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Citation for published version (APA):
Amsterdam: UvA-IBED

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Adaptive Learning in Arthropods: Spider Mites Learn to Distinguish Food Quality

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Submitted to Behavioral Ecology

Many herbivorous arthropods have been shown to possess learning capabilities, yet fitness effects of learning are largely unknown. In this paper, we test whether two-spotted spider mites (*Tetranychus urticae*) learn to distinguish food quality in choice tests, and whether this results in fitness benefits. Food consisted of cucumber plants with one of three degrees of feeding damage: undamaged (no mites), mildly damaged (infested by a mite strain adapted to tomato) and heavily damaged (infested by a mite strain adapted to cucumber). Mites were subjected to one choice test in a greenhouse and three sequential choice tests on leaf disks. Thereafter, individual mite performance was measured as oviposition rate over four days. In the course of the three small-scale choice tests, preference shifted towards less damaged food. The performance tests showed that learning was adaptive: mites learned to prefer the food type that yielded the higher oviposition rate. Interestingly, innate preferences in the greenhouse tests were close to those shown after learning in the small-scale tests. Given that both strains of mites had not experienced cucumber for several years, we hypothesise that the preference in the greenhouse was due to avoidance of mite odours rather than odours of damaged plants. Through its effect on foraging behavior, adaptive learning may promote the evolution of host plant specialisation in herbivorous arthropods.
For herbivorous arthropods, learning may play an important role in selection of host plants for feeding and/or oviposition (reviews in Bernays 1993, 1995a,b; Papaj and Prokopy 1986, 1989; Szentesi and Jermy 1990). Two distinct kinds of learning can be identified: non-associative and associative learning. Non-associative learning involves a gradual change in response to a (conditioning) stimulus. The response can either wane (habituation) or increase (sensitization). Both types of learning have been observed in herbivorous arthropods, e.g., habituation of Schistocerca gregaria to a feeding deterrent (Szentesi and Bernays 1984) and rejection by Locusta nymphs of unpalatable leaf material at a progressively earlier stage in food selection with experience (Blaney and Simmonds 1985). In associative learning, behaviour is changed due to an unconditioned stimulus which has become associated with a conditioned stimulus. Recently, studies have shown associative learning in several species of moths and one grasshopper species (Daly and Smith 2000; Daly et al. 2001; Dukas and Bernays 2000; Fan et al. 1997; Hartlieb 1996; Hartlieb et al. 1999).

Fitness effects of learning are not well documented (Dukas 1998; Maynard Smith 1987; Papaj and Lewis 1993), and this also holds for herbivorous arthropods (Dukas and Bernays 2000; Papaj and Prokopy 1989). Recently, Egas and Sabelis (Chapter 4) reported on the fitness effects of host-preference learning in the two-spotted spider mite (Tetranychus urticae Koch). Individual mites from two strains were repeatedly given a choice between two host plants, tomato and cucumber, and then subjected to a performance test on each. During the three consecutive choice tests, mites of both strains learned to prefer cucumber over tomato. The performance test showed that cucumber allowed for higher oviposition, higher survival, and faster development than tomato.

In this paper, we test whether the mite strains are also able to distinguish differences in food quality within one host. Specifically, we used cucumber plants with various degrees of feeding damage in tests of preference and performance, to investigate whether experience affects food choice, and whether such changes in food choice result in fitness benefits. When behaviour changes with experience such that reproductive performance is improved, we refer to this process as adaptive learning (Chapter 4; Papaj 1993a; Parmesan et al. 1995). We are not concerned here with mechanisms underlying the change in behaviour with experience (e.g., associative learning, sensitization etc.). Hence, throughout this paper we use the term learning in a general sense.
Material and methods

