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Rapid host-plant adaptation in the herbivorous spider mite *Tetranychus urticae* occurs at low cost

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The herbivorous spider mite *Tetranychus urticae* is a generalist world crop pest. Early evidence for host races, its fully sequenced genome resolved to the chromosome level, and the development of other molecular tools in this species suggest that this arthropod can be a good model to address host plant adaptation and early stages of speciation. Here, we evaluate this possibility by reviewing recent studies of host-plant adaptation in *T. urticae*. We find that evidence for costs of adaptation are relatively scarce and that studies involving molecular-genetics and genomics are mostly disconnected from those with phenotypic tests. Still, with the ongoing development of genetic and genomic tools for this species, *T. urticae* is becoming an attractive model to understand the molecular basis of host-plant adaptation.

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Introduction

The interaction between herbivores and plants is a textbook example for the evolution of ecological interactions [1,2]. Given the differential selection pressures that host plants exert upon herbivores, these may evolve to become specialized on a particular subset of hosts [3]. Such ecological specialization is expected when local adaptation to different hosts occurs through divergent selection, that is when phenotypes favored on a given set of hosts are selected against in others [4,5]. Moreover, organisms preferring particular host plants will be more often exposed to them, fostering evolution of specialization [6]. This results in trade-offs and fitness costs when the ability to feed and reproduce on a particular host leads to poorer performance on other host plants [4]. The high prevalence of specialist species among herbivorous arthropods [7] is in agreement with this hypothesis. Indeed, it is estimated that over 25% of all multicellular species are plant-feeding arthropods [2], and this extreme diversity is thought to result from increased opportunities for ecological speciation via host shifts [8,9].

Generalist plant-feeding arthropods challenge the prediction that host-plant adaptation leads to ecological specialization. Indeed, generalist species may be composed of individuals that are able to thrive on many hosts. Alternatively, they may represent a compilation of host races, that is genetically distinct populations specialized on particular host plants [10,11]. The occurrence of such races, especially among sympatric populations, suggests that costs of adaptation also occur within generalist species, which may be experiencing early steps of speciation.

Spider mites (Acari: Tetranychidae) are a family of haplodiploid herbivorous mite species that colonize a highly variable number of host plants [12]. A recent study suggests that most limitations in host range in this group stem from the lack of geographic co-occurrence of spider-mite and plant species, rather than from phylogenetic distance between host plants [13]. Additionally, equivocal evidence for the occurrence of host races within several species of this group has been documented [14••].

Tetranychus urticae is an extreme generalist spider-mite crop pest, colonizing more than 900 host plant species [15]. Given its high economic impact, understanding how it adapts to different host plants is highly relevant from an applied perspective [16••]. Most studies on host races in the Acari have been done with this species, providing mixed evidence for their occurrence [14••]. Moreover, experimental evolution studies under controlled laboratory conditions suggest that rapid adaptation to novel host plants occurs often, and that this process does not entail high costs [17]. The sequencing of the genome of this species [16••] opened the door to understand these processes at the genomic level. Since then, a great effort has been put into characterizing the genetic basis of the response to different host plants and to pesticides (e.g. [18,19••]). The evolution of pesticide resistance in *T. urticae* and its potential connection with wide host range has been reviewed recently [20]. Here, we review

our current knowledge on the evolution of host use in *T. urticae* based on studies from field populations and experimental evolution.

Evidence for host-plant driven divergence in field populations of *T. urticae*

In the field, *T. urticae* often occurs on several host plants even within small spatial scales, making it an excellent system to study early stages of ecological specialization [14••]. Ecological specialization is expected to reduce gene flow between locally adapted populations. This may lead to increased genetic differentiation and accumulation of incompatibilities between populations on different host plants, potentially forming host races [11]. For spider mites collected from the field, host races can be detected by measuring (a) genetic differentiation using genetic markers; (b) reproductive incompatibilities based on inter-population crosses; or (c) performance on different host plants based on common garden experiments.

Results from field studies reveal inconsistent evidence of specialization (Table 1). This could be explained by the fact that measures of ecological specialization can be confounded with isolation by distance (IBD), that is the increased genetic differentiation with geographic distance due to limited dispersal [21]. Thus, spider-mites from different hosts could be genetically distinct simply because they were collected from distant places. This effect is minimized by collecting individuals from different hosts in the same geographic area (e.g. [22]), or by accounting for IBD when evaluating genetic differences between populations [21].

