Journal of Chemical Ecology*, **33**, 1041-1048.

Li R, Weldegergis BT, Li J, Jung C, Qu J, Sun Y, Qian H, Tee C, van Loon JJA, Dicke M, Chua N-H, Liu S-S, Ye J (2014). Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance. *The Plant Cell*, **26**, 4991-5008.

Lill JT (2009). Selection on herbivore life-history traits by the first and third trophic levels: the devil and the deep blue sea revisited. *Evolution*, **55**, 2236-2247.

Marquis RJ, Alexander HM (1992). Evolution of resistance and virulence in plant-herbivore and plant-pathogen interactions. *Trends in Ecology and Evolution*, **7**, 126-129.

Matsuda T, Morishita M, Hinomoto N, Gotoh T (2014). Phylogenetic analysis of the spider mite sub-family Tetranychinae (Acari: Tetranychidae) based on the mitochondrial COI gene and the 18S and the 5' end of the 28S rRNA genes indicates that several genera are polyphyletic. *PLoS One*, **9**, e108672.

Matsushima R, Ozawa R, Uefune M, Gotoh T, Takabayashi J (2006). Intraspecies variation in the Kanzawa spider mite differentially affects induced defensive response in lima bean plants. *Journal of Chemical Ecology*, **32**, 2501-2512.

McGurl B, Orozco-Cardenas M, Pearce G, Ryan CA (1994). Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9799-9802.

Moreau J, Desouhant E, Louâpre P, Goubault M, Rajon E, Jarrige A, Menu F, Thiéry D (2017). How host plant and fluctuating environments affect insect reproductive strategies? *Advances in Botanical Research*, **81**, 259-287.

Musser RO, Hum-Musser SM, Eichenseer H, Pfeiffer M, Ervin G, Murphy JB, Felton GW (2002). Caterpillar saliva beats plant defences. *Nature*, **416**, 599-600.

Mutti NS, Louis J, Pappan LK, Pappan K, Begum K, Chen M-S, Park Y, Dittmer N, Marshall J, Reese JC, Reeck GR (2008). A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences USA*, **105**, 9965-9969.

Nylin S, Gotthard K (1998). Plasticity in life-history traits. *Annual Review of Entomology*, **43**, 63-83.

Ode PJ (2006). Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annual Review of Entomology*, **51**, 163-185.

Opitz SEW, Müller C (2009). Plant chemistry and insect sequestration. *Chemoecology*, **19**, 117-154.

Paulo JT, Godinho DP, Silva A, Branquinho C, Magalhães S (2018). Suppression of plant defences by herbivorous mites is not associated to adaptation to host plants. *International Journal of Molecular Sciences*, **19**, 15-19.

Pearse IS, Altermatt F (2013). Predicting novel trophic interactions in a non-native world. *Ecology Letters*, **16**, 1088-1094.

Peñaflor MFGV, Erb M, Robert CAM, Miranda LA, Werneburg AG, Dossi FCA, Turlings TCJ, Bento JMS (2011). Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize. *Planta*, **234**, 207-215.

Pires CSS, Price PW, Fontes EG (2000). Preference-performance linkage in the neotropical spittlebug *Deois avopicta*, and its relation to the phylogenetic constraints hypothesis. *Ecological Entomology*, **25**, 71-80.

Poelman EH, Broekgaarden C, van Loon JJA, Dicke M (2008). Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Molecular Ecology*, **17**, 3352-3365.

Price PW (1992). Plant resources as the mechanistic basis for insect herbivore population dynamics. In: *Effects of Resource Distribution on Animal – Plant Interactions* (eds: Hunter MD, Ohgushi T, Price PW). Academic Press, San Diego, USA.

Price PW, Cobb N, Craig TP, Fernandes GW, Itami JK, Mopper S, Preszler RW (1990). Insect herbivore population dynamics on trees and shrubs: new approaches relevant to latent and eruptive species and life table development. In: *Insect-Plant Interactions, volume II* (eds: Bernays E). CRC Press, Boca Raton, USA.

Raffel TR, Martin LB, Rohr JR (2008). Parasites as predators: unifying natural enemy ecology. *TREE*, **23**, 610-618.

Rhoades D (1985). Offensive-defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. *American Naturalist*, **125**, 205-238.

Riddick EW, Simmons AM (2014). Do plant trichomes cause more harm than good to predatory insects? *Pest Management Science*, **70**, 1655-1665.

Rodriguez-Saona C, Crafts-Brandner SJ, Cañas LA (2003). Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. *Journal of Chemical Ecology*, **29**, 2539-2550.

Root RB (1967). The niche exploitation pattern of the blue-gray gnatcatcher. *Ecological Monographs*, **37**, 317-350.

Sabelis MW (1991). Life-history evolution of spider mites. In: *The Acari: reproduction, development and life-history strategies* (eds Schuster P, Murphy PW). Chapman & Hall, London, UK.

