

DOES ARTIFICIAL SELECTION FOR FIXED PREY PREFERENCE AFFECT LEARNING IN A PREDATORY MITE? EXPERIMENTS TO UNRAVEL MECHANISMS UNDERLYING POLYPHAGY IN *HYPOASPIS ACULEIFER*

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ABSTRACT — Individual reproductive success in polyphagous arthropod predators critically depends on the prey species included in their diet. Hence, selection will act on traits that enable the predator to tune its preference to the best prey available. Such traits may be either rigid or flexible and are manifested as genetically fixed or learned preferences. Whether these two types of behaviour are mutually exclusive or manifest themselves in condition-dependent ways, is still an open question. We sought possible answers by studying a soil-dwelling predatory mite (*Hypoaspis* (*Gaeolaelaps*) *aculeifer* Canestrini), known to exhibit a genetic polymorphism in prey preferences within local populations. We had previously shown that 4 generations of artificial selection on the choice for either of two astigmatic mites (*Rhizoglyphus robini* and *Tyrophagus putrescentiae*) resulted in isofemale lines with contrasting prey preferences (*R*-line and *T*-line) and that the preference traits are inherited as though they are monogenic without dominance. In this article, we ask whether artificial selection has influenced the ability to switch preferences in a condition-dependent way. First, we conditioned the female predators of both isofemale lines by starving them in the presence of odour from each of the two prey species separately or in the absence of any prey odour. Then, at least half an hour later, we assessed their prey preference in two-choice tests. When starved in presence of odour from non-preferred prey, both lines show a slight but non-significant increase in preference for their preferred prey. However, when starved in presence of odour from the preferred prey, predators of both lines showed a clear and significant switch toward the alternative prey. This shows the ability of predatory mites to memorize the odour experienced during starvation, and to change their prey preference. It also shows that - despite four generations of artificial selection for a fixed prey preference - they retain the ability to exhibit a form of learning and to switch preferences. This result sheds new light on the impact of selection on fixed and flexible prey preferences in polyphagous arthropod predators that experience different dominant prey species in space and time.

KEYWORDS — learning; prey preference; artificial selection; predatory mite; *Hypoaspis aculeifer*; Laelapidae

INTRODUCTION

Animals make decisions about where to forage and which foods to consume (Stephens, 1991). Such decisions influence their contribution to the next generation and ultimately determine their long-term

growth rate, also referred to as fitness (Metz et al., 1992).

If food composition and supply is constant within and between generations, foraging decisions will not change either. In that case, genetically fixed food preferences will be favoured by selection, be-

cause this prevents errors and energy investments in exploring the environment. If, however, food composition and supply vary within and over generations, there will be a variation threshold above which it pays to tune food preference to the current conditions, despite the erroneous decisions that will inevitably result as a by-product of sampling the environment (Lavery and Plowright, 1988; Sullivan, 1988). Genetically fixed preferences may then still be selected, but their phenotypic expression is conditional upon 'the environment' experienced by the animal. Selection will then act on the sampling scenario and on fine-tuning the relation between food preference and experience. This ability to switch preferences depending on experience is a form of learning behaviour, often referred to as conditioning (Johnston, 1982; Stephens, 1991; Dukas, 1998). It may well have a genetic basis that is at least partially separate from that for the preferences. A similar mechanism may operate when the animal faces a novel food item, but then it is likely to test food quality first and to compare the result with some 'internal standard' before switching to prefer this novel food. If the novel food becomes a regularly encountered item in the environment, then selection will ultimately lead to a genetically fixed preference for the same reasons as explained above.

Preference may be expressed to various cues that are directly or indirectly related to the food item. When perceived upon first encounter, the animal may link the cue to this food item and use this cue as a guide to find more of it. This behaviour, referred to as associative learning (Johnston, 1982; Stephens, 1991; Dukas, 1998, 2008), will be favoured by selection provided the level of variability in cue and/or associated reward in time and space is not too high and too low. When variability is too high, the cue loses its meaning, and, if too low, then selection will favour genetically fixed cue preferences over learning (Kerr and Feldman, 2003). This is because, like conditioning, associative learning will involve costs in terms of the time needed to establish an association and the errors made in the process, as well as in terms of the neurological infrastructure (Johnston 1982; Mayley 1996; Mery and Kawecki, 2003, 2004a, 2005).

