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Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences

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Plants and herbivores are thought to be engaged in a coevolutionary arms race: rising frequencies of plants with anti-herbivore defences exert pressure on herbivores to resist or circumvent these defences and vice versa. Owing to its frequency-dependent character, the arms race hypothesis predicts that herbivores exhibit genetic variation for traits that determine how they deal with the defences of a given host plant phenotype. Here, we show the existence of distinct variation within a single herbivore species, the spider mite *Tetranychus urticae*, in traits that lead to resistance or susceptibility to jasmonate (JA)-dependent defences of a host plant but also in traits responsible for induction or repression of JA defences. We characterized three distinct lines of *T. urticae* that differentially induced JA-related defence genes and metabolites while feeding on tomato plants (*Solanum lycopersicum*). These lines were also differently affected by induced JA defences. The first line, which induced JA-dependent tomato defences, was susceptible to those defences; the second line also induced JA defences but was resistant to them; and the third, although susceptible to JA defences, repressed induction. We hypothesize that such intraspecific variation is common among herbivores living in environments with a diversity of plants that impose diverse selection pressure.

Keywords: jasmonate; plant defence; spider mite; *Tetranychus urticae*; adaptation; intraspecific variation

1. INTRODUCTION

Plants respond to herbivory by activating defences (Walling 2000; Kessler & Baldwin 2002; Kant & Baldwin 2007). Effective induced defences select for herbivores that resist or circumvent these defences, and, when frequent enough, these adapted herbivores in turn impose selection pressure on plants. This process of frequency-dependent reciprocal selection drives the coevolutionary arms race between herbivores and plants (Berenbaum & Zangerl 1998; Stahl *et al.* 1999; Bergelson *et al.* 2001*a*). Owing to its frequency-dependent character, the arms race hypothesis predicts, among other predictions, that herbivores will be genetically variable in how they deal with defences of their host plant.

To test for the existence of such intraspecific variation, we studied the spider mite *Tetranychus urticae*. This mite is a generalist herbivore since it is recorded on over 900 plant species or 124 different plant families (Bolland *et al.* 1998). Moreover, it is well known that spider mites easily adapt to a diversity of toxins (Jacobson *et al.* 1999; Konanz & Nauen 2004; Kono 2004; Nauen & Denholm 2005; Van Leeuwen *et al.* 2005) and host plants (Gotoh *et al.* 1993; Chatzivasileiadis & Sabelis 1998; Agrawal 2000; Agrawal *et al.* 2002; Egas *et al.* 2003). *Tetranychus urticae* males are haploid, females are diploid and reproduction is truly arrhenotokous. Hence, unfertilized females produce haploid sons that if mated with their mother produce a mixture of viable male and female offspring. By using mother–son matings, it is relatively simple to fix intraspecific variation in the form of nearly isogenic lines. Furthermore, we chose *Solanum lycopersicum* as a model host plant since *T. urticae* is a notorious pest species on tomato plants (Drukker *et al.* 1997). Moreover, the plant is a model system for the molecular biology of plant defences (Schilmiller & Howe 2005). Hence, its defence responses to the spider mite *T. urticae* have been described in detail (Li *et al.* 2002*c*; Ament *et al.* 2004; Kant *et al.* 2004) and several well-characterized defence mutants are available (Li *et al.* 2001).

Induced tomato defences depend on a small signalling protein called systemin and on the commonly occurring plant hormone jasmonic acid (JA; Schilmiller & Howe 2005). In response to herbivore feeding, for example, by spider mites, genes involved in JA biosynthesis and defence marker genes downstream of JA are induced (Li *et al.* 2002*a*,*c*; Ament *et al.* 2004; Kant *et al.* 2004). The best-studied group of spider mite-induced tomato defence genes comprises the wound-induced proteinase inhibitors (WIPI) I and II, which encode proteins that inhibit herbivore digestive proteases (Kant *et al.* 2004).

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Spider mites feeding on tomato cause an increasing emission of two plant volatiles, methyl salicylate (MeSA) and 4,8,12-trimethyl-1,3(*E*),7(*E*),11-tridecatetraene (TMTT); these volatiles, which are dependent on JA, have been shown to function in indirect defences (Ament *et al.* 2004). The transgenic tomato plant *35S::prosystemin* (*PS*) has constitutively activated direct defences and is highly resistant to thrips (*Frankliniella occidentalis*) that, like spider mites, feed on mesophyll cells. In contrast, the JA biosynthesis mutant *def1* neither accumulates WIPI transcripts nor elevates proteinase inhibitors in response to herbivory (Li *et al.* 2002*c*); moreover, *def1* is highly vulnerable to thrips and spider mites.

Li *et al.* (2002*c*) reported that spider mites that had been cultivated on bean plants produced more offspring and caused more damage on *def1* than on wild-type (wt) tomato plants. Interestingly, Ament *et al.* (2004) concluded that tomato-adapted spider mites performed well on *def1* but equally well on wt plants. These results prompted us to ask whether *T. urticae* exhibit such pronounced intraspecific variability that tomato defence-resistant and -susceptible individuals can be extracted from populations (or even a single population) of this species. Therefore, we selected isofemale lines from a natural population of spider mites that was collected from spindle tree *Euonymus europaea*, and assessed the reproductive performance of these strains on tomato. We found that two distinct intraspecific traits in spider mites determined whether and how spider mites deal with tomato JA defences. One trait determined whether the mite induces the defence response or not. The second trait determined whether reproductive performance was affected by JA defences or not. Furthermore, we investigated how these traits upheld on plant species other than tomato. We propose that, in nature, such traits are maintained by frequency-dependent selection pressure on generalist herbivores such as spider mites.