Sarmento, R.A., Lemos, F., Bleeker, P.M., Schuurink, R.C., Pallini, A., Oliveira, M.G.A., Lima, E.R., Kant, M.R., Sabelis, M.W. & Janssen, A. (2011a) A herbivore that manipulates plant defence. *Ecology Letters*, **14**, 229-236.

Sarmento, R.A., Lemos, F., Dias, C.R., Kikuchi, W.T., Rodrigues, J.C.P., Pallini, A., Sabelis, M.W. & Janssen, A. (2011b) A herbivorous mite down-regulates plant defence and produces web to exclude competitors. *PLoS One*, **6**, e23757.

Sato, Y., Alba, J.M. & Sabelis, M.W. (2014) Testing for reproductive interference in the population dynamics of two congeneric species of herbivorous mites. *Heredity*, **113**, 495-502.

Sato, Y., Staudacher, H. & Sabelis, M.W. (2016) Why do males choose heterospecific females in the red spider mite? *Experimental and Applied Acarology*, **68**, 21-31.

Schimmel BCJ (2016). From induction to suppression: how to manipulate plant defences. PhD thesis, University of Amsterdam, Amsterdam.

Schimmel BCJ, Ataide LMS, Chafi R, Villarroel CA, Schuurink RC, Kant MR (2017a). Overcompensation of defense suppression by herbivores in response to competition. *New Phytologist*, **214,** 1688-1701.

Schimmel BCJ, Ataide LMS, Kant MR (2017b). Spatiotemporal heterogeneity of tomato induced defense responses affects spider mite performance and behavior. *Plant Signaling & Behavior*, **12**, e1370526.

Schittko U, Hermsmeier D, Baldwin IT (2001). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. II. Accumulation of plant mRNAs in response to insect-derived cues. *Plant Physiology*, **125**, 2189-2202.

Schmid-Hempel P (2008). Parasite immune evasion: a momentous molecular war. *Trends in Ecology and Evolution*, **23**, 318-326.

Schwartzberg EG, Böröczky K, Tumlinson JH (2011). Pea aphids, *Acyrthosiphon pisum*, suppress induced plant volatiles in broad bean, *Vicia faba*. *Journal of Chemical Ecology*, **37**, 1055-1062.

Sigmon E (2015). Interspecific variation in aggressive fighting behavior of shelter-building caterpillars. *Journal of Insect Behavior*, **28**, 403-416.

Singer MS, Rodrigues D, Stireman IIIrd JO, Carrière Y (2004). Roles of food quality and enemy-free space in herbivory by a generalist caterpillar. *Ecology*, **89**, 470.

Stahl E, Hilfiker O, Reymond P (2018). Plant–arthropod interactions: who is the winner? *The Plant Journal*, **93**, 703-728.

Stam, J.M., Kroes, A., Li, Y., Gols, R., van Loon, J.J.A., Poelman, E.H. & Dicke, M. (2014). Plant interactions with multiple insect herbivores: from community to genes. *Annual Review of Plant Biology*, **65**, 689-713.

Takabayashi J, Shimoda T, Dicke M, Ashihara W, Takafuji A (2000). Induced response of tomato plants to injury by green and red strains of *Tetranychus urticae*. *Experimental and Applied Acarology*, **24**, 377-383.

Takai H, Ozawa R, Takabayashi J, Fujii S, Arai K, Ichiki RT, Koeduka T, Dohra H, Ohnishi T, Taketazu S, Kobayashi J, Kainoh Y, Nakamura S, Fujii T, Ishikawa Y, Kiuchi T, Katsuma S, Uefune M, Shimada T, Matsui K (2018). Silkworms suppress the release of green leaf volatiles by mulberry leaves with an enzyme from their spinnerets. *Scientific Reports*, **8**, 1-14.

Tooker JF, Rohr JR, Abrahamson WG, De Moraes CM (2008). Gall insects can avoid and alter indirect plant defenses. *New Phytologist*, **178**, 657-671.

Traw MB, Bergelson J (2003). Interactive effects of jasmonic acid, salicylic acid, and gibberelline on induction of trichomes in *Arabidopsis*. *Plant Physiology*, **133**, 2439-2440.

Tschumi M, Albrecht M, Entling MH, Jacot K (2015). High effectiveness of tailored flower strips in reducing pests and crop plant damage. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20151369.

van der Hoorn RAL, Kamoun S (2008). From guard to decoy: a new model for the perception of plant pathogen effectors. *The Plant Cell*, **20**, 2009-2017.

Van Leeuwen T, Dermauw W (2016). The molecular evolution of xenobiotic metabolism and resistance in chelicerate mites. *Annual Review of Entomology*, **61**, 475-498.

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. *Insect Biochemistry and Molecular Biology*, **40**, 563-572.