If learning ability and fixed preferences each have their own genetic basis and entail differential costs, then how will selection act on these traits when the food to be favoured is present for long periods covering many generations, yet may suddenly disappear and stay scarce for another long period? The naive answer will be that, given a long enough period of availability, genetically determined preferences will go to fixation and the ability to learn will disappear. However, this argument ignores the scenario in which learning ability may be expressed in a condition-dependent way. We hypothesize that long periods of different dominant food types are separated by a transition period in which scarcity of the initially preferred food and starvation will trigger the expression of learning ability. Learning and its costs will thus be suppressed in periods of constant availability, yet expressed when availability of one type of food declines and that of another increases. This leads to the prediction that selection for a genetically determined food preference will not go at the expense of expressing learning abilities under starvation.

For small arthropods, consecutive periods with different dominant food items are the rule, rather than the exception. Their populations usually exhibit a patchy structure with local dynamics that covers several generations before food quality declines and dispersal is triggered. Selection can thus act within the local population, and will favour a food preference matching the locally dominant food type. However, dispersal may bring the arthropod to other sites, where another food type prevails. If selection within the local population would result in a genetically fixed preference, then the arthropod would starve after dispersal unless it expresses an ability to learn under the new conditions. Thus, the time scale of selection for fixed preferences is determined by the generation time during local population dynamics, whereas the time scale of selection for learning is set by between-population dispersal. Condition-dependent expression of learning ability is therefore to be expected, implying that learning experiences may overrule the genetically fixed preferences evolved in response to the more frequent food type in the environment as a whole.

In this article, we test this hypothesis by performing experiments on learning ability of the soil-inhabiting predatory mite, *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) (= *Gaeolaelaps aculeifer*; Beaulieu, 2009), using isofemale lines selected for a fixed prey preference (Lesna and Sabelis, 1999, 2002). This predator is perfectly suited for this test because (1) it is known to feed on various prey species that differ in abundance in space and time (Inserra and Davis, 1983; Ragusa *et al.*, 1986; Sardar and Murphy, 1987; Zedan, 1988; Lesna *et al.*, 1995, 1996, 2000; Berndt *et al.*, 2004; Baatrup *et al.*, 2006; Heckmann *et al.*, 2007), (2) it builds up high numbers in response to a local prey population and goes through several generations before dispersing to new localities (Lesna *et al.*, 2000), and (3) it exhibits a genetic polymorphism in prey preferences (Lesna and Sabelis, 1999, 2002). The latter phenomenon was established by artificial selection on a strain collected from a single, small soil sample. After 4 generations of selection, isofemale lines were obtained with a preference for either of two astigmatic prey mite species, i.e. bulb mites or copra mites (without losing the capacity to feed on the non-preferred prey!). Testing for preference among F1-hybrids and the offspring of their backcrosses with males of each parental line showed that the preference trait is inherited as if determined by a single gene. The isofemale lines obtained in this way (Lesna and Sabelis, 1999, 2002) are used here to test their ability to switch or maintain preferences after experiencing the association between their starved state and the odour of their preferred prey or the non-preferred prey (kept on the same substrate).

MATERIALS AND METHODS

Prey mites

The bulb mite, *R. robini*, was collected from lily bulbs cv. Pollyanna, harvested in December 1991 from a field in Anna Paulowna (Province North-Holland, The Netherlands). They were reared in 8 cm ø Petri dishes provided with a bottom layer of moistened "Plaster of Paris" mixed with charcoal. The copra mite, *T. putrescentiae*, was obtained from

Koppert BV, Berkel en Rodenrijs (The Netherlands) and reared in open glass jars placed in a closed plastic box filled up to c. 1 cm with water (to maintain a humid environment and prevent escape of mites from the open jars). Both species of prey mites were fed with yeast flakes and kept in a dark climate box at c. 15°C.

Predatory mites

The predatory mite, *H. aculeifer*, was collected in September 1991 from lily bulbs grown in a field near Breezand (North-Holland). A sample of 20 bulbs infested by *R. robini* was taken from c. 0.25 m² of sandy soil. Approximately 150 individual predatory mites were collected from the bulbs. The population reared from these individuals in the lab has been referred to as strain "Breezand" in earlier publications (Lesna *et al.*, 1995, 1996, 2000, 2002), and served as the so called "Base population" for artificial selection for *R. robini* (prey R) preference and for *T. putrescentiae* (prey T) preference. The selection was carried out in the period from 1996 to 1997 and ultimately resulted in two lines with contrasting prey preferences, i.e. R-line and T-line (Lesna and Sabelis 1999, 2002). Predatory mites were reared in a climate room at 22°C and c. 70% RH in closed plastic vials (7 cm ø and c. 7 cm high) with a bottom layer of moistened Plaster of Paris (mixed with charcoal) to maintain a humid environment. The vials were closed by a lid that was provided with pin holes to allow air exchange. A cover of fine mesh gauze sealed under the lid prevented mite escape via the pin holes. In all cultures only *T. putrescentiae* was used as prey to rear the predatory mites (base culture of strain Breezand and all isofemale lines). The individuals used for our experiments (carried out in 2000) had thus been reared for 3–4 years on *T. putrescentiae*.

