

Do mites evolving in alternating host plants adapt to host switch?

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Abstract

A fluctuating environment may be perceived as a composition of different environments, or as an environment *per se*, in which it is the fluctuation itself that poses a selection pressure. If so, then organisms may adapt to this alternation. We tested this using experimental populations of spider mites that have been evolving for 45 generations in a homogeneous environment (pepper or tomato plants), or in a heterogeneous environment composed of an alternation of these two plants approximately at each generation. The performance (daily oviposition rate and juvenile survival) of individuals from these populations was tested in each of the homogeneous environments, and in two alternating environments, one every 3 days and the other between generations. To discriminate between potential genetic interactions between alleles conferring adaptation to each host plant and environmental effects of evolving in a fluctuating environment, we compared the performance of all lines with that of a cross between tomato and pepper lines. As a control, two lines within each selection regime were also crossed. We found that crosses between alternating lines and between pepper and tomato lines performed worse than crosses between lines evolving in homogeneous environments when tested in that environment. In contrast, alternating lines performed either better or similarly to lines evolving in homogeneous environments when tested in a fluctuating environment. Our results suggest that fluctuating environments are more than the juxtaposition of two environments. Hence, tests for adaptation of organisms evolving in such environments should be carried out in fluctuating conditions.

Introduction

Although the study of adaptation usually concerns organisms adapting to a particular, constant and homogeneous environment, in fact, organisms usually face environments that are both variable in time and spatially heterogeneous. Environmental heterogeneity has been first recognized by theoretical biologists, generating predictions for the maintenance of polymorphisms in spatially varying environments (Levene, 1953; Dempster, 1955). Since then, both models and experi-

mental tests of evolution in such environments have multiplied (Kassen, 2002; Ravigné *et al.*, 2009; Poisot *et al.*, 2011). These studies aim at generating or testing predictions concerning the conditions allowing for the evolution of specialist or generalist genotypes.

In environments that fluctuate over time, theory predicts that selection will favour the evolution of a generalist genotype, with the highest geometric mean fitness across environments (Gomulkiewicz & Kirkpatrick, 1992; Gavrillets & Scheiner, 1993), except in very specific cases in which a polymorphism can be maintained (Haldane & Jayakar, 1963; Levins, 1965; Gillespie, 1972; Hedrick, 1974). Indeed, empirical tests have shown that generalists usually evolve in temporally varying environments (Reboud & Bell, 1997; Kassen & Bell, 1998; Turner & Elena, 2000; Kassen, 2002; Buckling *et al.*, 2007; Cooper & Lenski, 2010; Venail *et al.*, 2011). Temporal heterogeneity may favour the

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evolution of phenotypic plasticity, especially with a positive autocorrelation in environmental changes (Slatkin & Lande, 1976; Gavrillets & Scheiner, 1993; Zhang, 2005). Moreover, the evolution of phenotypic plasticity is expected if the environment varies at a fast rate (Tufto, 2000), including within a single generation (Gomulkiewicz & Kirkpatrick, 1992). Indeed, a few studies have confirmed that a higher genetic or phenotypic variance is maintained in fluctuating environments, as compared to homogeneous ones (Reboud & Bell, 1997; Venail *et al.*, 2011). Alternatively, high fitness across environments may be achieved through homeostasis in the underlying traits (Gomulkiewicz & Kirkpatrick, 1992), which can be simply viewed as phenotypic plasticity at a different level of integration.

Populations evolving in a fluctuating environment may perform worse in each environment than populations selected in homogeneous environments, a phenomenon called the cost of generalism (Kassen, 2002). This cost may arise from two nonexclusive causes. First, populations evolving in a fluctuating environment spend less time in each environment, compared with populations evolving in the same environment (Kawecki, 1994; Whitlock, 1996). Moreover, a trade-off between adaptations to each environment may occur, resulting in populations evolving in both environments having an overall lower performance than each of the specialists (Levins, 1968). Evidence for such a cost is mixed: whereas some studies show that this is indeed the case (Reboud & Bell, 1997; Maeda *et al.*, 2006), others provide evidence against it (Turner & Elena, 2000; Buckling *et al.*, 2007).