2. MATERIAL AND METHODS

(a) *Plants and spider mites*

Seeds of wt tomatoes (*S. lycopersicum* cv. *Castlemart* and cv. *Moneymaker*), *PS* and *def1* (both in the genetic background of *Castlemart*) were germinated in soil and grown in a greenhouse compartment as described in Kant *et al.* (2004). *Arabidopsis thaliana* ecotype Columbia (Col-0 and *asLox*; Bell *et al.* 1995) and *Phaseolus vulgaris* seeds were germinated and grown in soil in a climate room (16/8 h light regime, 100 $\mu E\, m^{-2}\, s^{-1}$, 60% RH, 22°C). Experiments were performed on four-week-old *Arabidopsis* plants and on 10-day-old bean plants. Spindle trees (*E. europaea* Jacq.) of 2–3 years old were maintained in the greenhouse at 23–18°C and a 16/8 h light/dark regime. For spindle tree experiments, randomly chosen leaves were detached, placed with the abaxial surface onto wet cotton wool and maintained in the climate room.

Spider mites were reared on detached tomato leaves on wet cotton wool as described in Kant *et al.* (2004). By oedipal mating, nearly isogenic (inbred) lines were obtained. To fix the observed phenotypes, we allowed groups of five females with high or low fecundity to establish a new population as described in §2*b*.

(b) *Identifying spider mite lines and evaluating their performance*

Spider mites and their eggs were collected from a single European spindle tree (*Euonymus europea* L.), in the dunes near Santpoort (The Netherlands) during the spring of 2001, and transferred to the laboratory. Eggs were allowed to hatch on detached bean leaves placed on wet cotton wool, after which single 2-day-old adult females ($n=90$) were placed separately on tomato leaf discs (cv. *Moneymaker*) on wet cotton wool; the number of (haploid) eggs that each virgin female had produced was counted after 7 days. From these mites, five individual females that had produced high number of eggs were pooled to start new populations (henceforth called 'lines') and we did the same for females that had produced low number of eggs. In this way, we created eight lines; here, however, we report on results that used only two of those lines: the high-performing KMT line and the low-performing KMB line. We attempted to cultivate the KMB line on detached tomato leaflets, but the resulting population did not generate enough individuals per time interval to do experiments efficiently. Therefore, we maintained both KMT and KMB lines on detached bean leaves (on which both produced sufficient numbers of offspring for experiments). The KMT line was transferred to detached tomato leaves every four weeks (the time of one generation cycle) to maintain the selection pressure of tomato. In practical terms, this means that we grew one batch of the KMT line on tomato and two on bean, one of which was 'fresh' and one of which was four weeks old. Every four weeks, half of the tomato grown batch was transferred to fresh bean leaves; at the same time, the mites from the four-week-old bean culture were mixed back in with the remaining mites in the tomato culture. This procedure was repeated every four weeks so that we always had sufficient numbers of tomato-selected KMT mites on bean for experiments.

To compare the performance of these two selection lines on tomato with that of a tomato-adapted line, we used KOP (Ament *et al.* 2004). KOP had been cultivated on tomato for over 10 years as described in Kant *et al.* (2004). We assessed how much leaf area mites of each line damaged as described in detail in Kant *et al.* (2004).

(c) *Classifying resistance (R+) and susceptibility (R−) in three spider mite lines*

For the fecundity assays on wt (cv. *Castlemart*), *def1* and *PS* plants, mites were placed gently on the adaxial surface of the fully expanded leaflets of intact plants using a soft bristle paintbrush. We introduced five mites per leaflet on three leaflets per plant using 10 plants per genotype for each mite line. After 4 days, infested leaves were detached and eggs were counted using a stereomicroscope. All experiments were conducted simultaneously and repeated once such that, in total, 20 plants per genotype were analysed. The fact that we did not observe spider mites dispersing to adjacent leaflets during the period of the experiment was probably due to petiole hairiness. A line was only classified to be resistant if its performance on *def1* was not significantly different from its performance on *PS*.

(d) *Inducing (I+) and non-inducing (I−) classification of three spider mite lines*

To obtain leaf material for the PI measurements and RNA gel blot analysis, intact wt plants (cv. *Castlemart*) were

infested with 15 mites per leaflet on three leaflets per plant. To obtain RNA, leaflets were cut at the base and three leaflets per plant were pooled in 14 ml tubes, directly frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated with a phenol–LiCl-based method as described in Verdonk *et al.* (2003). For RNA gel blot analysis, we used a *WIPI-II* probe from nucleotides 19 to 620 (GenBank K03291). RNA gel blots were repeated three times with leaf material from independent experiments. The chymotrypsin PI activity assays were performed as described in Kant *et al.* (2004). Two plants per mite line were infested with 15 mites per leaflet on three leaflets per plant. After 4 days, leaves were detached, frozen in liquid nitrogen and stored at −80°C. This was repeated four times in independent experiments. All data from the independent replicates were pooled. PI inhibition was expressed relative to control samples (therefore called 'fold induction'). All values were log transformed to correct for unequal variances prior to performing ANOVA and the Dunnett *post hoc* test for comparing a control group with several treatment groups.