Predator conditioning

The predator females used for the experiments were all 1 day-old since their last moult. They were obtained from an egg wave from c. 50 mated females that were put together in closed plastic vials (7 cm ø, 7 cm high) with a 2 cm bottom of moistened Plaster of Paris and an ample supply of *T. putrescentiae*.

as prey. After c. 8 days female deutonymphs were collected and put in new vials with a fresh supply of prey. All females that emerged within a day were put together with prey for 24 hrs. Next, they were transferred in groups of 20 young females to small plastic vials (2 cm $\varnothing$, 2 cm high) with a 0.5 cm bottom of moistened Plaster of Paris, and without prey for 24 hrs starvation (20°C, 70% RH). The latter vials were provided with a lid that had an opening covered with gauze to allow air exchange, but with a mesh size small enough to prevent mites from moving in or out. These small vials were fixed in the gypsum bottom of a larger plastic vial (7 cm $\varnothing$, 7 cm high) and had their lid just above the Plaster of Paris surface. The starving predators were exposed to odours from their preferred prey or the alternative prey. This was achieved by releasing c. 200 individual prey (mobile stages only) on the Plaster of Paris bottom of the larger vial, so that their odours could reach the starving predators through the gauze-covered lid of the small vial. A few fresh yeast flakes were provided as well, to feed and arrest the released prey mites on the bottom of the large vial. The control treatment only differed in that the larger vial had no prey, nor yeast flakes, on the bottom.

The starvation procedure did not cause any mortality among the predators. The occasional loss of predators was due to the manipulations during transfer from one vial to the other. Selection of predators during the conditioning procedure can therefore be ruled out as a mechanism changing prey preference.

Prey choice experiments

The female predatory mites, starved as described above, were transferred first to a clean vial for collection (20 predators per vial, 3 cm $\varnothing$, 4 cm high with 1 cm bottom layer of moistened Plaster of Paris). After at least 30 minutes the female predatory mites were transferred individually to new, yet the same type of vials (3 cm $\varnothing$, 4 cm high; 1 cm bottom layer of moistened Plaster of Paris) that were provided with 2 individuals (1 nymph and 1 adult female) of each of the two prey species (*T. putrescentiae*, *R. robini*), prior to predator introduction. Successful at-

tack, defined as capture and actual feeding, always occurred within a few minutes. The species of the first prey attacked was then scored, as the result of the prey choice experiment. Per day, c. 20 individual predators of each of the three conditioning treatments were tested and the results of this series represent one replicate experiment. For all three conditioning treatments, four replicate prey choice experiments were carried out on four different days. The three conditioning treatments and subsequent prey choice tests were performed for each of the two lines, one selected for *R. robini* preference and one for *T. putrescentiae* preference according to a selection procedure described in detail by Lesna and Sabelis (1999, 2002).

Statistical analysis

Per treatment, the prey choices recorded on a single day were considered to be one replicate experiment, because the predator females under test originated from the same egg wave and received collectively the same conditioning treatment. In all other respects both the predator individuals tested and the prey individuals offered were independent. The four replicate experiments per treatment were analysed statistically by a replicated G-test for goodness of fit (Sokal and Rohlf, 1995) to two-choice distributions that are based on the assumption of random search and equal encounter rates with the two prey species (i.e. 1:1 ratio), or that are derived from the results under either of the two alternative conditioning treatments. The advantage of this G-test procedure is that it enables tests on pooled as well as per-replicate results, and - most importantly - allows for a separate test on heterogeneity among replicate experiments. If the heterogeneity test is not significant, then this provides a basis to decide for testing the pooled results, but if significant careful inspection of variability among the replicate experiments is required to interpret the results (e.g. does an overall deviation of the pooled results from the H_0 expectation hinge on the outcome of only a few replicate experiments?).