In all these studies, the heterogeneous environment is viewed as a congregation of two or more homogeneous environments. Organisms may thus adapt to one or several environments. However, a heterogeneous environment is more than the juxtaposition of homogeneous environments. Indeed, apart from adapting to each environment, organisms may also adapt to the environmental fluctuation in itself. Although a direct test of this hypothesis has never been sought, there are indirect lines of evidence that this may be the case. First, bacterial phenotypes that switch morphologies at a faster rate are selected for in a fluctuating environment (Beaumont *et al.*, 2009). Second, the gene expression profile of organisms that occur in varying environments differs from that of organisms in homogeneous environments. For example, mussels thriving in the intertidal zone (more subject to environmental fluctuations than either marine or terrestrial habitats) show higher rates of switching in their gene expression profiles than mussels from the other environments (Gracey *et al.*, 2008). Similarly, an Arg regulon of *Escherichia coli*, which promotes the switch between expression and repression, has a selective advantage when environmental fluctuations occur at a low rate, but high fluctuation levels favour a regulon promoting

constitutive expression (Suiter & Dean, 2005). Conversely, yeast evolving in constant environments accumulate mutations that lead to the loss of signalling pathways that sense a change in the environment (Kvitek & Sherlock, 2013).

Although these studies show that there is genetic variation for switching rates and that these can be adaptive in a fluctuating environment, no study so far has shown that specific adaptations to a fluctuating environment evolve. To test this, it is necessary to perform experimental evolution in a fluctuating environment, test the performance of those lines in that environment and compare it to their performance in each of the (homogeneous) environments that compose the fluctuating environment. In this way, it is possible to single out a possible adaptation to the fluctuation in itself from the physiological adaptations to each of the environments that compose the heterogeneous setting in which populations are evolving. The inclusion of a fluctuating environment in the fitness tests of experimentally evolving lines has been carried out in previous studies (Kassen & Bell, 1998; Cooper & Lenski, 2010). However, the authors did not compare the performance of lines in homogeneous vs. fluctuating environments. We propose to do so in the current study. Furthermore, we will compare the performance of lines evolving in fluctuating environments to a cross of lines evolving in each of the homogeneous environments, which will test whether the potential adaptation is due to a mix of alleles from each selection regime or to a specific adaptation in the fluctuating selection regime. Finally, a comparison between the performance in homogeneous environments of the cross between lines evolving in constant environments and the alternating lines will allow disentangling between a potential genetic trade-off between adaptation to each environment and a cost of evolving in a fluctuating environment *per se*.

The spider mite *Tetranychus urticae* occurs on more than 900 host plants (Navajas, 1998). Tests for the occurrence of host races have provided mixed results (reviewed in Magalhães *et al.*, 2007b). Indeed, two studies revealed no evidence for the occurrence of host races among several host plants (Tsagkarakou *et al.*, 1997, 1999), another showed host races in two separate data sets (Weeks *et al.*, 2000). Finally, a fourth study found that populations on citrus trees were genetically differentiated from those on other host plants, whereas populations from other host plants were genetically similar (Tsagkarakou *et al.*, 1998). This suggests that some populations have the potential to adapt to several host plants, whereas others do not. In this context, testing the consequences of evolving in a fluctuating environment seems particularly relevant in this species. Given the potential of *T. urticae* in experimental evolution studies (Belliere *et al.*, 2010), it would seem straightforward to address this in such a setting. However, this task has not been undertaken far.

Using a population of the spider mite *T. urticae* originally from cucumber and evolving on two novel hosts, tomato and pepper, we have previously shown that mites evolving on a single host plant (tomato or pepper) adapt to each host plant within 15 generations (Magalhães *et al.*, 2007a), and that this adaptation does not entail a cost in other environments (Magalhães *et al.*, 2009). In this study, we test whether lines evolving in an alternating regime of tomato and pepper evolve specific adaptations to this regime. Specifically, we ask the following: (i) are there genetic and/or environmental costs associated with evolving in a fluctuating environment? (ii) Are lines evolving in a fluctuating environment adapted to such fluctuation? To test whether a potential adaptation to a fluctuating environment can be generalized to different scales of fluctuation, we have tested for adaptation in two types of fluctuating environment, one among and the other within generations.