Mites and plant material
The two-spotted spider mite *Tetranychus urticae* is a polyphagous herbivore recorded from over 900 plant species in 478 genera from 124 plant families (data from Bolland *et al.* 1998) and a well-known pest of many crops (Helle and Sabelis 1985). Mites were collected from tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativa*) from two separate greenhouses (Gotoh *et al.* 1993), and are further referred to as the Tomato strain and the Cucumber strain. Both strains were maintained in our laboratory on bean leaves (*Phaseolus vulgaris*, a benign host for *T. urticae*) for more than two years prior to the experiments. To obtain adult females of equal age for the experiments, egg waves were produced, *i.e.*, eggs laid within an hour by many adult females on detached bean leaves, placed on cotton-wool covered sponges. Thirteen days after the egg waves were initiated egg-laying females were collected for the experiments.

Cucumber plants of the cultivar 'Ventura' were grown in the greenhouse under controlled conditions. To obtain cucumber plants with different degrees of damage, 12 four-week-old plants were each inoculated with 30 adult female spider mites from egg waves of either the Tomato strain or the Cucumber strain, and used for the experiments after three weeks of infestation. Because Tomato strain mites have a lower oviposition rate and development rate on cucumber than Cucumber strain mites (Chapter 4), cucumber plants with Tomato strain mites were only mildly damaged (with $620 \pm 110$ [avg. ± se] adult females per plant at the time of the experiments), whereas the plants with Cucumber strain mites were heavily damaged (with $956 \pm 152$ adult females per plant at the time of the experiments). Six other plants were left undamaged for seven weeks. Hence, we used three types of food: undamaged cucumber plants (denoted C for clean plants), mildly damaged cucumber plants (denoted R for the red Tomato-strain mites) and heavily damaged cucumber plants (denoted G for the green Cucumber-strain mites). For use in the greenhouse release-recapture choice test (see below), the mites were excluded from the first true leaf with a Tanglefoot® sticky substance barrier around the petiole; the same treatment was applied to the clean control plants. The experiments were performed in the greenhouse (greenhouse release-recapture choice test) and in a climate room (multiple-choice preference-performance test), at 23°C, 65% relative humidity, and 16:8 h light:dark period.
Greenhouse release-recapture choice test
To measure food preference in pairwise comparisons over a long distance, we used the set-up as detailed in Pallini et al. (1997). Six cucumber plants were arranged in a circle (Ø 80 cm), in a soil-filled plastic tray. Care was taken to level the soil inside and outside the plant pots. For each of two treatments, three plants were alternated in the circle, so that every test plant had two neighbours of the other treatment. The first true leaf of each plant, which was kept clean of mites with a Tanglefoot© barrier around the petiole, touched the soil with approximately the same contact surface and the same distance to the center of the circle. Mites from the egg wave (200 individuals) were starved for one hour in a Petri dish (Ø 8 cm) and then released from the Petri dish in the centre of the plant circle. As wind speed was too low to allow for wind dispersal, the spider mites could only disperse by walking over the soil. After 24 hours, the numbers of mites on the soil-touching leaf of all plants were counted. Because mites seldom leave a plant in this set-up, let alone arrive at a different plant within a day (Chapter 7), the numbers of mites found on the plants are not affected by differential arrestment on plants, but are a measure of the relative long-distance attractiveness of the plants.

With each of the two mite strains, three experiments were performed to test all three possible pairwise comparisons of the three plant treatments. In all six experiments, recaptured mites were collected from the plants, forming two groups according to plant treatment. Mites from these groups as well as the plants from their choice experiment were subsequently used for the small-scale multiple-choice preference-performance test.