Apart from the replicated G-test against a distribution expected under H_0 (e.g. 1:1), the pooled results for each of the different treatments per preda-

TABLE 1: Overall results (total number of predators tested, percentage choosing prey *R*, i.e. *Rhizoglyphus robini*) and replicated *G*-tests on preference scores obtained in 4 replicate experiments (see Figure 1) for predatory mites (*Hypoaspis aculeifer*) of the *R*-line (i.e. selected to prefer prey *R*). Three conditioning treatments were applied to the female predatory mites preceding the prey choice test: starvation (a) – in presence of odour from prey *R*, (b) – in presence of odour from prey *T* or (c) – in absence of prey odour. Goodness of fit is tested for two distributions expected under H_0 (preference for prey *R* equal to 50% or 75%; for explanation see text). G_H = *G*-value for heterogeneity; G_P = *G*-value for pooled data; G_T = Sum of *G*-values for heterogeneity and pooled data. *P*-values are summarized by *ns* ($P > 0.10$), *bs* ($0.10 \geq P > 0.05$), * ($0.05 \geq P > 0.01$), ** ($0.01 \geq P > 0.001$), *** ($P \leq 0.001$).

Treatment odour from	Total tested	% choosing prey <i>R</i>		G-statistics (degrees of freedom)		
		found	expected	G_H (3)	G_P (1)	G_T (4)
(a) prey <i>R</i>	39	36	50	4.13 <i>ns</i>	3.14 <i>bs</i>	7.27 <i>ns</i>
			75	4.13 <i>ns</i>	26.45 ***	30.58 ***
(b) prey <i>T</i>	47	83	50	5.26 <i>ns</i>	19.25 ***	24.51 ***
(c) none	44	80	50	0.5 <i>ns</i>	16.41 ***	16.92 ***

tor line (*R*- or *T*-line) were also tested against each other, using 2×2 contingency tables and a *G*-test of independence (Sokal and Rohlf, 1995).

RESULTS

As expected from their selection history, the predators from the *R*-line exhibited a significant preference for prey *R*, when starved in absence of prey-related odours prior to the prey-choice test (Figure 1c). The pooled results showed a prey choice significantly different from 1:1 and there was no significant heterogeneity among the replicate experiments (Table 1c). In fact, the predators chose prey *R* three times more frequently than prey *T*. When starved in presence of odours from prey *T*, the predators chose prey *R* on average four times more frequently than prey *T* (Figure 1b). Also, these results significantly differed from 1:1 and there was no significant heterogeneity among replicate experiments (Table 1b). However, when the *R*-line predators were starved in presence of odours of prey *R*, they exhibited a switch in prey preference (Figure 1a). Instead of choosing prey *R* more frequently (in a ratio of 3:1; Figure 1c and Table 1c), they chose prey *T* more frequently (in a ratio 1:2; Figure 1a and Table 1a). Whereas there was no significant heterogeneity among replicate experiments, the pooled results differed significantly from a 3:1 ratio, but the difference from a 1:1 ratio was bordering significance (Table 1a). We conclude that exposure to odours from

prey *R* during starvation causes *R*-line predators to switch from a preference for prey *R* to no preference for either of the two prey or a tendency to a prefer prey *T*.

Unexpectedly, the predators from the *T*-line exhibited no significant preference for prey *T*, when starved in absence of prey-related odours, prior to the prey-choice test (Figure 2c; Table 2c). At best, there was a weak tendency for these predators to prefer prey *T* over prey *R* in a ratio of c. 3:2. Thus, the expression of preference for prey *T* declined during the 4 years of maintenance of the *T*-line starting from selection to the prey-choice experiments described here. Possibly, this isofemale line harboured some variation with individuals exhibiting genetically fixed preferences initially dominating, but later decreasing in share of the cultured population. An alternative explanation may be that there was no shift in preference, but only a change in the method (and hence outcome) of prey preference assessment: in the experiments described here preference was assessed only once per individual, whereas in the selection experiment preference was estimated from 3 assessments per individual, carried out at intervals of 4 days (one day feeding on *T. putrescentiae* followed by 3 days starvation).

When starved under exposure to odours from prey *R*, the predators of the *T*-line chose prey *T* 2.5 times more frequently than prey *R* (Figure 2b). The pooled results significantly differed from 1:1,

TABLE 2: Overall results (total number of predators tested, percentage choosing prey *R*, i.e. *Rhizoglyphus robini*) and replicated G-tests on preference scores obtained in 4 replicate experiments (see Figure 2) for predatory mites (*Hypoaspis aculeifer*) of the *T*-line (i.e. selected to prefer prey *T*). Conditioning treatments and G-statistics as in Table 1, but preference for prey *R* expected under H_0 now equal to 50%, 40%, 30% (for explanation see text).