Evidence for fitness costs has come from reports of partial incompatible crosses between *T. urticae* populations from different hosts collected in the same location [23,24]. Early genetic studies based on allozymes reported higher differentiation between hosts [25,26], but when accounting for IBD by comparing differentiation patterns at small scales and across wider areas, geographic distance seemed to explain most of the genetic differentiation patterns [22,25,27]. More recent studies based on mitochondrial DNA (mtDNA), microsatellites and other nuclear markers confirm that population structure reflects mostly limitations to dispersal across space rather than host use [28–31], despite cases of host-related differentiation (e.g. [32]).

The evolution of specialization depends on environmental and ecological factors that vary across space (e.g. landscape structure, endosymbionts, parasites, predators), which affect demography and metapopulation dynamics. Moreover, seasonal changes in host availability can prevent specialization (e.g. [33]). Interactions between these factors are complex and their impact may vary, even within a single system. For instance, genetic differences between *T. urticae* populations from rosebay and other hosts (e.g. citrus, tomato) were found in the Western but not in the Eastern Mediterranean region [25].

So far, studies of field populations have not made use of the well-annotated reference genome of *T. urticae* [16••]. This is unfortunate, as genomic studies of field *T. urticae* populations hold the promise of uncovering genes responsible for host-adaptation, while accounting for the effects of isolation by distance and demographic history [21,34], as well as symbionts and cryptic species [35], as recently done to study ecological speciation in other phytophagous arthropods (e.g. *Rhagoletis* flies [36]).

Evidence for rapid host-plant adaptation from experimental evolution studies

Experimental evolution is a methodology that follows the phenotypic and/or genotypic changes of populations placed in different environments over multiple generations [37]. Its experimental power relies on (a) knowledge of the ancestral state of populations, (b) the ability to manipulate environmental variables under controlled settings and (c) having replicates at the population level (Figure 1). This allows causality to be inferred between the environmental variable being manipulated and the evolutionary change observed in populations [37,38]. Additionally, in experimental evolution designs, traits can be assessed in multiple environments, allowing correlated responses to selection to be assessed (e.g. costs of adaptation). The disadvantage of such set-ups are that they do not necessarily encompass all the relevant selection pressures that populations are exposed to in natural settings.

We found a total of 14 experimental evolution studies of host use in *T. urticae*, some of which were reported across several articles (Table 2). Most studies revealed rapid adaptation to novel hosts (usually tomato, but also broccoli, cucumber or cotton plants) with few costs of performance on ancestral hosts (mostly bean plants), while some studies showed no evidence of adaptation. However, several requirements must be met to draw such conclusions (Figure 2). First, rapid evolution rests upon the existence of genetic variation in the initial population. Although no study has explicitly measured the genetic variation upon collection in the field, some studies report the number of individuals used to form base populations in the laboratory, which can serve as an indication of such variation (Table 2). Second, and for the same reason, experimentally evolving populations should be initiated from a high number of individuals. Otherwise, genetic drift due to small populations and/or founder events can lead to loss of genetic diversity and mask the effects of natural selection (Figure 2). Third, having replicates at the population level ($n > 1$) is essential to disentangle adaptation by natural selection from drift (Figure 2, [37]). Finally, it is only possible to disentangle genetic from