Multiple-choice preference-performance test
To measure small-scale food preference of mites, individual choice arenas were made by putting together two leaf disk halves (each of a different treatment; arena Ø 1.5 cm), with an insect pin in the center. Mites, webbing and mite excretions were removed from the leaves by brushing off and washing with water (clean leaves were given the same treatment). To prevent leaf material from drying out, the arenas were placed on sponges covered with wet cotton-wool. Adult females were put on top of the pin with a fine brush. Pilot experiments with this set-up showed that the mites readily descend from the pin and inspect both leaf disk halves (by walking and occasionally probing). Generally, mites settle at one site within approximately one hour and do not change leaf halves for the next 24 hours. This is because they invest time and energy in colonising a site (Helle and Sabelis 1985).
In the choice experiments, adult females were placed on top of the pins and the leaf disk half initially colonised was scored. After 24 hours, the position of the mites was scored again, as well as the number of eggs per leaf disk half. Although in the vast majority of trials eggs were only found on one disk half, in <10% of the trials both disk halves contained eggs, in which case it was always observed that the mites had switched to the other food source since the start of the trial. We defined the choice made by the mites as the food source, corresponding to the disk half on which most eggs were found. The individual mites were then transferred to a fresh arena for a second and third choice test, and the same records were made. The few females that made no choice on one or more days were excluded from the rest of the experiment, as well as from the analysis of preference.

After the three choice experiments, the females were transferred to leaf disks (Ø 1.5 cm) of either type of food experienced in the choice tests. Care was taken to equally distribute females of similar choice history over the two types of food. After four days, the number of eggs was counted to calculate oviposition rate.

Results

Greenhouse choice test
The fractions of mites recaptured in the three choice experiments were comparable between the two mite strains (Fig 1). The mites generally preferred the lesser-damaged plants. In choice experiments with clean vs. infested cucumber plants, the mites tended to avoid plants infested with mites from the same strain relatively more than mites from the other strain did (Fig 1; compare left and right panel for both choice tests with a white bar). On the other hand, in choice experiments with two different types of infested cucumber, the mites tended to bias their preferences towards plants infested with mites from the same strain.

Multiple-choice tests
In all six experiments, the small-scale choices of individual females were not different between the two groups of individuals obtained per long-distance choice test (G-tests yielded p>0.2 in all cases). In four experiments, there was no clear preference in the first choice test (Fig 2). The Tomato strain showed a preference for mildly damaged cucumber (R) over undamaged (C; Fig 2a) and the Cucumber strain initially preferred heavily damaged cucumber (G) over R (Fig 2c). In all six experiments, though, the fraction of mites on the more damaged type of food decreased
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Fig 1 Fractions of mites on clean cucumber plants (white bars), cucumber infested by the Tomato strain (grey bars) or by the Cucumber strain (dark bars) in the three two-choice greenhouse tests for both the Tomato strain and the Cucumber strain.

over the choice tests (Fig 2a-c; 3-D contingency table $\chi^2$ test [Zar 1999]: Tomato strain C vs. G $G_6=37.0$, $p<0.001$; C vs. R $G_6=69.9$, $p<0.001$; G vs. R $G_6=20.5$, $p<0.005$; Cucumber strain C vs. G $G_6=50.7$, $p<0.001$; C vs. R $G_6=34.5$, $p<0.001$; G vs. R $G_6=28.9$, $p<0.001$).

To investigate how feeding experience affected the subsequent choice, we calculated the fraction of mites changing food choice from one test to the next. In both the second and the third choice test between e.g., G and C, this yields a fraction of mites that first preferred G and then C (G→C), and a fraction of mites that first preferred C and then G (G→C). Comparing the fractions switching in the third test with those in the second, is only justified if the third choice is independent of the first choice (mutual independence in a 3-D contingency table; Zar 1999). This appeared to be true for all six experiments (3-D contingency table $\chi^2$ tests yielded $p>0.1$ in all cases; Fig 3 a-f, right-hand panels).