Treatment odour from	Total tested	% choosing prey <i>R</i>		G-statistics (degrees of freedom)		
		found	expected	G_H (3)	G_P (1)	G_T (4)
(a) prey <i>R</i>	55	29	50	3.65 <i>ns</i>	9.92 **	13.57 **
(b) prey <i>T</i>	47	76	50	8.03 *	13.16 ***	21.19 ***
(c) none	44	43	50	2.42 <i>ns</i>	0.78 <i>ns</i>	3.20 <i>ns</i>

but there was marginally significant heterogeneity among replicate experiments (Table 2b). When the *T*-line predators were starved in presence of odours from prey *T*, they exhibited a switch in prey preference (Figure 2a). Instead of no preference or weak preference for prey *T*, they chose prey *R* three times more frequently. Heterogeneity among replicate experiments was not significant and the pooled results were significantly different from a 1:1 ratio (Table 2a). Finally, we compared the pooled results of the different treatments by G-tests of independence applied to the data arranged in 2×2 contingency tables. For the *R*-line and the *T*-line, the null hypothesis that prey choice is independent from the prey odour experienced during starvation can be rejected. Prior starvation while exposed to odour from the preferred prey instead of the alternative prey led to a significant shift in prey choice in favour of the alternative prey (for *R*-line $G = 18.8$ and $P = 1.4 \times 10^{-5}$; for *T*-line $G = 23.0$ and $P = 1.6 \times 10^{-6}$). Prey choice also significantly differed when previously exposed to odour from preferred prey instead of no prey odour at all (for *R*-line $G = 16.8$ and $P = 4.0 \times 10^{-6}$; for *T*-line $G = 10.4$ and $P = 0.00126$). No significant difference in prey choice was found when previously exposed to odour from the alternative prey instead of no prey odour at all (for *R*-line $G = 0.06$ and $P = 0.862$; for *T*-line $G = 2.3$ and $P = 0.131$).

DISCUSSION

Using young females of the predatory mite *H. aculeifer* from two lines reared on *T. putrescentiae*, one

line obtained by selection for preference of *R. robini* as prey (*R*-line) and another obtained by selection for preference of *T. putrescentiae* as prey (*T*-line), we showed that exposure to the odour from either the preferred prey, the alternative prey or no prey during a one-day starvation period had a significant effect on the subsequent choice for the two prey species in a test taking place at least 30 minutes after the treatment. Apparently, the duration of the starvation period was long enough for the female predators to memorize the odour experienced during the starvation period and subsequently to avoid capturing the prey species whose odours were present during starvation. Females of the *R*-line preferentially captured *R. robini* if starved in absence of prey odours or in presence of odours from *T. putrescentiae*, but they switched to capture *T. putrescentiae* if starved in the presence of odours from *R. robini*. Similarly, females of the *T*-line had a slight preference for *T. putrescentiae*, if starved respectively in absence of prey odours or in presence of odours from *R. robini*, but they switched to capture *R. robini* if starved in the presence of odours from *T. putrescentiae*. This demonstrates that an ability to learn is retained in lines that had been obtained by selection to prefer

Olfactory learning has been demonstrated earlier for a predator species from another family of mites, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), by Drukker *et al.* (2000), De Boer *et al.* (2005) and Wijk *et al.* (2008) and an ability to learn to make adaptive choices has been shown for the herbivorous mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), by Egas and Sabelis (2001) and for