Vidal MC, Murphy SM (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecology Letters*, **21**, 138-150.

Walling LL (2008). Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiology*, **146**, 859-866.

Weech M-H, Chapleau M, Pan L, Ide C, Bede JC (2008). Caterpillar saliva interferes with induced *Arabidopsis thaliana* defence responses via the systemic acquired resistance pathway. *Journal of Experimental Botany*, **59**, 2437-2448.

Ximénez-Embún MG, Ertego F, Castañera P (2016). Drought-stressed tomato plants trigger bottom–up effects on the invasive *Tetranychus evansi*. *PLoS One*, **11**, e0145275.

Yack JE, Gill S, Drummond-Main C, Sherratt TN (2014). Residency duration and shelter quality influence vibratory signalling displays in a territorial caterpillar. *Ethology*, **120**, 354-364.

Yang J-Y, Iwasaki M, Machida C, Machida Y, Zhou X, Chua N-H (2008). βCl, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. *Genes and Development*, **22**, 2564-2577.

Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology*, **143**, 866-875.

Zhang P-J, Zheng S-J, van Loon JJA, Boland W, David A, Mumm R, Dicke M (2009). Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *Proceedings of the National Academy of Sciences USA*, **106**, 21202-21207.

Zhao J-Z, Cao J, Li Y, Collins HL, Roush RT, Earle ED, Shelton AM (2003). Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nature Biotechnology*, **21**, 1493-1497.

Summary

Herbivores eat plants, and plants defend their tissues. To overcome these plant defences, herbivores evolved a variety of offensive strategies. Some herbivores use enzymes to detoxify defensive compounds of their hosts, other herbivores cut veins in the leaves of their hosts to prevent exposure to dangerous defensive latex, and yet others sequester the toxic compounds of their hosts as protection against predators. Why has this diversity of herbivore offensive strategies evolved? Understanding the evolution of herbivore offensive strategies requires insight into their costs and benefits, in relation to the environment in which they are employed. In this thesis, I studied costs and benefits of a recently described offensive strategy, defence suppression, in the herbivorous spider mite *Tetranychus evansi*.

Herbivores can suppress plant defences by manipulating a plant's physiological processes, for example by manipulating signalling pathways of plant hormones involved in defence, including jasmonic acid and salicylic acid. This allows herbivores to prevent expression of inducible plant defences, thereby increasing their own performance. How much do *T. evansi* spider mites benefit from suppressing the defence response of their tomato hosts? In CHAPTER 2 I quantitatively review evidence, published and unpublished, of the benefits of defence suppression conferred to *T. evansi* as measured by fecundity. I found that, on average, *T. evansi* increase their oviposition rate by approximately one egg per day on tissues previously infested by conspecifics, corresponding to an increase in fecundity of 9-12% each day. However, this benefit varied considerably among studies, ranging from strongly positive to none at all, or even slightly negative. Some of this variation could be explained by the time that spider mite populations had been cultured in laboratories before being used in experiments, such that weaker benefits were found when populations had been maintained in the laboratory for longer time. These results indicate that *T. evansi* adapted to culture conditions, and that the net effect of suppression of plant defence on the performance of spider mites in subsequent infestations is subject to phenotypic change over generations. Consequently, *T. evansi* populations harbour heritable variation for their plant-mediated interactions with other herbivores, and thus have the potential to adapt to plant-mediated ecological interactions.

The meta-analysis in CHAPTER 2 quantifies the average benefit of defence suppression for *T. evansi*, but also confirms previously published assertions regarding potential

ecological costs. Specifically, I found that induction of tomato defences by defence-inducing genotypes of the spider mite species *Tetranychus urticae* decreased the fecundity of spider mites in subsequent infestations by approximately one egg per day. This confirms that the presence of *T. urticae* can pose an ecological cost to *T. evansi* through its effects on plant defence. Moreover, I found that *T. urticae* also attained an increase in fecundity of one egg per day from defence suppression by *T. evansi*. This shows that lowered tomato defences are profitable for spider mites in general, and that, on average, defence suppression increases not only the performance of *T. evansi* itself, but also that of its competitors.

The phenotypic change in plant-mediated interactions with other spider mite populations observed in CHAPTER 2 provides indirect evidence for intraspecific variation in defence suppression within *T. evansi* populations. To measure intraspecific variation in defence suppression more directly, in CHAPTER 3 I investigate *T. evansi* populations from several locations around the world. Because the costs of defence suppression may depend on biotic interactions with competitors and natural enemies, I expected that *T. evansi* from different environments would suppress plant defences to varying levels. By measuring the expression of several marker genes for tomato defence induction, I found significant variation among *T. evansi* populations in the degree to which they suppress jasmonic acid-dependent and salicylic acid-dependent defence pathways. This complements the findings of CHAPTER 2, by showing that variation for defence suppression is not only present within, but also among *T. evansi* populations.