For the Tomato strain in the undamaged vs. heavily damaged (C vs. G) tests, the results of these calculations show that most mites switched to undamaged cucumber after having experienced heavily damaged cucumber, but hardly any mite switched from undamaged to heavily damaged cucumber (Fig 3a). In the undamaged vs. mildly damaged (C vs. R) tests, few mites switched from undamaged to mildly damaged cucumber, but, when having experienced mildly damaged cucumber, mites were indif-
Fig 2 The preferences of mites in the three subsequent choice tests for both mite strains. a-b: Fractions of mites choosing mildly damaged cucumber (dashed lines and squares) or heavily damaged cucumber (solid lines and circles) instead of undamaged cucumber; a) Tomato strain, and b) Cucumber strain. c: Fractions of mites choosing heavily damaged cucumber instead of mildly damaged cucumber (dashed line and triangles: Tomato strain; solid line and diamonds: Cucumber strain).
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**Tomato strain**

- Fraction of mites switching food type in subsequent choices.
- a-c: Tomato strain, choosing a) between undamaged cucumber (C) and heavily damaged cucumber (G), b) between C and mildly damaged cucumber (R), and c) between G and R.
- d-f (overleaf): Cucumber strain, choosing d) between C and G, e) between C and R, and f) between G and R.
- In the left-hand panels, the fractions making different choices in two subsequent choice tests are shown. White bars: mites switching food type in the second choice test; dark bars: mites switching food type in the third choice test (e.g., G→C: first choosing heavily damaged cucumber, then undamaged cucumber; C→G: vice versa).
- In the right-hand panels, the fractions of individuals switching in the third choice test are shown for all combinations of the previous two choices (e.g., GG→C is the fraction switching to undamaged cucumber after choosing heavily damaged cucumber twice).
- Numbers of individuals are indicated above the bars, above the graph.

**Fig 3** Fractions of mites switching food type in subsequent choices. a-c: Tomato strain, choosing a) between undamaged cucumber (C) and heavily damaged cucumber (G), b) between C and mildly damaged cucumber (R), and c) between G and R. d-f (overleaf): Cucumber strain, choosing d) between C and G, e) between C and R, and f) between G and R. In the left-hand panels, the fractions making different choices in two subsequent choice tests are shown. White bars: mites switching food type in the second choice test; dark bars: mites switching food type in the third choice test (e.g., G→C: first choosing heavily damaged cucumber, then undamaged cucumber; C→G: vice versa). In the right-hand panels, the fractions of individuals switching in the third choice test are shown for all combinations of the previous two choices (e.g., GG→C is the fraction switching to undamaged cucumber after choosing heavily damaged cucumber twice). Numbers of individuals are indicated above the bars, above the graph.
ferent in the next choice test (Fig 3b). In tests with the two types of damaged food, almost all mites switched from heavily damaged to mildly damaged cucumber, but not vice versa (Fig 3c). For the Cucumber strain, mites switched from heavily damaged to undamaged cucumber but not from undamaged to heavily damaged cucumber (Fig 3d). Mites also switched from mildly damaged to undamaged cucumber but less so from undamaged to mildly damaged cucumber (Fig 3e), and they were fairly indifferent to mildly damaged vs. heavily damaged cucumber (Fig 3f).

Performance
Oviposition rate was independent of previous choice history (Kruskal-Wallis tests yielded $p>0.1$ in all cases). For Tomato-strain mites (Fig 4a), oviposition rate was higher on undamaged than on heavily damaged cucumber (ANOVA: $F_{1,17}=15.4, p<0.001$), higher on undamaged than on mildly damaged cucumber (ANOVA: $F_{1,28}=14.0, p<0.001$), but not significantly different on mildly damaged and heavily damaged cucumber (ANOVA: $F_{1,28}=2.44, p=0.130$). For Cucumber-strain mites (Fig 4b), oviposition rate was higher on undamaged than on heavily damaged cucumber (ANOVA: $F_{1,28}=8.25, p=0.008$), but not significantly different on undamaged and mildly damaged cucumber (ANOVA: $F_{1,35}=1.70, p=0.201$), and on mildly damaged and heavily damaged cucumber (ANOVA: $F_{1,38}=2.88, p=0.098$).