Materials and methods

Stock cultures

Plants were sown once *per week* and cultured in a herbivore-free room under controlled conditions (25 °C). Cucumber plants (variety Ventura) were provided by Rijkzwaan France, tomato plants (variety Moneymaker) by Gebroeders Eveleens in the Netherlands, and pepper plants (variety pikante reuzen) were obtained from the University of Wageningen.

Two-spotted spider mites (*T. urticae* Koch) were cultured in large numbers (> 10 000) on cucumber plants (approximately 4 weeks old, provided twice *per week*) under controlled conditions (25 °C) in a separate room. Spider mites were originally collected from a cucumber greenhouse (variety: Ventura) in Pijnacker, The Netherlands, in May 1994 and kept on that same variety in a climate chamber at the University of Amsterdam. The culture at the University of Montpellier was established in April 2004 from approximately 10 000 individuals of the population from the University of Amsterdam.

Selection lines

Selection lines of spider mites were established in March 2005 by placing 300 adult mated females from the base population on a detached leaf of cucumber, tomato or pepper. The petiole of each leaf was placed in a small vial (*circa* 5 cm diameter and 3 cm high) with water. The vial was covered with a plastic lid with a few holes for the petioles. The leaf and the vial were maintained in a larger plastic box (*circa* 20*20*10 cm) closed with a lid with a central hole covered with gauze, to allow ventilation. The lid was sealed with parafilm and each box was placed in a tray containing

water with a small quantity of soap, to isolate the selection lines from one another. New leaves were added twice *per week*, and old leaves were discarded when completely deprived of mites. We created three selection regimes, each with five lines: tomato (T-lines), pepper (P-lines) and an alternation of tomato and pepper (A-lines), which occurred approximately every generation (15 days). Experiments were carried out after mites had evolved for more than 45 generations under each selection regime.

Experimental procedure

An outline of the experimental procedure is given in Fig. 1.

To test whether evolving in a fluctuating environment was comparable to the accumulation of genetic changes in each of the two homogeneous environments, we crossed individuals from one P-line with those from one T-line (30 males and 30 females of each line were reciprocally crossed). We then compared the performance of that cross (hereafter T×P) with that of a cross between two A-lines (A×A) and with a cross between populations from the homogeneous selection regimes (P×P and T×T). All these crosses were generated in the same way as the T×P crosses. The two lines involved in each cross were randomly chosen among the five replicate lines within each selection regime. Hence, each cross type is represented by only one cross between two lines of the same or different selection regime(s).

The performance of individuals resulting from these crosses was tested in four environments: tomato, pepper and two environments in which tomato and pepper were alternating: (i) every 3 days until the death of the individuals and (ii) every generation, during two generations. In the latter case, only the data on the second generation were used. The control for this alternation is a homogeneous environment across generations, that is, individuals which had experienced either tomato or pepper during two consecutive generations. An environment with alternation between generations was chosen because this fluctuation time roughly corresponds to that imposed in the selection process. The environment in which host plants alternate within a single generation was added because it allows for a within-individual expression of plasticity, which may also play a role in the selection regime imposed. In each environment, and for each cross, we measured juvenile survival and oviposition rate, the traits that had been shown to be modified during experimental evolution on either pepper or tomato (Magalhães *et al.*, 2007a, 2009). All individuals spent one generation on bean before the onset of the experiment, to minimize environmental effects specific of each selection environment (Magalhães *et al.*, 2011).