(e) *Inducing volatile emission by the three different spider mite lines*

Volatiles of the cv. *Castlemart* wt plants were collected on Tenax TA and identified and quantified using gas chromatography/mass spectrometry (GC/MS) and based on synthetic external standards of known concentration; benzyl acetate was used as an internal standard as described in Kant *et al.* (2004). In short, volatiles were collected from groups of three plants, each containing three leaflets infested with 15 mites per leaflet. We sampled all volatiles produced during three consecutive days over 24 hour intervals and summed the three samples of each set of plants, after GC/MS analysis, prior to performing statistics using ANOVA and the Dunnett *post hoc* test for comparing a control group with several treatment groups. We report on four volatiles: β-phellandrene and β-caryophyllene, which are not induced by spider mites, and MeSA and TMTT, which are both induced by spider mites and whose emission depends on induced JA signalling (Ament *et al.* 2004). Four different treatments (plants infested with (i) KOP, (ii) KMB, (iii) KMT and (iv) uninfested plants as control) were sampled for volatiles simultaneously. The treatments were repeated four times in four independent experiments. After each experiment, the total leaf fresh weight of the plants was determined.

(f) *Cross-talk experiment*

To test whether the fecundity of one line could be affected by another, we prepared a set-up that enabled us to monitor the performance (eggs per mite per 4 days) of two lines separately on one leaflet (figure 4*a*). For this purpose, we created an approximately 1 mm thin spider mite-proof barrier of lanolin paste, which we applied using a syringe, perpendicular to the mid-vein, around the abaxial and adaxial surfaces of the leaflet. The reason for not putting the lanolin along the central vein is that mites tend to attach the silk to this vein when producing the web within which they lay their eggs. Fifteen adult female spider mites (the inducer group) were introduced to the part of the leaflet connected to the petiole, and five adult female spider mites (the receiver group) were introduced onto the leaflet on the other side of the barrier; henceforth, these will be referred to as inducer and receiver mites. The unequal number of inducer and receiver mites was

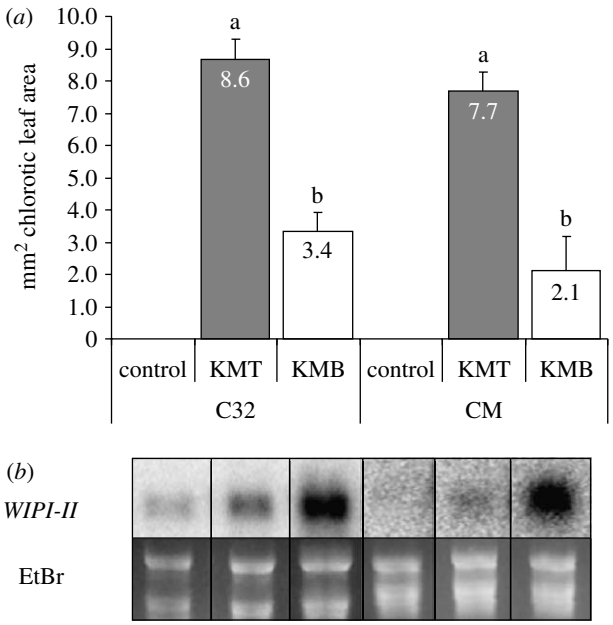

Figure 1. The amount of spider mite feeding damage negatively correlates with induced levels of defence genes. Tomato plants (cv. *Moneymaker* (C32) and cv. *Castlemart* (CM)) were infested with five adult female spider mites of lines KMT or KMB. (*a*) After 4 days, the extent of leaf area damaged by feeding was measured. Vertical bars indicate the means and standard errors (s.e.). Different letters above the bars indicate significant differences as reported in §3. (*b*) The same tomato cultivars were infested with 15 adult female spider mites of lines KMT and KMB for 1 day after which leaflets were collected for RNA isolation and assayed for *WIPI-II* mRNA levels by RNA gel blot analysis. One representative experiment ($n=3$) is shown. Ethidium bromide (EtBr) staining is shown to illustrate equal loading.

due to the fact that although 15 mites per leaflet were used for induction, 15 mites produce more eggs every 4 days than can be counted. Therefore, fecundity was always assessed for groups of five mites (Kant *et al.* 2004). After 4 days, leaflets were detached and eggs of the receiver group were counted using a stereomicroscope. Each treatment was repeated 30 times in three independent series. Data were pooled for analyses.

(g) *Spider mite performance on other host plants*

Performance during 4 days on *Castlemart* tomato ($n=20$ per mite line), detached spindle tree leaves ($n=30$ per mite line) and bean plants ($n=12$ per mite line) was assessed as described previously. Spider mite performance on intact *A. thaliana* Col-0 and *A. thaliana* antisense lipoxygenase-2 (*asLox*; Bell *et al.* 1995) was determined on four-week-old plants in separate pots. Plants were infested with five adult female spider mites (placed on separate leaves) per plant ($n=12$ per mite line). After 4 days, all plants were cut off at the base and intact rosettes were inspected for eggs on both sides under a stereomicroscope.

3. RESULTS

To select spider mite lines that perform differently on tomato plants, we sampled a natural spider mite population from European spindle trees (*E. europaea* Jacq.) and created nearly isogenic lines (referred to here as KMT, KMB and KOP) from the females that