Discussion
In the greenhouse choice tests, mites tended to avoid the more damaged food plants, and this effect was stronger when the damaged plants were infested by conspecifics (Fig 1). However, in the first small-scale choice test, the same mites generally exhibited no preference, or (in two cases) preferred the more damaged (and conspecifically infested) food type. There are two possible, not mutually exclusive explanations for these differences. First, they could be due to odour sources in the greenhouse test that were absent in the leaf disk test (e.g., mite odours indicating the presence of competitors on a plant). Second, they could be due to the difference in scale. In the greenhouse, mites cannot taste and inspect the food until they have reached a plant. Hence their choice is based on other cues, e.g., odours. Moreover, once they have found a plant, mites hardly ever decide to leave that plant again in this set-up (Chapter 7). In the small-scale choice test, in contrast, mites can and do inspect and taste both food types before settling on one of them. The differences could not be attributed to experience of the food types in the greenhouse test affecting the
Fig 4 Average oviposition rates (± 1 s.e.) of a) the Tomato strain and b) the Cucumber strain on undamaged cucumber (C; white bars), mildly damaged cucumber (R; light gray bars) or heavily damaged cucumber (G; dark gray bars). Numbers of individuals are indicated above the bars, above the graph; n.s.: not significant, **: p<0.01; ***: p<0.001.

choice in the small-scale test. First, all leaves for recapture were not damaged (although the food quality could have differed due to systemic direct defense of the plant). Second, in all six experiments the small-scale choices of individual females were not dependent on the choice made in the greenhouse test.

In the course of the three small-scale choice tests, preference shifted towards the less damaged food (Fig 2), as manifested by (1) a larger than expected fraction of mites switching from the more damaged food to the less damaged food (e.g., from heavily damaged (G) to undamaged cucum-
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number (C), G→C) and (2) a smaller than expected fraction switching from the less damaged food to the more damaged food (e.g., from C to G, C→G; see Fig 3a,d). This provides compelling evidence for a learned preference, where mites learn to avoid the more damaged food and to prefer the less damaged food. In the two tests where mites did show a preference in the first choice test (the Tomato strain in the undamaged vs. mildly damaged (C vs. R) test (Fig 2a) and the Cucumber strain in the mildly vs. heavily damaged (R vs. G) test (Fig 2c)), they preferred the more damaged, conspecifically infested food. In these cases, the switching behaviour showed no clear preference of mites that had experienced the more damaged food. Given that they preferred that food type before, this also led to a decrease in the number of mites on the more damaged food over the choice tests.

The performance results showed that the Tomato-strain mites had the highest oviposition rate on undamaged cucumber and the lowest on heavily damaged cucumber (Fig 4a). The Cucumber-strain mites had a higher oviposition rate on undamaged and mildly damaged cucumber than on heavily damaged cucumber (Fig 4b). Hence, learning was adaptive: mites learned to prefer the food type that yielded the higher oviposition rate. The mites do not immediately perceive plant quality in the small-scale set-up, but need feeding experience on the plants to adjust their preference. Such learned responses are by no means self-evident. First, previous tests of adaptive learning in herbivorous arthropods yielded positive (Chapter 4; Dukas and Bernays 2000) but also negative results (Parmesan et al. 1995). Second, learning is not the best response to every type of variable environment (Stephens 1987, 1993). In this study, we have only measured one fitness component – oviposition rate. However, measurements on other fitness components indicate that juvenile mortality rate of *T. urticae* is higher on damaged than on undamaged cucumber leaves but offspring development rate is similar (M. Montserrat, unpublished results). Hence, oviposition rate appears to be a good fitness correlate.