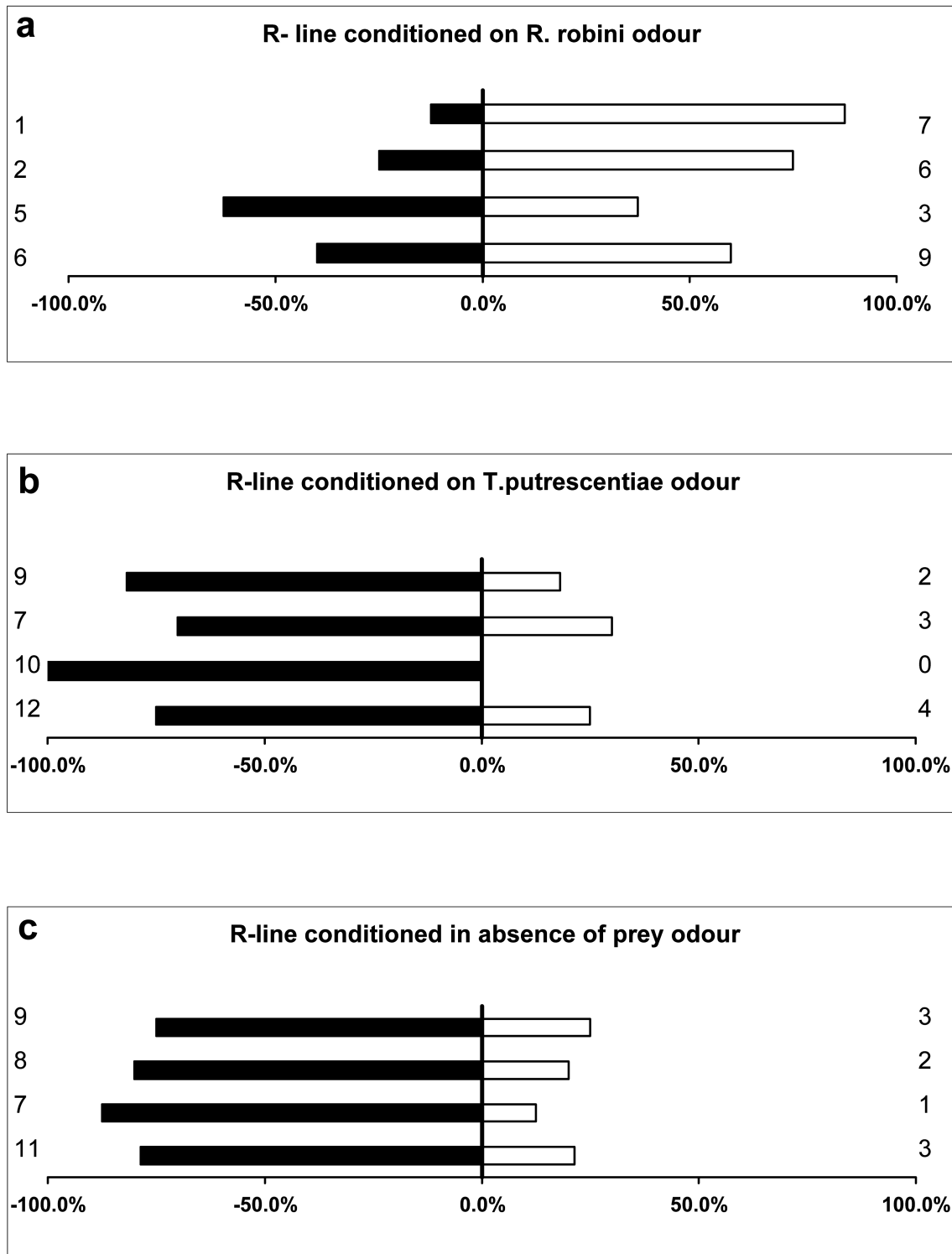


FIGURE 1: Preference of *R*-line predators, expressed as percentages (horizontal bars) of individuals choosing prey *T* (0 to 100%) or prey *R* (0 to -100%) for four replicates and three starvation treatments: (a) – in presence of odour from prey *R* or (b) – prey *T* or (c) – in absence of prey odour. Numbers shown left and right of the horizontal bars represent number of predators choosing prey *R* (left) or prey *T* (right) for each replicate experiment.

the predatory mite, *P. persimilis*, by Rahmani *et al.* (2009). In this article, we show olfactory sensing of prey-related odours by *H. aculeifer* for the first time. However, it should be noted that Hall and Hedlund (1999) earlier showed that *H. aculeifer* exhibits olfactory responses to odours from fungi. They argued that this response to fungal odours helps the predators to search for fungivorous mites as prey.

More experiments are needed to elucidate which of modes of learning are exhibited by *H. aculeifer* (Papaj and Prokopy, 1989; Dukas, 1998, 2008). Because the predators memorized the odour experienced during starvation for at least 30 minutes, sensory adaptation is unlikely to be the mechanism, but habituation is a fair possibility since this enables long-term memory. During starvation the predators may have been repeatedly triggered to search upon perceiving prey odour of variable intensity, but each bout of search activity was in vain as there were no prey to be captured. This may cause habituation of the search response to the prey odour experienced during starvation and may explain why the search response in the choice experiment is more likely to be triggered by the other prey (i.e. whose odour was not present during starvation). However, associative learning, a more advanced mode of learning, cannot be ruled out as a possibility. In particular, repeated sensations of hunger may reinforce an aversion of the odour experienced during starvation. The learned aversion may then trigger the predator to actively suppress a search response toward the prey releasing the same odour, but not toward prey releasing another odour. In absence of evidence of such behavioural suppression, however, aversion learning remains conjectural and a more elaborate experimental approach is needed to demonstrate this beyond doubt (Papaj and Prokopy, 1989). However, it is reasonable to expect predatory mites to be capable of aversion learning, because it is considered to occur most likely in arthropods that are polyphagous, highly mobile and relatively long-lived (Dukas, 1998), which are all characteristics shared by these predators. In addition, soil-dwelling predatory mites may be particularly predisposed to associating an olfactory or gustatory cue with a negative experience because they

live below-ground or in the litter and must therefore heavily rely on olfactory or gustatory cues to determine suitable prey.