CHAPTERS 2 and 3 demonstrate that *T. evansi* populations harbour intraspecific variation for defence suppression within and among populations. I then hypothesised that manipulating the benefits of defence suppression through experimental evolution could expose its costs. Specifically, in environments where inducible plant defences are either absent or constitutive, I expected *T. evansi* to lower its level of defence suppression through drift or through selection against metabolic costs. Experimental evolution, however, requires a source population with sufficient genetic variation. I hypothesized that a cross between *T. evansi* mites from genetically differentiated lineages would produce such a genetically diverse population. Therefore, in CHAPTER 4, I perform reciprocal crosses between *T. evansi* from two genetically differentiated lineages. First, I confirmed that offspring suffered substantial post-zygotic hybrid breakdown, by showing that only ~5% of hybrid F2 offspring hatched. Then, by genotyping viable and inviable hybrid offspring at eight microsatellite loci, I showed that Bateson-Dobzhansky-Muller incompatibilities underlie hybrid breakdown among these lineages. Moreover, I also found that viable hybrids contained recombined genetic material, and that heterosis contributes to their viability.

After establishing a genetically diverse hybrid *T. evansi* population, in CHAPTER 5 I expose this population to experimental evolution on host plants with an altered

inducible jasmonic acid-dependent defence response. I used *defenseless-1* (def-1) tomato mutants in which JA-dependent inducible defences are absent, the *35S::prosystemin* (PS) tomato genotype in which these defences are constitutively overexpressed, and *Phaseolus vulgaris* bean as a distantly related host with different defences. I expected that in the absence of inducible defences (def-1), when suppression of plant defences is not possible because they are constitutively overexpressed (PS), or because they are regulated differently than in tomato (bean), suppression is not necessary and would erode through drift or be selected against because of metabolic costs. I maintained replicate populations of the hybrid *T. evansi* base population for approximately 60 generations in these three environments and on wildtype controls, and then measured the performance of these mites on wildtype tomato, def-1, PS and bean. I found that mite fecundity showed a general response to selection, but that fecundity on wildtype tomato remained high and was similar among all evolved strains. In line with these results, the degree to which wildtype tomato plants infested with these mites accumulated phytohormones and expressed defence gene markers was also similar among these strains. This shows that evolved strains retained their level of tomato defence suppression, and that metabolic costs of defence suppression are likely low.

In conclusion, I found that *T. evansi* can attain a considerable fecundity benefit by suppressing the inducible defences of their tomato hosts. However, competing herbivores can impose considerable costs on this offensive strategy, because they can also benefit from the suppressed defences of a shared host plant, and can subsequently induce these defences to the disadvantage of *T. evansi*. Furthermore, suppression of plant defences is variable within and among *T. evansi* populations, and metabolic investments required to produce defence-suppressing effectors are likely low. Although these conclusions help to better understand the evolution of defence suppression by herbivores, several open questions remain. In CHAPTER 6, I highlight gaps in our knowledge of defence suppression by *T. evansi*, such as its effect on the recruitment of natural enemies, the range of host plant species in which *T. evansi* can suppress defences, and whether aspects of plant quality other than defence, such as nutrient concentration, are also manipulated. Lastly, I provide three detailed suggestions for further research that could answer some of these open questions.

Author contributions

Chapter 2 – Meta-analysis reveals intraspecific variation for plant-mediated interactions among herbivores

BK and ME conceived the ideas and designed methodology; BK, JMA, LMSA, TAB, AMGB, RC, CRD, MVAD, DPG, FL, DL, JM, EFdO, FRR, BCJS, PAdS, NRdSJ and MSV collected the data; BK analysed the data; BK, ETK and ME led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 3 – *Tetranychus evansi* spider mite populations suppress tomato defences to varying degrees

All authors designed the research, BK and TTM performed experiments, BK and TTM analysed data, BK wrote the manuscript. All authors contributed to and approved the final version of the manuscript.

Chapter 4 – Detection of genetic incompatibilities in non-model systems using simple genetic markers: hybrid breakdown in the haplodiploid spider mite *Tetranychus evansi*

BK and ME designed the research. BK, TP, NAP, YS, HS, and BCJS performed experiments. BK and TP analysed data. BK wrote the manuscript. All authors contributed to and approved the final version of the manuscript.

Chapter 5 – Spider mites maintain defence suppression after 60 generations of experimental evolution on tomato lines that lack inducible jasmonic acid defences

BK and ME designed the research. BK performed experiments, analysed data, and wrote the manuscript. All authors contributed to and approved the final version of the manuscript.