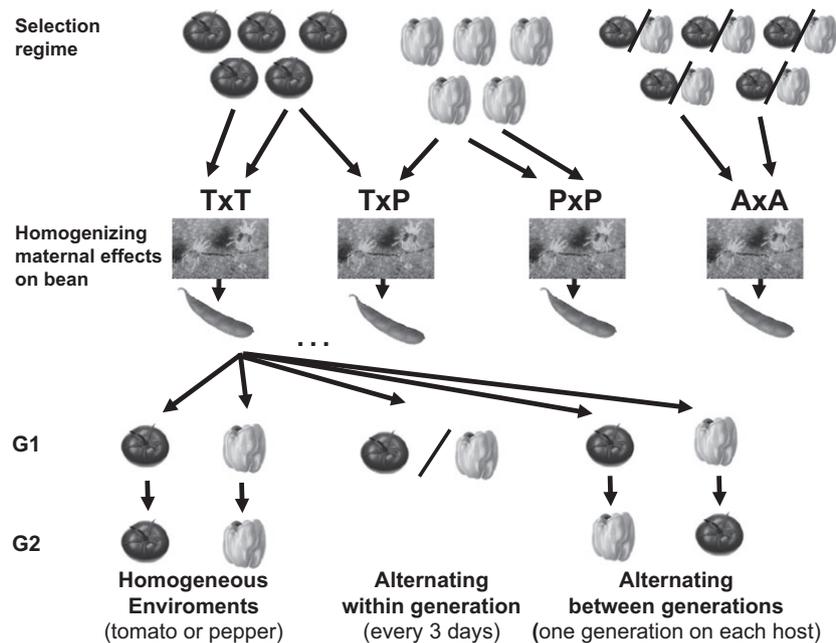


Fig. 1 Experimental design. All lines (each initiated with 300 females) stemmed from a population that had been on cucumber for about 400 generations. Individuals evolved either on pepper (P-lines), tomato (T-lines) or a regime in which pepper and tomato alternated approximately every generation (A-lines). Five lines per regime were created. After 45 generations, we performed crosses among lines of the same selection regime (T×T, P×P and A×A for crosses among tomato, pepper or alternating lines, respectively) or between one pepper and one tomato line (T×P). Individuals from these crosses spent one generation in a common environment (bean) and those from the subsequent generation were assigned to three environmental conditions: homogenous (tomato or pepper), alternating within generations or alternating between generations. The performance of such individuals (survival and oviposition rate) was then measured.

Statistical analysis

All analyses were performed in R (v. 3.0.36-; www.r-project.org). Juvenile survival was analysed using a multifactorial survival analysis (coxph), whereas differences in oviposition rate were analysed with a general linear model with a Poisson error distribution. Multiple comparisons were followed by a sequential Bonferroni correction. A sequential Bonferroni correction was applied to significance values of multiple comparisons. Whenever such correction modifies the conclusion, it is stated in the text.

Analyses in homogeneous environments

We first performed a global model with the fixed factors environment (tomato or pepper), cross (T×T, P×P, T×P and A×A), generation (1 or 2) and their interactions. Because we found no significant effect of the interaction between generation and cross, and a significant interaction between cross and environment, for both traits, we continued the analysis within each environment but grouping both generations. We then performed contrast analyses to address specific questions: (i) Is there local adaptation? (i.e. comparison between trait values of T×T and P×P); (ii) Is A×A comparable to a cross in which only the genetic interactions between

T and P are present? (i.e. comparison between A×A and T×P); (iii) Are interactions costly? (i.e. comparison of A×A and T×P with T×T and P×P, which was carried out either together or separately, depending on whether (i) and (ii) yielded significant results; in case both (i) and (ii) were significant, we performed a Tukey's test instead of contrasts, as this involved comparing all crosses among each other).

Analysis in alternating environments

To test whether spider mites adapt to an alternating environment, we compared the performance of all populations when tested in homogeneous vs. heterogeneous environments. This model included a factor cross (T×T, P×P, T×P and A×A), a factor environment (alternating or homogeneous, see below) and the interaction between these factors. Whenever the interaction was significant, we tested whether the performance of each cross differed significantly between environments by performing an analysis of performance per cross with environment as a factor.

Alternation within a single generation. We grouped the data from the homogeneous environments (tomato or pepper) into one factor and called it 'homogeneous environment'. Then, we compared the performance of

the populations in that environment to their performance in the alternating environment. Hence, in each environment, each population had experienced both host plants in similar proportions, only in the homogeneous environment each individual experienced either tomato or pepper and in the alternating environment each individual experienced both environments. We used only the data of the homogeneous environments in the first generation (cf. Fig. 1) to ensure a meaningful comparison with the performance in this alternating environment.

Alternation among generations. We grouped into 'homogeneous environment' the data for individuals that were tested in the same environment (either tomato or pepper) during two consecutive generations. In 'heterogeneous environments', we grouped the data of populations that had experienced pepper in the first generation and tomato in the second and populations that had experienced tomato in the first generation and pepper in the second. We then compared the performance of each population in homogeneous vs. heterogeneous environments in the second generation.