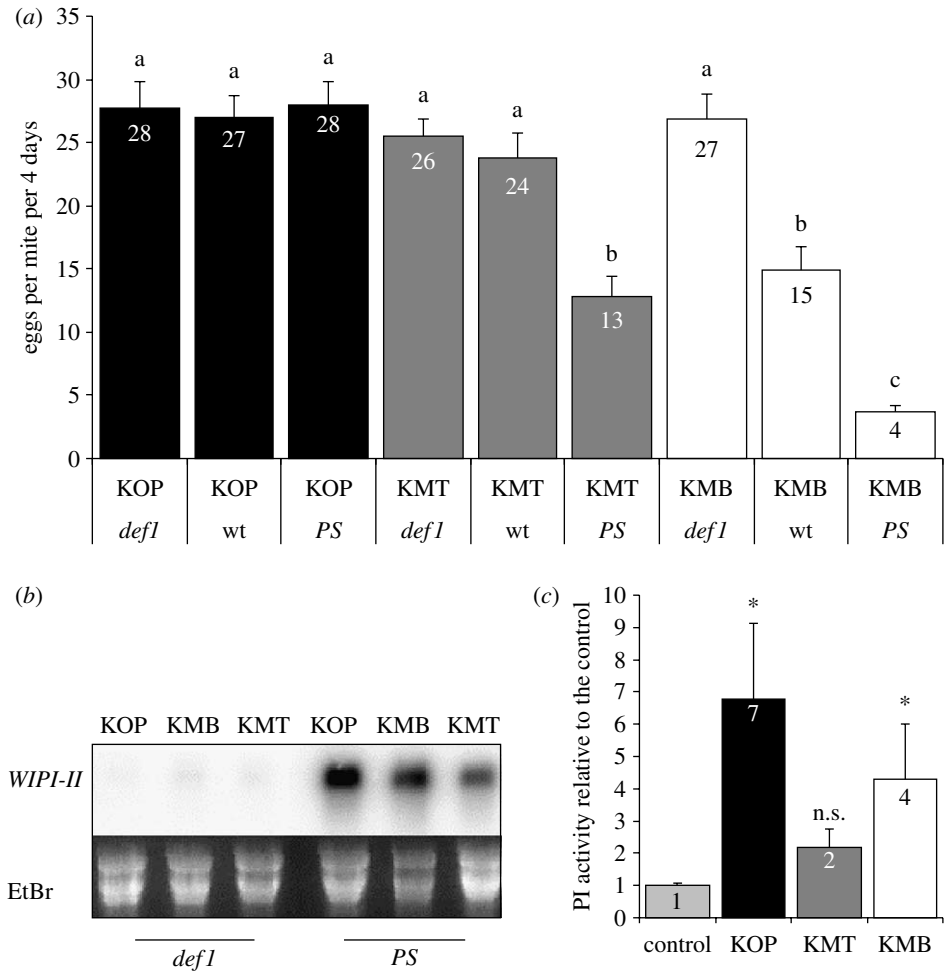

Figure 2. Resistance and susceptibility of spider mites to tomato defences do not always depend on induced JA defences. (*a*) Cultivar *Castlemart* (wt), *def1* and *35S::prosystemin* (*PS*) tomato plants were infested with five adult female spider mites of lines KOP, KMT and KMB, and after 4 days we assessed the average number of eggs produced per female per 4 days on each tomato genotype. Vertical bars indicate the means and s.e. and different letters above the bars indicate significant differences. (*b*) Tomato plants (*def1* and *PS*) were infested with 15 adult female spider mites of the lines KOP, KMT and KMB, and after 4 days leaflets were collected for RNA isolation and assayed for *WIPI-II* mRNA levels. EtBr staining is shown to illustrate equal loading. (*c*) *Castlemart* tomato plants (wt) were infested with 15 adult female spider mites of lines KOP, KMT and KMB, and after 4 days leaflets were collected to determine PI activity relative to the control (uninfested plants). Vertical bars indicate the means and s.e. and an asterisk denotes a significant difference compared with the control while 'n.s.' stands for 'not significantly different from the control'.

had performed differently due to (unknown) genotypic differences.

(a) *Identifying spider mite lines and evaluating their performance*

KMT and KOP caused approximately two to three times more feeding damage than KMB did, but KMB and KOP induced *WIPI-II* expression more than KMT did. KMT caused 8–9 mm^2 feeding damage to cv. *Moneymaker* tomato leaves per 4 days (figure 1*a*), while KOP caused approximately 6.0 ± 0.8 mm^2 feeding damage on cv. *Castlemart* tomato (Ament *et al.* 2004; Kant *et al.* 2004) and 6.9 ± 0.7 mm^2 on cv. *Moneymaker* tomato (data not shown in the figure) during the same amount of time. KMB caused 2–3 mm^2 feeding damage per 4 days on tomato (figure 1*a*). KMT feeding caused lower levels of JA-dependent *WIPI-II* transcripts to accumulate compared with both KMB (figure 1*b*) and KOP, as was previously shown by means of microarray (a 10- to 20-fold upregulation of WIPI expression; Kant *et al.* 2004) and RT-PCR (Ament *et al.* 2004) on mRNA from KOP-infested cv. *Castlemart* tomato leaves.

(b) *Classifying resistance (R+) and susceptibility (R−) in three spider mite lines*

To assess the impact of the JA-dependent defences in tomato on the fecundity of our mite lines, we determined their performance on wt, *def1* and *PS* tomato plants. Line KOP performed equally well on all three tomato lines (ANOVA: $F=0.2/p=0.8$), whereas KMT performed equally well on wt and *def1* (ANOVA: $F=8.5/p<0.001$; Tukey's HSD: $p=0.7$) but less well on *PS* (Tukey's HSD: $p<0.001$ for both comparisons; figure 2*a*). Line KMB performed just as well on *def1* as KMT and KOP but less well on wt plants (ANOVA: $F=25.3/p<0.0001$; Tukey's HSD: $p=0.001$) and poorest on *PS* (Tukey's HSD: $p=0.004$). None of the spider mite lines induced accumulation of WIPI-II transcript in *def1*, while, in *PS*, this gene was highly expressed (figure 2*b*). These results justify classifying KOP spider mites as resistant (R+) and both KMB and KMT lines as susceptible (R−) to JA defences.