Samenvatting

Herbivoren eten planten, en planten verdedigen hun weefsels tegen vraat. Bij herbivoren is daarom een veelvoud aan aanvalsstrategieën geëvolueerd om die verdediging van planten te doorbreken. Sommige herbivoren gebruiken bijvoorbeeld enzymen om de giftige verdedigingsstoffen van planten te neutraliseren, andere herbivoren snijden de bladnerven van hun waardplant door om niet in aanraking te hoeven komen met gevaarlijke latex, en weer andere verzamelen juist de giftige verdedigingsstoffen van planten als bescherming tegen vijanden. Waarom bestaat er zo'n grote variëteit aan aanvalsstrategieën onder herbivoren? Om meer inzicht te krijgen in de evolutie van deze aanvalsstrategieën is het noodzakelijk om hun voor- en nadelen te begrijpen binnen de context van de omgeving waarin ze worden toegepast. In dit proefschrift beschrijf ik de voor- en nadelen van een recentelijk ontdekte aanvalsstrategie, namelijk het onderdrukken van plantenverdediging, voor de spintmijt *Tetranychus evansi*.

Sommige herbivoren kunnen de verdediging van planten onderdrukken door fysiologische processen in de plant te verstoren, zoals de regulering van de plantenhormonen jasmonzuur en salicylzuur, die een grote rol spelen in de totstandkoming van plantenverdediging. Zo voorkomen deze herbivoren dat ze worden blootgesteld aan een tegen hen gerichte verdedigingsreactie van de plant. In welke mate bevordert het onderdrukken van plantenverdeding de eileg (aantal eitjes per vrouwtje per dag) van *T. evansi* spintmijten? In HOOFDSTUK 2 geef ik een kwantitatieve analyse van eerder werk, zowel gepubliceerd als niet-gepubliceerd, waarin de consequenties van het onderdrukken van plantenverdediging voor de eileg van *T. evansi* zijn onderzocht. Uit deze meta-analyse blijkt dat *T. evansi* mijten gemiddeld één ei per dag méér leggen wanneer ze eten van plantenweefsel dat eerder door soortgenoten is aangevallen, dan wanneer ze eten van onaangetaste planten. Dit is een toename in eileg van 9 tot 12% per dag.

De verschillen tussen de onderzochte studies zijn echter groot: sommige studies vermelden een sterke toename in eileg, terwijl andere juist een licht negatief effect rapporteren. Een deel van deze variatie hangt samen met de tijd die de spintmijtenpopulaties in het lab hebben doorgebracht sinds ze uit het veld zijn verzameld. De mijtenpopulaties die langer in het lab verbleven bewerkstelligden een kleinere toename in eileg dan mijten die korter in het lab verbleven. Deze resultaten geven aan dat *T. evansi* zich aanpast aan de omstandigheden in het lab, en dat de invloed van het onderdrukken van

plantenverdediging op de eileg van spintmijten verandert over generaties. Ik concludeer daarom dat *T. evansi* populaties erfelijke variatie met zich meedragen die de mate waarin zij andere herbivoren beïnvloeden, door plantenverdediging te manipuleren, mede bepaalt. *T. evansi* populaties hebben dientengevolge dus ook de mogelijkheid om zich aan te passen aan indirecte, door plantenverdediging vormgegeven ecologische interacties.

Behalve dat de meta-analyse uit HOOFDSTUK 2 de voordelen van het onderdrukken van plantenverdediging voor *T. evansi* kwantificeert, bevestigt het ook dat er ecologische kosten verbonden kunnen zijn aan deze aanvalsstrategie. De meta-analyse laat namelijk zien dat de eileg van spintmijten met gemiddeld één ei per dag afneemt, wanneer zij eten van plantenweefsel dat eerder is aangevallen door plantenverdediging-inducerende genotypes van de spintmijtensoort *Tetranychus urticae*. Bovendien kon deze concurrerende spintmijtensoort net zo goed profiteren van een door *T. evansi* onderdrukte plantenverdediging als *T. evansi* zelf. Een onderdrukte plantenverdediging is dus gunstig voor spintmijten in het algemeen, en bevordert niet alleen de eileg van *T. evansi*, maar ook die van haar concurrenten.

De verandering die de spintmijtenpopulaties in HOOFDSTUK 2 over de generaties ondergaan, geeft aan dat er variatie aanwezig is *binnen T. evansi* populaties voor het onderdrukken van plantenverdediging. Zou er *tussen T. evansi* populaties ook dergelijke variatie te vinden zijn? Om dat op een directere manier zichtbaar te maken heb ik in HOOFDSTUK 3 verschillende *T. evansi* populaties van over de wereld bestudeerd. Mijn verwachting hierbij was dat de populaties zouden verschillen in de mate waarin zij plantenverdediging onderdrukken, aangezien de voor- en nadelen hiervan van plek tot plek zouden kunnen verschillen, bijvoorbeeld door de aanwezigheid van verschillende concurrenten en natuurlijke vijanden. Na de activiteit van verschillende markergenen voor tomatenverdediging te hebben gemeten, bleken de *T. evansi* populaties significant te verschillen in de mate waarin zij de jasmonzuur- en salicylzuur-afhankelijke verdediging van de tomatenplant onderdrukken. Dit laat zien dat er niet alleen binnen *T. evansi* populaties, maar ook tussen populaties fenotypische variatie aanwezig is voor het onderdrukken van plantenverdediging.