Results

Performance in homogeneous environments

Juvenile survival

On pepper, the analysis of juvenile survival revealed a significant effect of cross (Fig. 2a, Cox Model,

$\chi^2_3 = 8.07$, $P = 0.045$) and no significant effect of the generation or its interaction with cross type (Cox Model, $\chi^2_1 = 2.47$, $P = 0.116$ and $\chi^2_3 = 6.59$, $P = 0.086$, respectively). Contrast analysis showed no significant difference between P×P and T×T ($|z| = 0.06$, $P = 0.95$); hence, there was no local adaptation for this trait. Also, no difference was found between A×A and T×P crosses ($|z| = 0.39$, $P = 0.69$); hence, there is no particular effect of evolving in an alternating environment. Individuals from both these crosses had lower survival than those from P×P or T×T ($|z| = 2.26$, $P = 0.024$), but this result is no longer significant after Bonferroni correction.

On tomato, juvenile survival was significantly affected by cross (Fig. 2b, Cox Model, $\chi^2_3 = 15.05$, $P = 0.002$), generation ($\chi^2_1 = 4.35$, $P = 0.037$), but not their interaction ($\chi^2_3 = 3.71$, $P = 0.296$). Individuals from the two generations differ in the maternal environment they are exposed to, as in the first generation, mothers were on bean and in the second they were on tomato. Hence, this result suggests that maternal environments affect survival on tomato in all lines. Contrasts revealed local adaptation, as individuals from T×T crosses had higher survival than those from P×P crosses ($|z| = 2.81$, $P = 0.005$). Again, no difference was found between A×A and T×P crosses ($|z| = 0.07$, $P = 0.43$). The juvenile survival of individuals from both these crosses was significantly higher than that of individuals from P×P crosses ($|z| = 3.77$, $P = 0.0002$), and similar to that of individuals from T×T crosses ($|z| = 0.241$, $P = 0.81$). Therefore, there is no negative interaction between alleles selected in pepper vs. tomato on this trait.

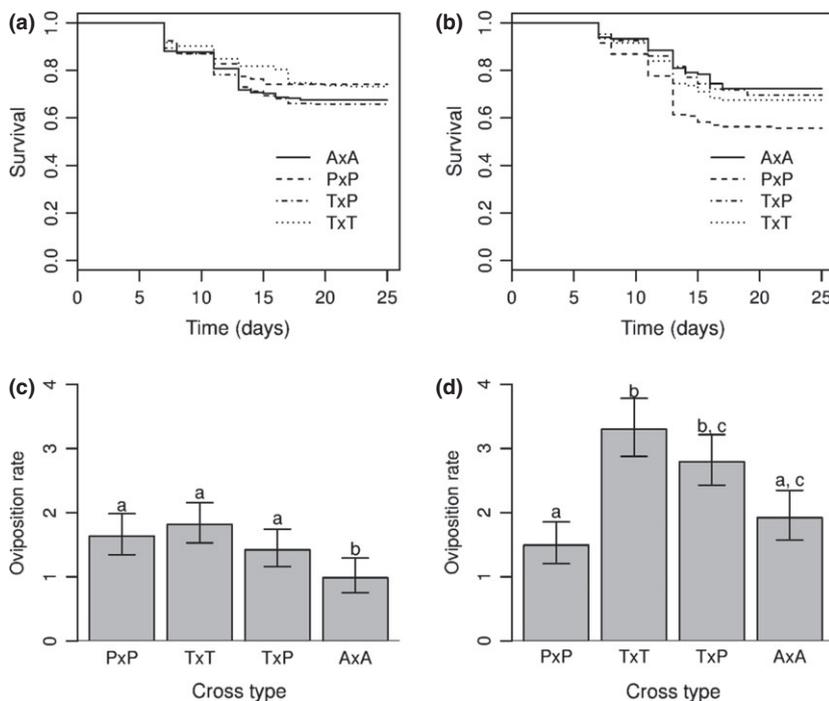


Fig. 2 Life history traits (a, b: juvenile survival; c, d: oviposition rate) of crosses between individuals from populations evolving in an alternating environment (A×A), on pepper (P×P), tomato (T×T) or of crosses between individuals from pepper or tomato populations (T×P), when tested on pepper (a, c) or on tomato (b, d).