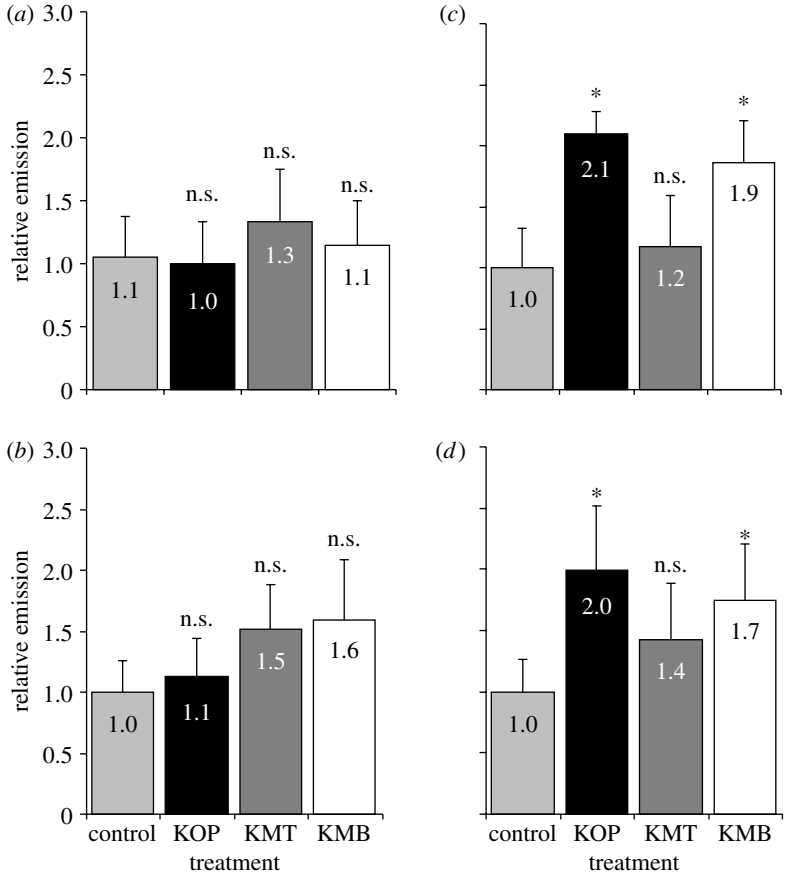

Figure 3. Differential induction of tomato leaf volatiles by individual spider mite lines. Cultivar *Castlemart* tomato plants (wt) were infested with 15 adult female spider mites of lines KOP, KMT and KMB on three leaflets (45 mites per plant), and during 3 days tomato volatiles were collected and analysed using GC/TOF-MS. Shown is the average emission of (*a*) β-phellandrene, (*b*) β-caryophyllene, (*c*) MeSA and (*d*) TMTT per day, expressed relative to the treatment that gave the lowest emission of that compound ('fold emission'). The 1.0 relative emission corresponds to 2.6 μg of β-phellandrene, 0.32 μg of β-caryophyllene, 10.7 μg of MeSA and 41.8 μg of TMTT per day per gram fresh weight. Vertical bars indicate the means and s.e. and an asterisk denotes a significant difference compared with the control while 'n.s.' stands for 'not significantly different from the control'.

(c) *Classifying inducing (I+) and non-inducing (I−) spider mite lines*

Mite-induced proteinase inhibitor activity was significantly higher in wt plants infested with KMB and KOP than in plants with KMT (figure 2*c*). KOP induced a significant sevenfold increase (ANOVA: $F=9.3/p<0.001$; Dunnett's test (control versus KOP): $p=0.009$) and KMB a significant fourfold increase (Dunnett's test (control versus KMB): $p=0.027$) in PI activity in wt tomato plants, relative to the untreated control. The twofold induction of PI activity by KMT was not significant (Dunnett's test (control versus KMT): $p=0.27$; figure 2*c*). Based on induced PI activity, the KOP and KMB lines are classified as I+ ('I+'=PI inducing in wt) and the KMT line as I− ('I−'=not significantly PI inducing in wt).

(d) *Inducing volatile emission by the three different spider mite lines*

The mite-induced emission of a monoterpene (β-phellandrene; figure 3*a*) and a sesquiterpene (β-caryophyllene; figure 3*b*) was previously shown not to be affected by KOP feeding (Kant *et al.* 2004) or jasmonate (Ament *et al.* 2004), but the emission of MeSA (figure 3*c*) and TMTT (figure 3*d*) was shown to be induced by KOP spider mites depending on JA (Ament *et al.* 2004). None of the spider mite lines induced significant increases in β-phellandrene or β-caryophyllene emission (ANOVA: $F=0.76$ ($p=0.52$) and $F=1.2$ ($p=0.30$), respectively). The KMB and KOP lines induced a significant twofold increase in the emission of MeSA (ANOVA: $F=3.0/p=0.05$; Dunnett's test (KMB versus control): $p=0.03$) and of TMTT (ANOVA: $F=2.7/p=0.04$; Dunnett's test (KMB versus control): $p=0.022$), whereas the KMT line did not (Dunnett's test (KMT versus control): $p=0.61$ for MeSA; $p=0.62$ for TMTT). Hence, only the KMT line did not induce the JA-dependent emission of volatiles. The statistical differences in volatile emissions of JA-dependent MeSA and TMTT confirm that KMT mites induce a weak JA response in tomato plants.