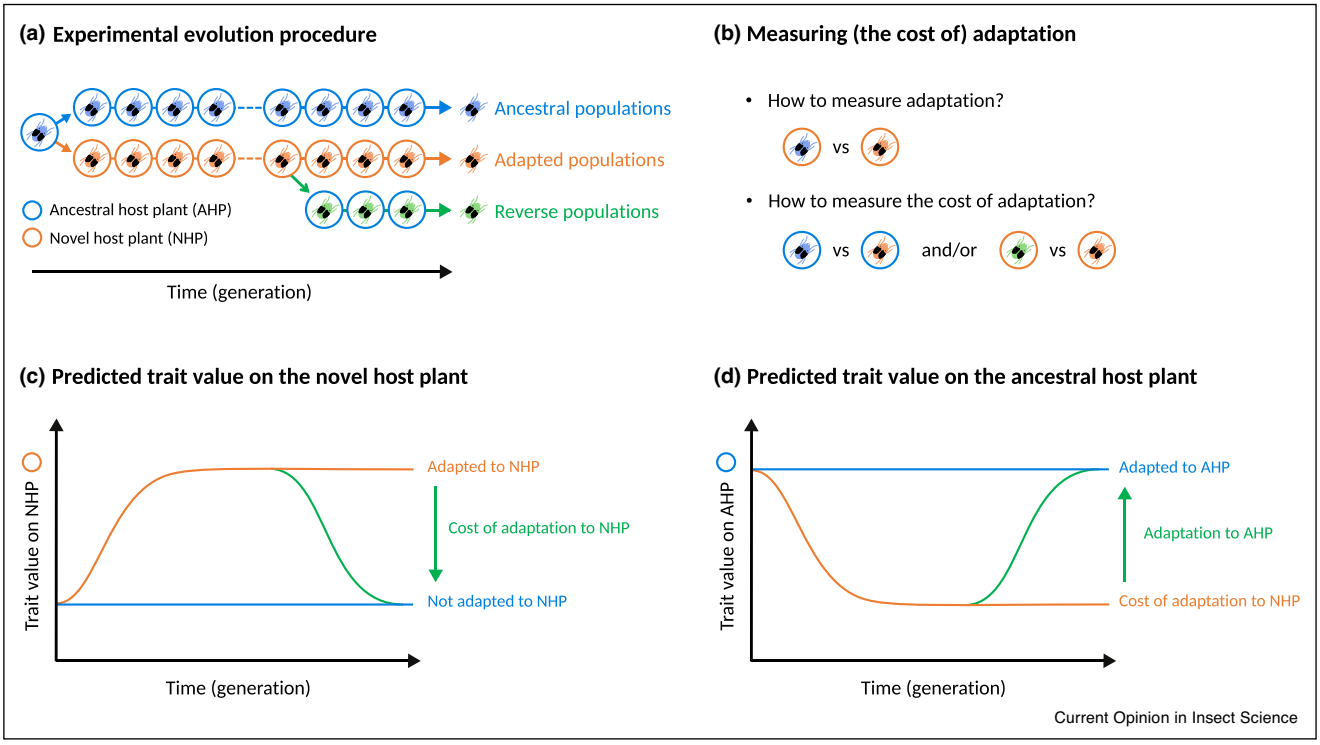
Table 1

Studies addressing host-plant specialization based on field populations of *T. urticae*.

Host plants	Measures of fitness costs[a]			Molecular makers		Account for symbiont infection?	Account for isolation by distance?	Geography explains patterns?	Host speciali-zation?	Refs.
	Perf. in the lab	Reprod. Incomp.	Genetic diff.	Type of marker	# loci					
Chrysanthemum, rose		✓		n/a	n/a	No	Yes	n/a	Yes	[23]
Rose, gerbera, ivy, croton, rhododendron		✓		n/a	n/a	No	No	No	No	[24]
Tomato, cucumber		✓	✓	Allozymes	1	No	No	n/a	Yes	[52]
Lemon, citron, lichwort, pumpkin, tomato, okra			✓	Allozymes	4	No	Yes	Yes	No	[27]
Rose, carnation, lemon, amaranth, eggplant, tomato, pumpkin, cucumber, bean, setaria, watermelon, melon, malva			✓	Allozymes	4	No	Yes	Yes	Yes/No[d]	[26]
Rose, field bindweed, European black nightshade, carnation, violet, red-root amaranth, eggplant, tomato			✓	Allozymes	4	No	Yes	Yes	No	[22]
Common spindle, common honeysuckle			✓	AFLP	53	No	Yes	n/a	Yes	[53]
Rosebay compared to several other plants		✓[b]	✓	Allozymes	4	Yes[c]	No	Yes	Yes/No[e]	[25]
Eggplant, french bean			✓	Microsatellite	5	No	Yes	n/a	No	[29]
rosebay, lemon, tomato	✓			n/a	n/a	No	No	No	Yes/No[f]	[39••]
Lemon, annual mercury		✓		n/a	n/a	No	Yes	n/a	Yes	[54]
Clementine, tall fescue			✓	Microsatellite	18	No	Yes	n/a	Yes	[32]