The main novelty of our results is that the ability to learn is retained despite artificial selection for fixed prey preferences. Thus, given that the predators are not punished for this ability during the selection process (as is likely under natural conditions and as was the case in the selection experiments carried out by Lesna and Sabelis (1999, 2002)), they retain the ability to cope with environments that harbour another prey composition. This feature is likely to be adaptive for a predatory mite, like *H. aculeifer*, because it lives in patchy populations with strong intra-patch, but weak inter-patch interactions. This so-called metapopulation structure implies that any given patch may offer widely different abundances and ratios of *R. robini* and *T. putrescentiae*. Thus, selection for a preference for one prey during several generations in one patch (or a series of similar patches) does not go at the expense of an ability to alter preferences once the predator invades a new patch with entirely different abundances and/or ratios of prey species. Retention of learning ability despite local selection for fixed preferences may therefore be an adaptation of polyphagous predatory mites in metapopulations.

It is important to realize that a history of selection may also result in the reverse phenomenon, i.e. where innate feeding preferences override an arthropod's experience. For example, larvae of the polyphagous Asian armyworm *Spodoptera litura* and fall armyworm *Spodoptera frugiperda* maintain their preference for castor leaf discs and corn over other plants, after recovering from morbidity induced by feeding on insecticide-treated samples of these plants (Raffa, 1987; Ghumare and Mukharjee, 2005) and adults of the polyphagous Japanese beetle *Popillia japonica* maintain their preference for - morbidity-inducing - geranium flowers over suitable alternative hosts, after recovery from morbidity induced by this plant. The failure of these polyphagous insects to learn to avoid morbidity-inducing food sources may result from internal limitations to their learning ability or from the nature of the conditioned stimulus (e.g. insecticide) (Dukas,