(e) *Cross-talk experiment: is KMT a 'non-defence-inducing' or a 'defence-repressing' line?*

To investigate whether the high-performing KMT line is a 'non-inducer' or a 'repressor' of JA defences, we designed a cross-talk experiment (figure 4*a*) based on the assumption that local feeding triggers leaflet-wide induction of defences. This is supported by the observation that PI induction in uninduced (unwounded) parts of a tomato leaflet results in the accumulation of similar amounts of transcript in the induced (wounded) part of the same leaflet (Lee & Howe 2003) as in *Nicotiana attenuata* (Wu *et al.* 2007). We reasoned that if KMT mites do not induce a defence response in a tomato leaflet, induction of defences by KMB mites feeding on the same leaflet will

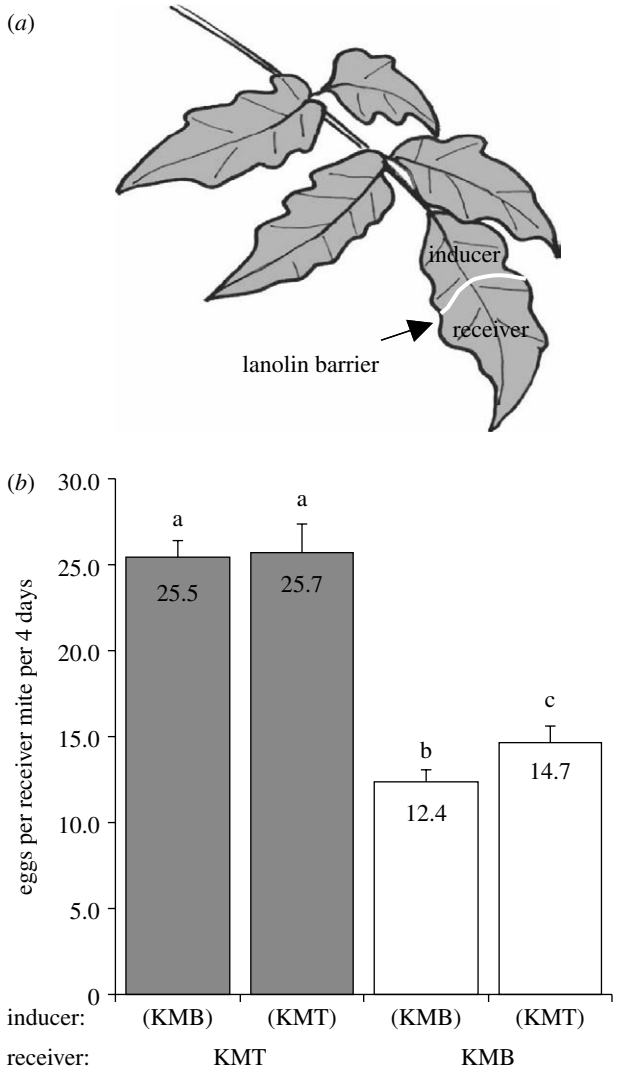

Figure 4. Introducing KMT spider mites onto KMB-infested tomato leaflets positively affects KMB fecundity. (*a*) A tomato leaf was divided into two by a thin lanolin barrier. Fifteen inducer mites (in brackets) were placed near the petiole and five receiver mites near the leaf tip. Shown in (*b*) are the performances of KMB and KMT (eggs per 4 days) in the presence of the other phenotype (KMB with KMT and KMT with KMB) on the same leaflet or in the presence of mites with the same phenotype (KMB with KMB and KMT with KMT) on the same leaflet as controls. Vertical bars indicate the means and s.e. and different letters above the bars indicate significant differences as reported in §3.

reduce the performance of KMT mites. Alternatively, if KMT mites actually repress defences, KMB mites on the same leaflet will perform better. The high performance of KMT appeared independent of the presence of KMB (25.5 versus 25.7 eggs; ANOVA: $F=9525/p<0.0001$; Tukey's HSD (KMB→KMT versus KMT→KMT): $p>0.99$; figure 4*b*), whereas the performance of KMB increased in the presence of KMT (12.4 versus 14.7 eggs; Tukey's HSD (KMB→KMB versus KMT→KMB): $p=0.055$; figure 4*b*). Hence, it is more likely that KMT represses JA defences than that it does not induce them.

(f) *Performance of three different lines on other host plants*

To investigate whether the selected spider mite lines exhibit traits specific to tomato, we compared their performance on tomato, spindle tree, bean and *Arabidopsis* (figure 5). As shown previously (figure 2*a*), the KOP line exhibited high fecundity on wt tomato (27 eggs per mite per 4 days), similar to KMT (24 eggs per mite per 4 days; ANOVA: $F=11.1/p<0.001$; Tukey's HSD (KMT versus KOP): $p=0.4$), whereas KMB exhibited significantly less fecundity (15 eggs per mite per 4 days; Tukey's HSD (KMT versus KMB): $p=0.005$; figure 5*a*). On bean plants (figure 5*d*) and spindle tree leaves (figure 5*b*), the KMT line always had significantly higher (with p-values <0.035 after Tukey's HSD) egg production (24 and 19, respectively) compared with KOP (15 and 9, respectively) and KMB (11 and 11, respectively). On *Arabidopsis* (figure 5*c*), the difference between KOP and KMT was not significant (16 versus 20; ANOVA: $F=8.7/p=0.001$; Tukey's HSD (KOP versus KMT): $p=0.25$), whereas the difference between KMB and KMT was significant (11 versus 20; Tukey's HSD (KMB versus KMT): $p=0.001$). Thus, the KOP phenotype only performed well on tomato, the host plant on which it had been reared for more than 10 years (Kant *et al.* 2004). Moreover, by analogy with its performance on *def1*, the performance of KMB on the JA-deficient *A. thaliana* antisense lipoxygenase-2 (*asLox*; Bell *et al.* 1995) plants increased to the level of performance seen for KMT on wt (from 11 ± 2 on wt to 16 ± 2 on *asLox* for KMB compared with 20 ± 2 for KMT on wt). This indicates that the difference in performance between KMB and KMT on *Arabidopsis* is JA dependent as well. Thus, the spider mite lines studied here have the characteristics of, or are, host plant specialists (KOP) or generalists (KMT).