– no effect found.
✓ – significant difference found.
– numbers.
n/a – not applicable.
[a] Measures of fitness costs in field populations vary across studies and include: quantification of reproductive incompatibilities (*Reprod. Incomp.*) with inter-population crosses, quantification of genetic differentiation (*Genetic diff.*) with molecular markers, and assessing performance in controlled laboratory conditions *(Perf. in the lab)*.
[b] Crosses between rosebay from different locations and a laboratory strain.
[c] Tested for *Wolbachia* infection in inter-population crosses.
[d] Yes for populations collected on lemon trees in open-field habitats only. No for all populations sampled in greenhouses.
[e] Yes on rosebay in Western Mediterranean region, no in Eastern Mediterranean region.
[f] Yes for populations collected on tomato and lemon, no for populations collected on rosebay.

Figure 1



Set-up to unravel adaptation and its potential costs using experimental evolution. **(a)** In experimental evolution studies, adaptation (and the cost of adaptation) is tested by transferring and allowing populations to evolve on a novel host plant (in orange) from populations that are maintained on a control host plant ('ancestral populations' in blue). 'Reverse populations' (in green), derived from those already adapted to the novel host (in orange), can also be placed back in the ancestral host plant. **(b)** Adaptation is then measured by comparing trait values (e.g. fecundity, survival, acceptance) of the adapted populations with those of the ancestral populations on the novel host plant, while the cost of adaptation is measured by comparing trait values of adapted and ancestral populations on the ancestral host plant, and/or by comparing adapted and reverse populations on the novel host plant. **(c)** The assumption here is that populations adapted to the novel host reach a plateau and do not continue increasing their performance on the novel host plant, while the performance of reverse populations decreases with time if such adaptation is costly to maintain (i.e. cost of adaptation). **(d)** If adaptation entails a cost, the performance of the populations evolving in the novel host should decrease in the ancestral host plant, and the cost of adaptation should disappear in the reverse populations while they are re-adapting to the ancestral host.

plastic responses if individuals are placed in a common environment before being tested for adaptation.

Nine out of the 14 studies tested for the occurrence of costs of adaptation. Out of these, three found a cost. Two of these [39••,40] show inconsistencies in the different measures used to test for costs: Fry [40] found a cost when using reverse lines but not when testing mite performance in the ancestral environment, whereas Fellous *et al.* [39••] found a cost of rosebay adapted mites when tested on tomato that was not recapitulated in tests involving field populations. The third study found that populations evolving on the novel host performed worse than control populations on both the novel and the ancestral host [41]. These results are more consistent with an overall poor performance of those populations, possibly due to inbreeding depression instead of a cost of adaptation, as those populations were initiated with two individuals only. Therefore, overall, evidence of costs of adaptation are, at most, equivocal.

Only six studies so far have exploited molecular-genetic methods to unravel host-plant adaptation in *T. urticae* [42–44,45•], and of those six, only two combine such techniques with phenotypic measurements [19••,46•]. Although some studies found a link between the response to host plants and pesticides, most of these studies focused on transcriptome profiles or differential expression of particular genes. Hence, molecular-genetic and genomic approaches have been largely disconnected from phenotypic studies [42–44,45•]. Still, the recent study of Wybouw *et al.* [19••] illustrates the potential of combining experimental evolution with genomic data. Their results suggest that adaptation to new hosts is likely polygenic, involving several genes and phenotypic traits [19••]. Hence, although studies combining genomics and phenotypes are still in their infancy [19••,46•], this will be required for a clear identification of the genetic basis of adaptation to different host plants in *T. urticae*.