Aangezien er variatie aanwezig is binnen en tussen *T. evansi* populaties voor het onderdrukken van plantenverdediging, zouden kosten die verbonden zijn aan het onderdrukken van de verdediging zichtbaar moeten kunnen worden gemaakt in een selectie-experiment. Wanneer de voordelen van het onderdrukken van plantenverdediging zouden wegvallen, zouden metabolische kosten van het onderdrukken of genetische drift ervoor kunnen zorgen dat *T. evansi* een zwakkere mate van onderdrukken evolueert. Dit zou bijvoorbeeld plaats kunnen vinden in experimenten waar planten zich ófwel altijd verdedigen, of er nou herbivoren zijn of niet, ófwel wanneer planten überhaupt geen verdedigingsreactie op poten zetten. Zulk experimenteel evolutionair onderzoek heeft

echter een bronpopulatie nodig met voldoende genetische variatie. Kruisingen tussen genetische gedifferentieerde *T. evansi* populaties zouden een dergelijk genetisch diverse populatie kunnen opleveren. Daarom beschrijf ik in HOOFDSTUK 4 reciproke kruisingen tussen twee van zulke *T. evansi* populaties. Slechts 5% van de F2 nakomelingen van deze kruisingen was levensvatbaar, wat aangeeft dat er sterke post-zygotische hybrid breakdown optreedt. Vervolgens heb ik van zowel levensvatbare als niet-levensvatbare F2 hybriden het genotype bepaald op acht microsatelliet loci, waaruit bleek dat de hybrid breakdown wordt veroorzaakt door Bateson-Dobzhansky-Muller incompatibiliteiten. Daarnaast bevatten de levensvatbare F2 nakomelingen gerecombineerd genetisch materiaal, en droegen heterotische effecten bij aan hun overleving.

Nadat ik deze genetisch diverse, hybride *T. evansi* populatie had verkregen, heb ik deze populatie blootgesteld aan experimentele evolutie op waardplanten met een aangepaste jasmonzuur-afhankelijke verdedigingsreactie (HOOFDSTUK 5). Specifiek heb ik gebruik gemaakt van *defenseless-1* (def-1) tomatenmutanten zonder jasmonzuur-afhankelijke (induceerbare) verdediging, *35S::prosystemin* (PS) tomatengenotypes die hun jasmonzuur-afhankelijke verdediging altijd tot expressie laten komen, en *Phaseolus vulgaris* bonenplanten (boon) als waardplanten met een andere verdediging dan tomaat. Mijn verwachting was dat zonder (induceerbare) verdediging (def-1), wanneer het onderdrukken ervan onmogelijk is (PS), of wanneer de verdediging sterk verschilt (boon), het onnodig of onmogelijk zou zijn voor *T. evansi* om de verdediging te onderdrukken, en dat de onderdrukking daardoor zou verminderen. Ik heb de hybride *T. evansi* populatie ongeveer 60 generaties lang in deze drie proefopstellingen en op wildtype controle behandelingen laten evolueren, en vervolgens de eileg bepaald op wildtype tomaat, def-1, PS en op boon. Uit deze metingen volgde dat er een algemene respons was op de selectie, maar dat er geen verschil was in eileg van mijten uit de verschillende behandelingen. Er was bovendien ook geen verschil in de hormoonconcentraties of expressie van verdedigingsmarkers van wildtype planten die werden blootgesteld aan deze mijten. Dit geeft aan dat de mate waarin de *T. evansi* mijten de plantenverdediging onderdrukken gelijk is gebleven, en dat de metabolische kosten van het onderdrukken van plantenverdediging waarschijnlijk laag zijn.

Concluderend kan ik stellen dat het onderdrukken van plantenverdediging de eileg van *T. evansi* aanzienlijk bevordert. Concurrerende herbivoren kunnen echter aanzienlijke ecologische kosten teweeg brengen, omdat ze kunnen meeprofiteren van door *T. evansi* onderdrukte plantenverdediging, en omdat ze die verdediging zelf juist kunnen induceren ten nadele van *T. evansi*. Daarnaast heb ik gevonden dat er fenotypische variatie is voor het onderdrukken van plantenverdediging binnen en tussen populaties, en dat de metabolische kosten om plantenverdediging te onderdrukken waarschijnlijk laag zijn. Deze conclusies geven ons meer inzicht in de evolutie van het onderdrukken van plantenverdediging door herbivoren, maar laten ook een aantal belangrijke vragen onbe-

antwoord. In HOOFDSTUK 6 geef ik aan welke aspecten van het onderdrukken van plantenverdediging verder onderzoek verdienen, zoals mogelijke effecten op het aantrekken van natuurlijke vijanden, en of andere aspecten van plantenkwaliteit voor herbivoren, zoals concentraties van voedingsstoffen, ook door herbivoren worden beïnvloed. Tenslotte doe ik drie gedetailleerde suggesties voor verder onderzoek, die een deel van de resterende vragen zouden kunnen beantwoorden.