4. DISCUSSION

We have shown that local spider mite populations harbour a degree of variability in traits that determines (i) the levels of induced *WIPI-II* transcript, (ii) the levels of induced PI activity, (iii) the total amount of JA-dependent volatiles released by infested plants, and (iv) to which extent their reproductive performance is affected by those plants' defences. Based on induced PI activity, we classify the KOP line as R+I+('R+'=JA defence resistant; 'I+'=PI inducing in wt), the KMB line as R−I+('R−'=JA defence susceptible) and the KMT line as R−I− ('I−'= not PI inducing in wt). In the rest of the article, this phenotypic annotation will be used in superscript as follows: $KOP^{(R+I+)}$; $KMT^{(R-I-)}$; and $KMB^{(R-I+)}$. Such phenotypic variation might explain why spider mites, as a species, are able to adapt so fast to new host plants (Agrawal 2000).

Our selection lines revealed three mite phenotypes composed of two traits, one that accounts for the induction and the other for the impact of tomato defences. All three lines perform equally well on *def1*, showing that their reproductive potential is equal; however, their performance on wt and *PS* differed. First, the $KMB^{(R-I+)}$ line, which suffers from JA defences, as shown on wt and *PS* tomatoes (figure 2*a*), clearly performs better in the absence of JA defences (on *def1*). This phenotype confirms that JA can be essential in plant defence against herbivorous mites (Li *et al.* 2002*c*; Schweighofer *et al.* 2007). Second, the $KMT^{(R-I-)}$ line, which also suffers from JA defences, as shown on *PS* tomatoes (figure 2*a*), does not induce JA defences in wt plants (figures 1–3).

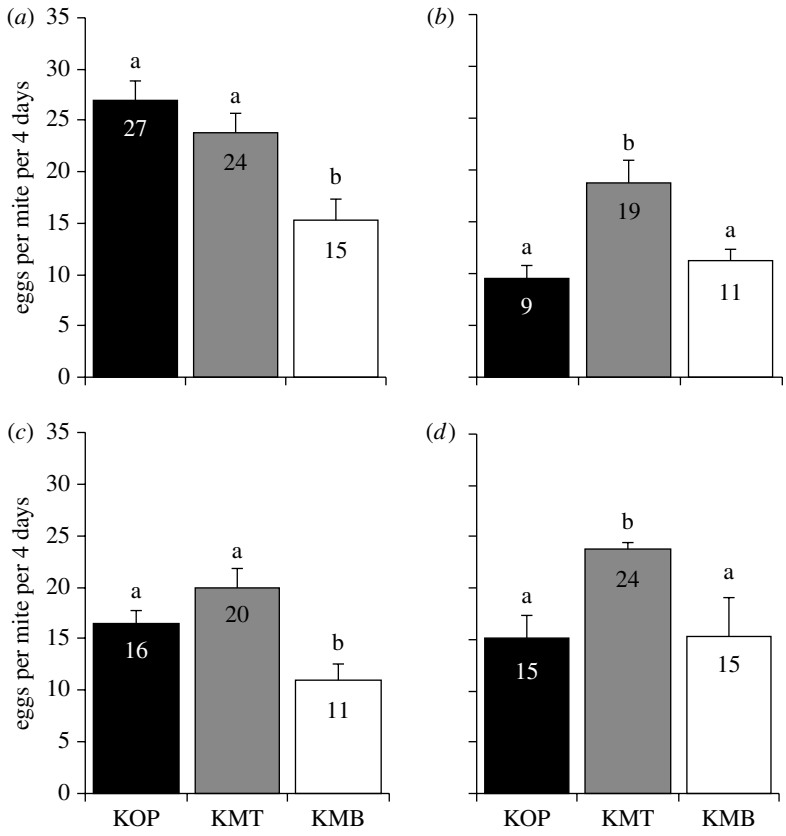

Figure 5. The R+I+ phenotype of KOP manifests itself only on tomato whereas the R−I− phenotype of KMT manifests itself on four different plant species. Shown is the spider mite fecundity per 4 days on (*a*) tomato (*S. lycopersicum* cv. *Castlemart*), (*b*) spindle tree (*E. europaea* L.), (*c*) mouse-ear cress (*A. thaliana* Col-0) and (*d*) broad bean (*Phaseolus vulgaris*). Vertical bars indicate the means and s.e. and different letters above the bars indicate significant differences.

Third, the KOP$^{(R+I+)}$ line, which performs equally well on all the three tomato genotypes (*def1*, wt and *PS*), is resistant to JA defences (figure 2*a*). Taken together, KOP$^{(R+I+)}$ and KMT$^{(R-I-)}$ have traits that enable them to cope with inducible JA defences in tomato but in fundamentally different ways: the former adaptively resists JA defences; the latter adaptively foregoes induction of JA defences.