Interestingly, innate preferences in the greenhouse tests were close to those shown after learning in the small-scale tests (compare Fig 1 with Figs 2 and 3). Hence, in the greenhouse choice test the mites preferred the plant types yielding higher oviposition rate without prior experience with cucumber. This was contrary to our expectation, because Pallini et al. (1997) reported a weak but consistent preference of *T. urticae* for infested cucumber over clean cucumber, using the same set-up with the same cucumber variety and roughly the same damage level but a differ-
ent mite strain. Both strains of mites we used had not experienced cucumber for several years, unlike the strain of Pallini et al. (1997) which was maintained on cucumber. Moreover, the mites did not prefer undamaged leaf disks over damaged ones in the first small-scale choice test, where potential odors of damaged and undamaged plants were present but mite odors were not (because mites, webbing and mite excretions were washed away). Therefore, we hypothesise that the preference in the greenhouse was due to avoidance of mite odors rather than avoidance of damaged plant odors. Whether *T. urticae* may also employ switching behaviour from the worse host plant to the better host plant in the greenhouse set-up is currently under investigation.

**The scope for learning in the field**

Since the experiments with *T. urticae* reported here and in Chapter 4 were performed in the laboratory, one may wonder whether adaptive learning is an ecologically relevant trait. However, there are good reasons to believe that learning in herbivorous arthropods is favoured by selection in the field. On the smaller scale within one plant or even one leaf, differences in food quality will arise due to the colonising habit of *T. urticae* spider mites live under a webbing and local groups gradually expand over the leaf and eventually the whole plant. Mites may then benefit by learning to find an undamaged part of a leaf or an undamaged leaf on the plant through association of food quality and taste or their internal physiological state. On the larger scale between plants, they enter novel environments (plants) while dispersing, unable to assess instantly what the environment has to offer. Moreover, given their small size relative to the plant, they are bound to visit new hosts sequentially, and hence build up experience in the process. Spider mites disperse aerially, land and reach a plant by walking, whereupon they probe its food quality and have to decide whether to stay, to move on to a neighbouring plant, or to become airborne again. At this stage, mites experience the association between plant odours and food quality. Whether *T. urticae* actually can learn associatively, however, is still to be demonstrated experimentally.

On theoretical grounds, the life history of *T. urticae* and its host plants provide almost perfect conditions for the evolution of learning. Stephens (1987, 1991; review in Stephens 1998) has shown that learning is most likely to evolve in systems with a high level of predictability within the lifetime of individuals (*i.e.*, generations), and a low between-generation predictability (although learning may occur under many other condi-
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tions, among which near constant between-generation conditions!). For spider mites, the environment is easily envisaged as predictable within generations and changing between generations due to increased damage to the plant and eventually overexploitation. The evolution of learning is also promoted in situations where there is a high value of learning (i.e., a high potential of an experience to change behaviour [Stephens 1989, 1993]). Judged from the probabilities of switching hosts after one day of experience (Fig 3), the value of learning may be high for *T. urticae*. This makes sense, because females invest time and energy (e.g., webbing) in colonizing part of a leaf, and are not likely to colonise a new part very often in their lifetime.

**Adaptive learning and the evolution of host specialisation**

Given that *T. urticae* is shown to adapt its behaviour to the local environment during its lifetime, one may ask how this affects local host specialisation. It has been proposed that learning may promote the speed of adaptation, guiding evolution (e.g., Hinton and Nowlan 1987; Maynard Smith 1987; see also Robinson and Dukas 1999). This so-called Baldwin effect may, in herbivorous arthropods, facilitate the evolution of host plant specialisation and eventually host race formation (Jaenike and Papaj 1992). Moreover, adaptive learning allows foragers to distribute themselves over the resources in a fashion closer to the optimal distribution, which also favours host specialisation and host race formation (Chapter 3). Indeed, specialisation and host race formation are repeatedly observed in the polyphagous spider mite *T. urticae* (Fry 1992; Gotoh et al. 1993). However, phenotypic plasticity due to adaptive learning may also have the effect of masking fitness differences among individuals. This so-called hiding effect (Papaj 1998b; Mayley 1997) hinders local specialisation provided phenotypic plasticity has sufficiently low costs. Thus, future investigations should elucidate which costs are involved to enable adaptive learning.

**Acknowledgements**

We thank Marta Montserrat, Sara Magalhaes, Merijn Kant, Steph Menken and Jan Bruin for comments on the manuscript.

**References**


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