Acknowledgements

Writing a thesis was a splendid adventure. Diving into a highly specialised subject for a substantial amount of time, becoming a world expert in an infinitely small niche, and not knowing beforehand whether the endeavour would yield, were all aspects that gave it a romantic appeal. I remember quite clearly how eager I was to engage in such a project, and to see if I could make a worthwhile contribution to understanding evolution in plant-herbivore contexts. As in any good adventure, though, obstacles appeared at unpredictable intervals, and frustrated the progress of designing experiments, collecting data, or writing. I have more than once condemned myself for another day that was seemingly wasted on a fruitless literature search or yet another experiment that failed to confirm my hypotheses. However, in retrospect, acknowledging these difficulties was often the first step to turn such obstacles into stepping stones towards what became the better parts of this thesis. I am therefore proud to see this thesis completed, and would like to thank the many people that supported me personally, scientifically or in any other way. Without their help, this thesis would not have matured.

First of all, Martijn, thanks for your supervision and mentoring over these past years. Throughout the whole trajectory, I found our collaboration pleasurable and productive. I could always come to you for some calm and supportive words, and you expressed a strong confidence in my ability to take good decisions, pursue interesting questions, and bring this project to an end. I cannot recall a single occasion in which you explicitly adjusted the course of my research, but perhaps your supervision was more subtle than that? You also often offered a helping hand with practical matters, such as supervision of students or caretaking of mite cultures when I was away for a course or conference. Your work ethic definitely is an example for me, and I hope for many others as well. I already miss your roars of laughter that readily tone down the serious and sometimes stressed atmosphere at Science Park!

Toby, thanks for stepping in as my promotor. We already knew each other from my MSc. internship, and I believe we simply continued to work together smoothly and efficiently. I was surprised to find out that the UvA allows PhD graduations with a VU promotor, and vice versa. Although on the level of institutions the UvA-VU collaboration perhaps not fully became what was anticipated, I'm glad that our small scale version was very fruitful. Thanks for your support while writing my chapters! No more sentences in

passive voice will be formulated by me. Your good advice on how to structure my texts, as well as how to emphasise the interesting parts of my results rather than the puzzling, have considerably improved the clarity of this thesis. You are an expert in positive feedback, which greatly motivated me to continue writing. Of course, the immediate cause for our collaboration was a very sad event. Maus, thanks for your confidence, some good discussions, and for forging the nice research group within which I could perform this research.

Over the years at Science Park, I was lucky enough to work together with some outstanding students. Nigel, thanks for all your hard work, long train rides, and summer internship. Your perseverance produced important data for our Heredity paper. Tomos, the same goes for you, and I admire the independence you showed already during your MSc. internship. Thanks for the endless egg counting, and for figuring out a way to genotype mite eggs. This was a major step towards bringing home the main message of our paper! Tomas, you were the last internship student that I supervised, and it really was a great pleasure to have you in the team. Your social skills are impressive, and you very quickly surrounded yourself with people that were willing to help with your experiments, or to explain another protocol. You fitted neatly into the group, and I was glad to see you work together with not just me, but also many others from population biology. Thanks for all your hard work, and for your major part in our Ecology & Evolution paper. Should I faint while defending this thesis, I'm sure that you will step in perfectly.

My interest in science was of course no *generatio spontanea*, and I owe thanks to the people that encouraged me to learn, ask questions and discuss. First and foremost, my parents, Jette and Robert, thanks for appreciating critical questions, and for enjoying discussions about positivism, determinism, and who should do the dishes. I'm happy that you supported me to find my own way, even though biology is no social science. Daniël, as my older brother, you were always a few years and steps of thinking ahead. Trying to keep up with you in discussions has undoubtedly sharpened my mind, and perhaps also my tongue. Your ability to ridicule literally any statement is unsurpassed, and a welcome toning down of heated debate. Thanks for at times lending an ear, and for your hospitality in the many places where you lived. Gijs, I don't know for how long we have discussed the nature of consciousness, but it was fun all the time. It's great to have a friend with the same interests, be it football, traveling or philosophy. Even though I'm still convinced that all our effort comes forth from an illusion, it is real enough to me, and I'm glad that we share our curiosity.