How do herbivores deal with (induced) plant defences? Many strategies describe how insects either avoid plant defences, i.e. through diet choice (De Moraes & Mescher 2004), or cope with plant defences, i.e. through target site insensitivity (Feyereisen 1995), or use plant defences to their own benefits, i.e. through sequestration (Hartmann *et al.* 2005; Narberhaus *et al.* 2005) or defensive regurgitation (Sword 2001). Moreover, herbivores have often evolved means of detoxifying components in their diet, such as plant secondary metabolites (Li *et al.* 2002*b*; Francis *et al.* 2005) and pesticides (Nauen *et al.* 2001; Bates *et al.* 2005). The latter is probably the case for KOP$^{(R+I+)}$ since it performs well on tomato despite JA defences. Interestingly, rather than detoxifying defence products, some herbivores (Bede *et al.* 2006; Zarate *et al.* 2007) and pathogens (Shiraishi *et al.* 1994; Nomura *et al.* 2005) have evolved strategies to directly interfere with the *in planta* establishment of the defence response, using suppressors to delay defence responses and/or activating the plants' own negative defence regulators (Zhao *et al.* 2003; Block *et al.* 2005; Nomura *et al.* 2005). For example, the compound glucose oxidase found in *Helicoverpa zea* regurgitant appeared to suppress plant defences (Musser *et al.* 2005; Bede *et al.* 2006). KMT$^{(R-I-)}$ may produce substances that interfere with defence responses, given the fact that the fecundity of KMB$^{(R-I+)}$ increases significantly in the presence of KMT$^{(R-I-)}$. Although the effect is small in an absolute sense (figure 4), the KMT$^{(R-I-)}$ repression, just like the KMB$^{(R-I+)}$ induction, of defences could wane as distance to the feeding site increases. Whether the fitness benefit of KMB$^{(R-I+)}$ mites in the presence of KMT$^{(R-I-)}$ also occurs under natural conditions remains to be seen; clearly, the KMB$^{(R-I+)}$-induced response is not completely repressed by KMT$^{(R-I-)}$ since KMB$^{(R-I+)}$ performance (figure 4*b*) still is much lower than on *def1* (figure 2*a*) and the effect might depend on the ratio in which KMB$^{(R-I+)}$ and KMT$^{(R-I-)}$ co-occur on a leaf.

Takabayashi *et al.* (2000) reported a 'red-coloured' and a 'green-coloured' spider mite line that, respectively, did and did not induce attraction of predatory mites. As in our experiments, this difference did not correlate with the amount of damage inflicted by the spider mite lines (figure 1). Moreover, the KMB$^{(R-I+)}$ and KMT$^{(R-I-)}$ lines did not only differ in the induction of volatile emission (figure 3*c*,*d*) but also in the induction of defence gene expression (figure 1*b*) and proteinase inhibitor activity (figure 2*c*). Takabayashi *et al.* (2000) suggested that some spider mite phenotypes might secrete salivary enzymes that prevent indirect defences from being established. Recently, Matsushima *et al.* (2006) identified 'white' and 'red' forms of the Kanzawa spider mite (*Tetranychus kanzawai*) that differently induced both salicylic acid responses and the emission of two isoforms

of volatile (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) in lima beans (*Phaseolus lunatus*). Taken together, these instances of discrete intraspecific variability suggest that herbivorous spider mites and plants are engaged in an arms race (Berenbaum & Zangerl 1998; Bergelson *et al.* 2001*a*,*b*; Christeller 2005); accordingly, frequency-dependent selection rewards plants for recognizing pests in time while pushing herbivores to mask their presence or to interfere with the establishment of proper plant defences.

The ecologically most interesting feature of the results presented here is that the genotypic variation in the mode of resistance to direct and indirect plant defences has been found within one herbivore species. Our results confirm that a generalist species does not necessarily consist of generalist individuals (Fox & Morrow 1981) but may well exhibit a genetic polymorphism. We think that the R+I+ type reflects the phenotype of a specialized herbivore (a specialist is here defined as having high reproductive success on a relatively limited number of different host plants) since it thrives only on tomato (figure 5). Specialization is more likely to emerge in populations confined to a particular host plant genotype for a long period of time (as happens with inbred laboratory cultures) and so exposed to a constant selection regime. On the contrary, we hypothesize that generalist herbivores tend to adapt (Stearns & Hoekstra 2000) to the lowest common denominators of the different defence arsenals of different plant genotypes/species. In line with this hypothesis, the ability of the I− type (KMT) to interfere with the host plant's defence signalling cascade—such as the JA cascade—may well be a common characteristic of generalist herbivores.

Why do not all the mites we investigated have R−I− and/or R+I+ phenotypes? We can only hypothesize on the reasons but the variation we found is in line with the prediction that disruptive selection is not only necessarily followed exclusively by phenotypic specialization through directional selection, but also by phenotypic diversification through balancing selection (Egas *et al.* 2005; Rueffler *et al.* 2006). A genetic mosaic of plant environments with different selection pressures (the kind of habitat one would expect for generalist rather than specialist herbivores) offers ample conditions for such diversification (Thompson & Cunningham 2002) as well as for the balanced protection of polymorphisms through frequency-dependent selection (Fitzpatrick *et al.* 2007). In line with this, selection of the R−I− trait might be a consequence of plant defence variability among different hosts and selection of the R+I+ trait a consequence of plant defence similarity among different hosts. However, trade-offs between the costs (Herms & Mattson 1992; Mole 1994; Bergelson *et al.* 2001*b*) of the different types of mite resistance and the fact that in sexual populations the phenotypic distribution is constrained by the processes of segregation and recombination can cause individuals to have maladapted or intermediate phenotypes (Burger & Gimelfarb 2004), such as R−I+ (susceptible type) and R+I− (resistant-but-not-inducing type). Having established the occurrence of distinct plant defence-related intraspecific variation in a herbivore species, we can now search for the underlying genetic and molecular mechanisms. A challenge for future research will be to figure out whether—and if so, which—trade-offs prevent mites from becoming 'jacks of all trades' (Richards *et al.* 2006) and to elucidate the driving forces behind the temporal dynamics of intraspecific variation.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number K03291 (*WIPI-II*).

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