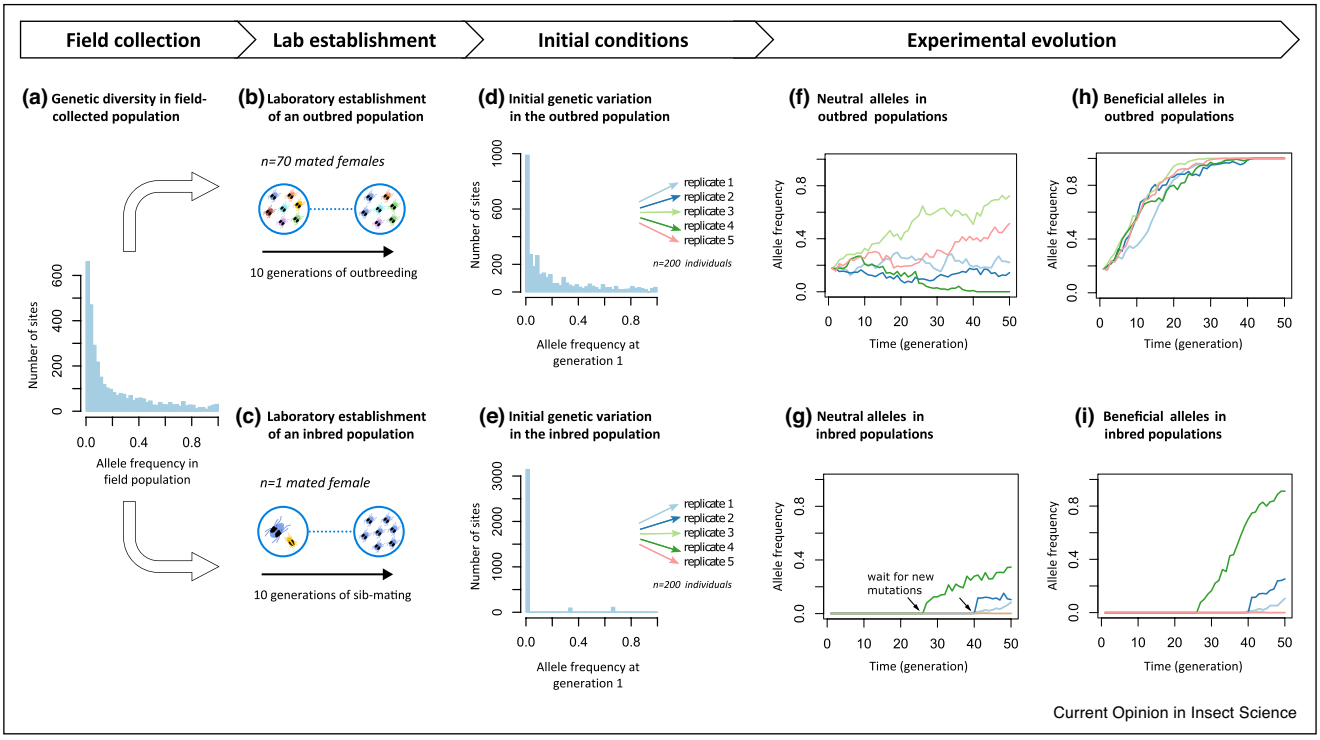
Table 2

Studies addressing the host range of *T. urticae* using experimental evolution.

Field		Laboratory and experimental evolution					Evolved adaptation?	Evolved costs of adaptation?	Molecular biology?	References
Field host	# Initial collected	Rearing host	Novel host plant	Replicate number	# Initial per replicate	Generations				
Peach	200	Bean	Cucumber	1	200	5, 42	Yes	No	n/a	[55]
Corn	1000	Bean	Tomato, broccoli	1	300	14, 50	Yes	Yes	n/a	[40,56,57]
Rose	500[b]	Bean	Bean with mycorrhizal fungi, bean with nematodes	1	>10 000	15	Yes	n/a[c]	n/a	[58]
Tomato, nerium, citrus	5–30	Bean	Tomato, nerium, citrus	4, 2 or 1	20	4–8	Yes	Yes	n/a	[39••]
Cucumber	5000[b]	Cucumber	Tomato	4	220–400	24	Yes	n/a	n/a	[59•]
n/a	n/a	Mexican Cotton	Cotton tree	6 10	2 100	10 6	No No	n/a Yes	n/a n/a	[41]
n/a[a]	1	Bean	Tomato	3	200	30–35	Yes	No	Yes	[46•]
n/a[a]	1	Bean	Tomato	7	3	20	Yes	No	n/a	[60]
n/a[a]	1[b]	Bean	Tomato	15	500	50	Yes	n/a	Yes	[19••]
Cotton, bean, rose, morning glory	>100	Cotton	Cucumber	1	>100	5, 13, 20	Yes	No	n/a	[61]
Cucumber	10 000[b]	Cucumber	Tomato, pepper	5	300	15, 25, 35, 45	Yes	No	n/a	[17,62,63]
Rose	n/a	Bean	Lima, bean, tomato, cotton, burclover	3	250	6	n/a	n/a	Yes	[42]
n/a[a]	1	Bean	Cotton, maize, soy, tomato	3–4	250	5 or 30	n/a	n/a	Yes	[43,44,45•]