From the start of my studies and onwards, I have met many people that made the academic trajectory a gratifying experience. Hanna, Hedwig and Setareh, thanks for being the best classmates I could have. We shared so much fieldwork, lab work, lectures and excursions. I have only very good memories of these years. It was great to join the Nijmeegse Vierdaagse together (and with Lotte!), will we ever voluntarily walk 50 km

again? Paulien, thanks for hosting me in 'your' lab during the first days of my PhD, and for helping me on my way during the first years. Your presentations definitely were an example for me, and it was always fun to talk about children, cars, homes, cooking, and other typical young adult's topics.

To Alexandra, Karen, Felipe, Saioa, Yukie, Fernando, Cleide, Nina, Paul, Josinaldo, Ernesto, Marcus, Dan Li, and Rizwan, thanks for making the population biology group such a welcoming place to work, and for sharing all the ups and downs of PhD / postdoc life. Thanks for all the coffee breaks, lab hours, and paper discussions. Bart, Joris, Rachid, Juan, Joséphine, Sabina, Livia, Jie, Nicky, Alessandra, Tom, Flor, Jacques, Kat, Rik, Naomi, and Heike, the same goes for you! It was great to attend and organise courses and conferences together, and to have a few beers at the end of the day. Vincent, Louise, Floor, Catalina, Silke, and all other members from the theoretical ecology groups, it was cool to have some shared dinners and parties, as well as paper discussions.

Merijn and Arne, my research was always quite close to your research lines. I more than once regretted walking into your offices, because I always came out again with a lot more work to do. Now that the work is done, though, I'm glad that I did (most of) it. Thanks for all the good advice, and for keeping a critical attitude all along. Hans, thanks for putting me on the right track for some basic spider mite biology (holokinetic chromosomes!), and for taking me on the one day of field work in my entire PhD. Isabel, thanks for the good paper discussions and for your career advice. Thomas Van Leeuwen, and Sabrina and Eric from WUR, thanks for helping me to reify my ideas for a postdoc proposal. Michiel and Astrid, thanks for stepping in as guest opponents at my defense.

I would also like to thank several people who helped me with various practical issues during my time at Science Park. Betsy, Peter, and Lin, thanks for all the help and support in the lab. Louis, thanks for taking care of my mite cultures. Climate rooms are good for many things, even brushing teeth. Ludek, Harold, Thijs and Fernando, thanks for sowing countless tomato seeds, and for handling my often quite complex sowing schemes. Tanya, Saskia, Mary, Pascale, and Amber, thanks for all your help with administrative issues, and for the always friendly atmosphere at the secretariat. Jan van Arkel, thanks for designing the cover of this thesis, and for some outstanding music sessions at various occasions. Jan Bruin, thanks for taking up the task to design the lay-out of this thesis.

Besides working on my thesis, I have also tried to make the faculty and the institute a better place, through being an active member of PhD councils. It was a sometimes frustrating, sometimes rewarding experience, but it was fun all the time. Anne, Soraya, Smriti, Olga, Rogier, Iris, Reuben, and Giovanni, thanks for the shared efforts, especially for bursary PhDs. Catalina, thanks for taking over my position as IBED representative. Floor, Wouter, Nora, Karen, Louise, and later Sabina and Livia, thanks for putting together IBED's PhD council, and for organising the quite successful P(h)D events.

Although writing a thesis requires a lot of time, it was great to sometimes *not* work and spend time with friends instead. Tom, Thomas, Donald, David, Djao, Olivier, Jaap, and Mark, thanks for some heroic tennis matches, some magnificent trips to Belgium, and for just having a good time together. Coen and Tim, I'm glad that we look out for each other every now and then, and I really enjoy our various discussions. Coen, I wouldn't go as far as saying that writing a PhD in evolutionary biology is as close as one can get to committing evolutionary suicide, but I'm sure that there must be a way to keep man from extinction. Mattanja, we went in quite different directions during our studies, but right now we are both teachers, both parents, and both getting married. Thanks for all the good conversations!

Already before this thesis was completed, I started teaching applied biology at Aeres Universty of Applied Sciences in Almere. This was, and is, a great opportunity and a very inspiring place to work. Linda, thanks for helping me find the time to finish my writing, and all other members of the TB team and students for their interest and enthusiasm.

During my time as a PhD candidate, two extremely fortunate events put everything into perspective. Jonas and Joeri, when I come home after work, and you run towards me while cheering 'papa!' makes me extremely happy and proud. Few things are as rewarding, inspiring, and relaxing as to have a welcoming home and family. Thanks for all your love and affection. Finally, there is one person to whom I always look out for after a full day. Anne Rose, my love, fiancée, and – at the time I defend this thesis – my wife. Sharing my life with you makes me happy, enthusiastic, cheerful and positive. We have so much fun together, in daily life, during our traveling some years ago, and also now as busy young parents. On top of that, you often ask excellent questions about my work, and are not easily convinced. Talking with you about my thesis has greatly improved my clarity. I'm looking forward to all the good times we will have, and I can't wait to spend the rest of our lives together.