Oviposition rate

Analysis of the oviposition rate on pepper revealed a significant effect of the cross (Fig. 2c, $F_{3,244} = 7.97$, $P < 0.0001$) but not of generation ($F_{1,244} = 1.23$, $P = 0.727$) or its interaction with cross ($F_{3,244} = 2.35$, $P = 0.073$). Contrast analysis showed no significant difference between P×P and T×T crosses ($|z| = 1.15$, $P = 0.251$), hence no local adaptation for this trait. A significant difference was found between A×A and T×P crosses ($|z| = 2.57$, $P = 0.011$), with A×A crosses having significantly lower oviposition rate than the homogeneous crosses ($|z| = 3.51$, $P = 0.0005$), which was not the case for T×P crosses ($|z| = 0.44$, $P = 0.662$). This analysis thus revealed costs in A×A crosses which do not emanate from negative genetic interactions among alleles selected in pepper vs. tomato.

On tomato, the oviposition rate was significantly affected by cross (Fig. 2d, $F_{3,288} = 16.97$, $P < 0.0001$) but not by generation ($F_{1,288} = 3.43$, $P = 0.065$) or its interaction with cross ($F_{3,288} = 0.82$, $P = 0.485$). Individuals from T×T crosses had higher oviposition rate than those from P×P crosses (Tukey's *post hoc* test, $|z| = 4.94$, $P < 0.001$), indicating local adaptation for this trait. Oviposition rate of A×A crosses was not significantly different from that of P×P crosses ($|z| = 1.78$, $P = 0.276$), but it was significantly lower than that of T×T crosses ($|z| = 3.41$, $P = 0.003$). In contrast, T×P crosses had similar oviposition rate to T×T crosses ($|z| = 1.24$, $P = 0.595$), and significantly higher than that of P×P crosses ($|z| = 4.07$, $P < 0.001$). However, A×A crosses had only marginally lower oviposition rate than

T×P crosses ($|z| = 2.34$, $P = 0.075$). This indicates that A×A and T×P crosses have oviposition rates intermediate to those of P×P and T×T.

Performance in alternating environments

Juvenile survival

When alternation occurred within a single generation, all crosses had significantly higher survival when tested in an alternating environment, as compared to homogeneous environments (Fig. 3a, Cox Model, $\chi^2_1 = 45.66$, $P < 0.0001$). However, no significant effect of crosses or their interaction with environments was found ($\chi^2_3 = 2.53$, $P = 0.469$ and $\chi^2_3 = 4.35$, $P = 0.226$, respectively).

When alternation was among generations, we found a significant effect of the interaction between cross and environment on juvenile survival (Fig. 3b, Cox Model, $\chi^2_3 = 9.08$, $P = 0.028$, respectively). An analysis per cross revealed that A×A, P×P and T×T crosses had similar survival in both environments ($\chi^2_1 = 0.48$, $P = 0.49$; $\chi^2_1 = 0.04$, $P = 0.85$ and $\chi^2_1 = 2.59$, $P = 0.108$, respectively). T×P crosses, in contrast, had higher survival in the alternating environment ($\chi^2_1 = 6.27$, $P = 0.012$). This difference, however, is not significant after Bonferroni correction.

Oviposition rate

When alternation occurred within a single generation, a significant interaction between cross and environment was found (Fig. 3c, $F_{3,364} = 3.5$, $P = 0.016$).