– numbers.
n/a – not applicable or no data.
[a] London strain.
[b] transferred from another laboratory.
[c] Probably a cost, but no statistical test performed.

Figure 2



Genetic diversity and experimental evolution sampling design. Maximizing the probability of detecting and quantifying responses to selection in experimental evolution requires a careful experimental design. Here, we illustrate this by simulating a 100 000 bp genetic region in a haplodiploid species with a female biased sex-ratio (70% females), mimicking *T. urticae* populations, using SLIM v2.6 [50]. We assumed that the ancestral population established in the laboratory was sampled from an outbred population of 100 000 individuals **(a)**. Establishment in the laboratory affects the initial genetic diversity upon which selection can act, which depends on whether individuals are kept in an outbreeding population **(b)** or in a small inbreeding population with 10 generations of sib-mating **(c)**. Indeed, following the theoretical predictions that most variants are rare in a stationary population, establishment with a population of $n = 100$ individuals (i.e. 70 mated females (b) retains much of the genetic variation in the ancestral field population **(d)**. In contrast, 10 generations of sib-mating (inbred) as a starting population ($n = 2$; i.e. 1 mated female (c) results in very low initial genetic diversity, with most alleles having a frequency of 0% **(e)**. After this, replicated populations start evolving in the new environment in the laboratory with a size of $n = 200$ individuals ('Experimental evolution' panels). We simulated a case where a random neutral allele with a frequency between 1% and 25% becomes beneficial in the new environment during experimental evolution, with a strong selective coefficient of $s = 0.25$. Also, we assumed a high mutation rate of 1.0×10^{-8} mutations/site/generation and a recombination rate of 1.0×10^{-8}/site/generation. In both cases, performing replicated experiments is crucial to detect selection as changes in allele frequencies and in phenotypic traits across generations are not necessarily evidence for selection. Changes due to genetic drift can be detected as neutral alleles are expected to show different trajectories in different replicate populations **(f)**. In contrast, selected beneficial alleles are expected to show similar evolutionary trajectories in all replicates **(h)**. When starting experimental evolution from a population with little initial diversity (e.g. inbred line), some generations are required until new mutations appear (even with a high mutation rate, as used in our simulations), and different mutations are likely to appear independently in different replicates, making it harder to distinguish genetic drift from selection **(g, i)**. Note that in experimental evolution designs with spider mites (and other arthropods) population sizes and mutation rates are likely lower than in these simulations used to illustrate principles. Hence, new mutations are less likely to occur during the experiment, as opposed to experimental evolution using bacteria or yeast, in which population sizes can be many orders of magnitude larger [51].

Future perspectives

T. urticae is a good model to address the evolution of host range in herbivorous arthropods, because (a) field populations are easy to collect, (b) *T. urticae* populations are amenable to experimental evolution and (c) genomic tools are available. However, progress on several fronts is needed to fully realize the potential of this model system. Using several approaches in the same study is needed to enable a more complete description of the mechanistic basis of specialism/generalism. Indeed, several factors have been suggested to underlie host race formation associated with local adaptation, including differential host chemistry [47] or morphology [48], and the community of natural enemies on different plants [49]. A future challenge will be to investigate whether such factors operate in this system. Second, it will be important to develop genetic tools (e.g. genome editing methods such as CRISPR-Cas9) that allow functional validation of candidate genes arising from genomic studies. Finally, a complementary approach that should be explored is the

investigation of the evolution of host range in related spider mite species. This is attractive as there is wide variation in this family along the generalist to specialist host range spectrum [13].

Conflict of interest statement

Nothing declared.

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