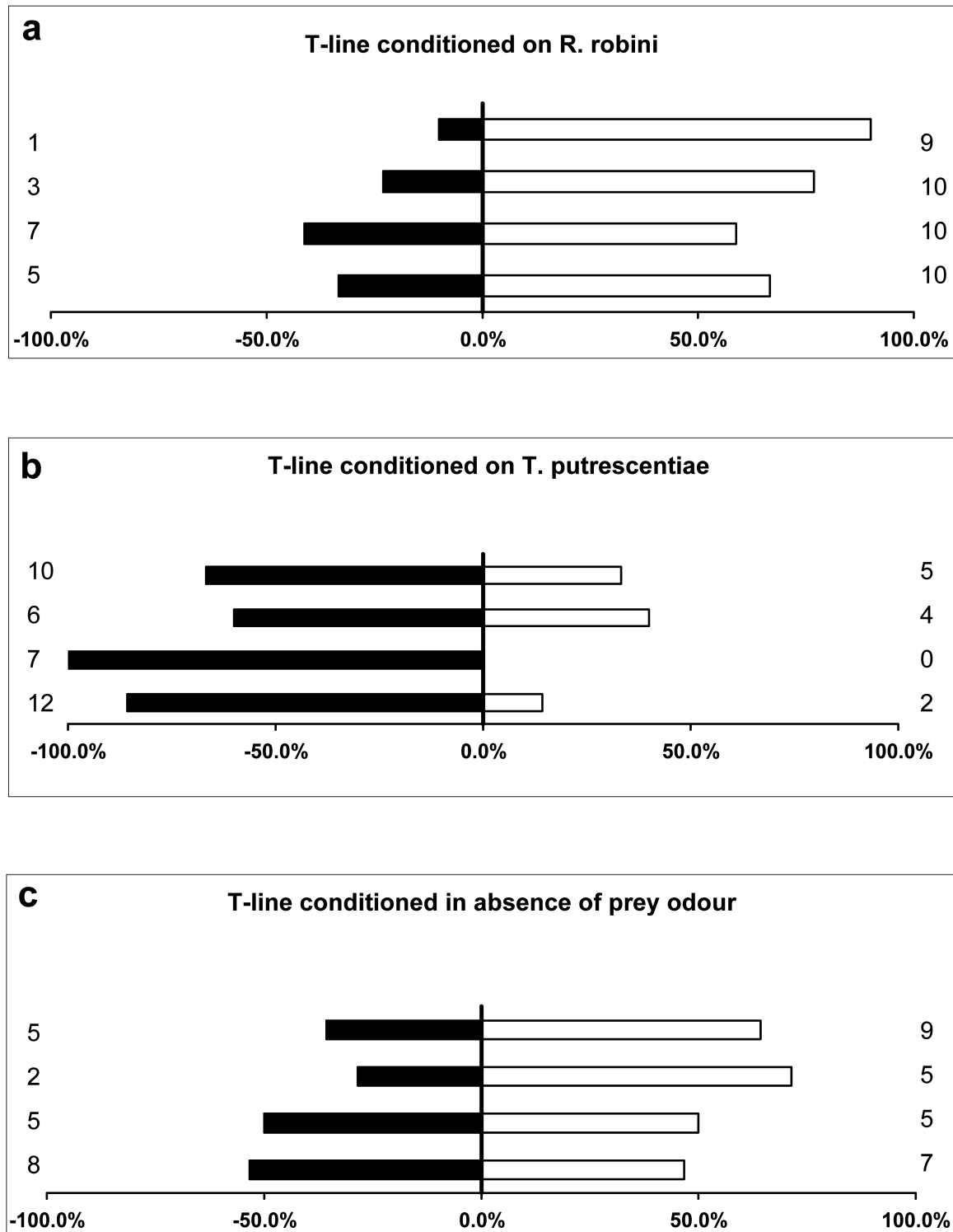


FIGURE 2: Preference of *T*-line predators, expressed as percentages (horizontal bars) of individuals choosing prey *T* (0 to 100%) or prey *R* (0 to -100%) for four replicates and three starvation treatments: (a) – in presence of odour from prey *R*, (b) – in presence of odour from prey *T* or (c) – in absence of prey odour. Numbers shown left and right of the horizontal bars represent number of predators choosing prey *R* (left) or prey *T* (right) for each replicate experiment.

1998). Thus, it is not self-evident that polyphagous arthropods retain an ability to learn.

Behavioral responses of an animal depend on an interaction between innate and learned components. While the innate, heritable component reflects the selection history of the population, the learned component is affected by experience accumulated within the individual's lifetime. The ability to modify behaviour based on experience (i.e., learning ability) is itself a product of evolution, with notable genetic differences between related species or even conspecific populations (Gould-Beierle and Kamil 1998; Girvan and Braithwaite 1998; Jackson and Carter 2001; Kawecki and Mery 2003). It has been predicted that learning would be advantageous in variable environments, i.e., when the fitness consequences of a given behavioral action change from generation to generation, or even within an individual's lifespan (for reviews see Johnston 1982; Stephens 1991; Dukas 1998). On the other hand, gaining experience is often costly and error-prone (Lavery and Plowright 1988; Sullivan 1988), and the energy spent on information processing and the maintenance of underlying physiological structures may also entail fitness costs (Johnston 1982; Mayley 1996; Mery and Kawecki 2003, 2004a, 2005). Hence, innate, ready-to-use behavioral responses are expected to be favoured if their fitness consequences change little from generation to generation. Learning is thought to be adaptive in variable environments, while constant (predictable) environments are supposed to favour unconditional, genetically fixed responses. However, such a dichotomous view of behaviour as either learned or innate ignores the possibility that the genes coding for learning ability and genes coding for fixed preferences are at least partially different and that the expression of one or the other set of genes may be condition-dependent. This possibility represents a hypothesis that is consistent with our data on *Hypoaspis aculeifer*: artificial selection for fixed preferences (Lesna and Sabelis 1999, 2002) resulted in two lines with contrasting prey preferences, but the females from either of the two lines retained an ability to learn after starvation. Whereas consistent with the hypothesis, our data do not provide proof of

it. Future research requires artificial selection of lines with different abilities to learn (conditioning, sensitization, habituation, associative learning) and with different preferences (not only positive, but also neutral and negative) preferences. Crossbreeding between these lines in combination with various preference tests may then reveal the genetic architecture of these traits and the interactions between the relevant genes.

Our results get a new perspective in the light of results published earlier by Mery and Kawecki (2002, 2004b). They carried out experiments to select preferences of *Drosophila melanogaster* for a substrate medium (either pineapple or orange) under two regimes, one that allowed for learning ability and one that did not (Mery and Kawecki, 2004b). When selecting for oviposition on the pineapple medium, a stronger innate preference for pineapple evolved when learning was allowed, than when it was not. When selecting for the orange medium, however, the reverse effect on preference was obtained. Thus, an opportunity to learn facilitated the evolution of innate preference under selection for a preference for pineapple, but hindered it under selection for preference for orange. How the learned and innate components of olfactory responses jointly evolve, is still an open question for future research.

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