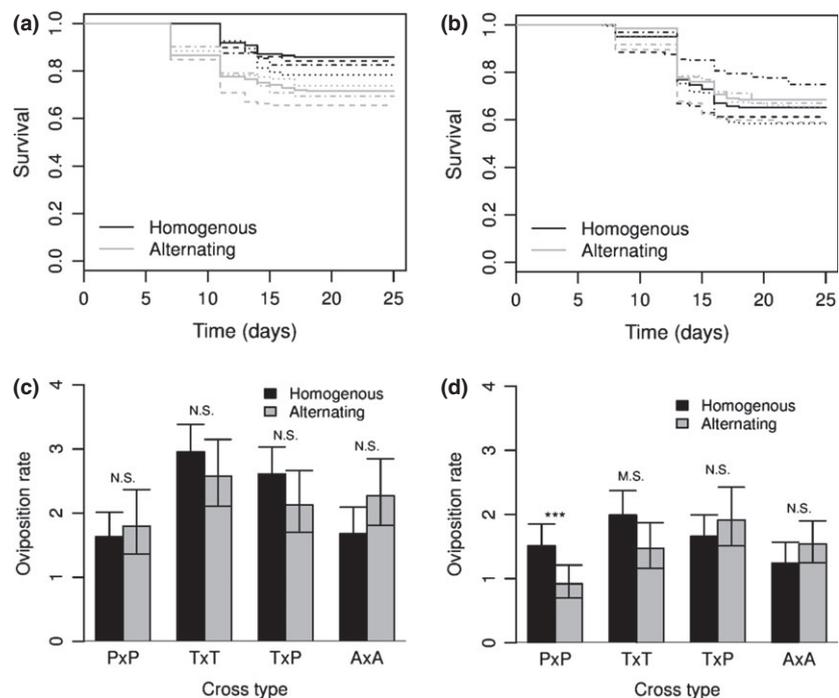


Fig. 3 Life history traits (a, b: juvenile survival; c, d: oviposition rate) of crosses between individuals from populations evolving in an alternating environment (A×A), on pepper (P×P), tomato (T×T) or of crosses between individuals from pepper or tomato populations (T×P), when tested on a homogeneous environment or on an environment that alternates within (a, c) or among generations (b, d).

Analyses per cross revealed that A×A crosses had higher oviposition rate in the alternating than in the constant environment ($F_{1,78} = 14.35$, $P = 0.04$), although this difference was not significant after Bonferroni correction. No difference was found for P×P, T×T or T×P crosses ($F_{1,80} = 10.56$, $P = 0.453$; $F_{1,106} = 11.69$, $P = 0.197$ and $F_{1,100} = 13.55$, $P = 0.062$, respectively).

When alternation occurred among generations, a significant interaction between cross and environment was found (Fig. 3d, $F_{3,435} = 5.88$, $P = 0.0006$). The oviposition rate of the P×P cross was significantly lower in the alternating than in the homogeneous environment ($F_{1,116} = 15.56$, $P = 0.0001$). The same tendency was found for the T×T cross ($F_{1,106} = 3.97$, $P = 0.049$), although this result was not significant after Bonferroni correction. No significant difference was found for the other crosses ($F_{1,111} = 2.54$, $P = 0.11$ and $F_{1,102} = 1.07$, $P = 0.304$ for the A×A and the T×P crosses, respectively).

Discussion

In this study, we performed experimental evolution of spider mites on tomato, pepper or an alternation of these host plants. We tested whether evolving in alternation resulted in specific adaptations to that environment. To test whether such adaptation relied on a mixture of alleles selected during adaptation to each of the homogeneous environments, we also analysed the performance of a cross between populations evolving in each of the homogeneous environments (T×P crosses). To make this cross comparable with the other populations, we also crossed two populations evolving in the alternating, tomato or pepper environments (A×A, T×T and P×P crosses, respectively). We also tested whether evolving in a fluctuating environment entailed a cost in homogeneous environments. We found local adaptation for populations evolving on tomato (T×T crosses), as they had higher juvenile survival and oviposition rate than populations evolving on pepper (P×P crosses). No local adaptation was found for the pepper populations on pepper. Moreover, evolving in an alternating environment entailed a cost in terms of oviposition rate in each of the homogeneous environments, as A×A crosses had lower performance than crosses from populations evolving in these homogeneous environments. This cost cannot be solely attributed to genetic interactions, as T×P crosses did not show this lower performance. Finally, A×A crosses had a slight advantage in oviposition rate when tested in an alternating environment.

In a previous study using these populations, we found that, at generation 25, populations evolving on tomato and those evolving on pepper both showed a local adaptation pattern in oviposition rate, but not in juvenile survival (Magalhães *et al.*, 2009). Here, at

generation 45, we found local adaptation in crosses involving these same populations evolving on tomato for both traits, and no such pattern in P×P crosses on pepper. Moreover, the absolute value of the oviposition rate on tomato of populations evolving on tomato increased by 50% relative to that of generation 25, whereas that of pepper populations did not change. Our aim in this study was not to detect adaptation in these lines; hence, we did not include control lines in our experiments (i.e. lines evolving in the ancestral host, cucumber). Thus, this pattern could be explained by differences in environmental conditions, or by heterosis. Still, the difference obtained between T×T and P×P patterns suggests that tomato populations continued to evolve, whereas those on pepper did not.

A test for a local adaptation pattern is also necessary for our interpretation on the performance of A×A and T×P crosses in homogeneous environments. Indeed, local adaptation implies the occurrence of Genotype*Environment interactions. In the absence of Genotype*Genotype interactions, we expect the performance of T×P crosses to be intermediate to that of P×P and T×T crosses. Whenever the alleles selected in one environment are dominant relative to those selected in the other, we expect the performance of T×P crosses to become more similar to that of a cross between lines selected in that environment. Genetic interactions among alleles selected in each environment (i.e. epistasis) need to be invoked if the performance of T×P cross is higher than the locally adapted populations, or lower than the nonlocally adapted populations. Again, a difference between trait values of A×A and T×P crosses indicates the effects of alleles selected in the alternating environment on the performance in homogeneous environments.

On tomato, the survival of A×A, T×P and T×T crosses was similar, and higher than that of P×P crosses. This suggests that (i) alleles selected in tomato are dominant, (ii) there are no negative interactions between alleles selected on pepper or tomato, and (iii) selection in an alternating environment does not affect survival on tomato. For the oviposition rate on tomato, the results are compatible with having codominant alleles, with no particular cost for A×A. On pepper, the absence of local adaptation simplifies the interpretation. Indeed, both A×A and T×P crosses had lower survival than T×T and P×P crosses, suggesting a trade-off between alleles selected in each environment. A×A crosses had lower oviposition rate than the other crosses, suggesting that, for this trait, the costs are not attributable to genetic interactions among alleles selected in tomato or pepper, but rather to a cost of evolving in an alternating environment.

We found no evidence that lines evolving in an alternating environment became specifically adapted to an alternating environment. Indeed, trait values of A×A crosses were similar when tested in an alternating or a

constant environment. This conclusion holds irrespective of whether this alternation occurred within or between generations. Hence, lines selected in an alternating environment are not specifically adapted to the temporal scale at which alternation occurs. The same absence of adaptation to the scale of variation (within vs. between generations) was found by Kassen & Bell (1998). However, we find that P×P and T×T crosses had lower oviposition rates in alternating than in constant environments. This suggests that evolving in a constant environment entails costs if the environment fluctuates. This cost is overcome in populations evolving in such fluctuating environment.

Overall, our data suggest no particular adaptation of lines evolving in an alternation environment to that environment. In contrast, these populations pay a cost when tested on pepper. Conversely, lines evolving in constant environments perform worse in fluctuating than in constant environments. This hints at a possible adaptation of each population to their environment. The lack of a clear adaptation pattern in populations evolving in the alternating environment may pertain to the low number of generations in the experiment. We found that 15 generations were sufficient to detect adaptation in the homogeneous environment (Magalhães *et al.*, 2007a). However, the response we set out to detect here is a much more subtle one than adapting to a novel host plant. Possibly, adapting to the fluctuation in an environment is a slower process than that of adapting to a host plant. Moreover, we here tested a single cross within each selection regime, which clearly represents a lower power than testing each of the five lines composing each selection regime individually. The response could also be stronger if environments were more antagonistic. Indeed, we have previously shown that there was a positive correlation between adaptation to tomato and to pepper (Magalhães *et al.*, 2009). This may account for the fact that no particular adaptation to the switching between environments that are relatively similar is necessary.

Despite the weak response, our study does suggest that experimental evolution in temporally varying environments can be exploited in order to understand whether specific adaptations to a fluctuating environment have evolved. If this is the case, one may then test whether differences in gene expression profiles account for this adaptation, thereby validating comparative studies in variable vs. constant environments (Suiter & Dean, 2005; Gracey *et al.*, 2008; Kvitek & Sherlock, 2013).

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