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The interplay between genetic and learned components of behavioural traits

Olfactory responses of predatory mites to signals contained in a herbivore-induced plant volatile



Beata Sznajder


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ACADEMISCH PROEFSCHRIFT

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aan de Universiteit van Amsterdam
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1

Introduction

This thesis deals with the evolution of behavioural traits that have a genetic basis, yet can be modified by learning in response to environmental influences. It is the tenet of Darwinian theory that natural selection will drive evolution of a trait if there is heritable and selectable variation for a phenotypic trait. Variation is selectable when phenotypes differ in survival chances and reproductive success, i.e., individuals that vary in the trait differ in terms of the number of their offspring. Variation is heritable when each phenotype has, at least partially, a genetic basis. Thus, phenotypic variation arises from differences in the genetic make-up of different phenotypes. Together with recombination, mutation is regarded as the process creating variants of the trait, whereas natural selection is the process eroding genetic variation.

The presence of selectable variation for a phenotypic trait determines its evolutionary potential to respond to natural selection (Houle 1992; Falconer and Mackay 1996). However, selectable phenotypic variation may arise from differences in the genetic make-up of different phenotypes as well as from the way these phenotypes respond to environmental influences (i.e., phenotypic plasticity). If the ability to adaptively respond to environmental influences by producing certain phenotypes has a genetic basis (and is therefore heritable) then this ability can be shaped by natural selection and evolve as well.

Therefore, there is an evolutionary interplay between the innate (i.e., genetically determined) phenotype and the ability to modify it in response to environmental influences. In particular, phenotypic plasticity may change fitness of individuals, and thus it may influence how natural selection acts on the selectable phenotypic variation for the innate trait. At the same time, both the innate phenotype and its plasticity may have genetic bases. Hence, both these two traits may be shaped by natural selection and thus jointly evolve. Thus, evolutionary pathways are possible where the trait evolves primarily via the innate component, or primarily via improved plasticity or a mixture of both (as illustrated by Papaj 1994). Moreover, phenotypic plasticity may play a role in creating novel selectable forms that are entirely environmentally induced when there is not any

genetic basis for such a variant as, e.g., in populations that colonize a novel environment (ten Cate 2000; Price et al. 2003; West-Eberhard 2005; Crispo 2008).

Learning can be considered as a special form of phenotypic plasticity of behavioural traits because its effect may be reversible; a learned behavioral response may wane if the environmental stimulus that triggered it is no longer present, or it may be modified if a new environmental stimulus occurs. Some mechanisms of learning result in an adaptive change of behaviour, i.e., a change that allows the modified phenotype to obtain higher fitness. An example is associative learning where animals learn the association between stimuli and an environmental state that may affect fitness (Dukas 1998), such as the presence or the lack of food (Dukas 1998; Dukas and Bernays 2000; Egas and Sabelis 2001), or the presence of predators or competitors (Dukas 1998; Nomikou et al. 2003; Dukas 2004).

Other forms of learning may be adaptive in some ecological situations, but not in other situations. Suppose a herbivorous arthropod feeds on a certain plant resource of good quality (due to varying profiles of secondary metabolites in different plant species herbivores may be well equipped to feed on some plant species but less on other species). If it learns through sensitisation (Kandel et al. 1993; Kandel 2001) then it learns to respond to a (otherwise neutral) stimulus following an experience with another stimulus that was intense (or noxious). In this hypothetical example, the herbivorous arthropod acquires an increased responsiveness to a variety of herbivore-induced plant volatiles, after an experience with a specific volatile (or a blend of volatiles). Thus, as long as these volatiles are coupled with abundance of food the increased responsiveness acquired by such learning is adaptive, i.e., allows the animal to find more resource of better quality. If, however, conditions change such that, e.g., more conspecifics start utilizing this resource the fitness benefits from remaining on this plant resource will diminish. However, the sensitized response may lead the herbivore to remain on the same resource until the sensitized response wanes.

The effects of adaptive learning are special in that they mimic the outcomes of adaptive evolution (Papaï 1994). Adaptive learning allows individuals to modify their behaviour such that their fitness increases. Therefore, learning ability may mask genetic differences among different phenotypes and such an effect weakens natural selection and slows down the evolution of the innate trait (Falconer and Mackay 1996). An alternative hypothesis, put forward by Mark Baldwin (1896) and hence known as the Baldwin effect, holds that adaptive learning may in fact accelerate evolution of innate behaviour in novel environments, i.e., where genetically determined adaptations to the new environment have not yet evolved and the behavioural trait is under directional selection to reach a distant fitness peak. Adaptive learning not only improves the survival of

the population (thus providing the time for the evolution of a genetic basis for optimal behaviour); there is also selection for improved learning (provided heritable variation for the ability to learn the behaviour associated with higher fitness exists). If learning confers a larger fitness increase to those phenotypes (as well as underlying genotypes) that are relatively closer to the fitness peak (as postulated by Baldwin 1896) then selection for improved learning will be associated with selection for innate behaviour. According to the Baldwin effect, in a non-plastic population selection of fitter genotypes proceeds slower because there is no learning that confers additional fitness benefits to genotypes that are already closer to the fitness peak.

The Baldwin effect spurred numerous theoretical models. However, the predictions of these models did not lead to a consensus: some lent support for an accelerating effect of adaptive learning on evolution (Hinton and Nowlan 1987; Fontanari and Meir 1990; Ancel 1999; 2000: the norm of reactions model; Mayley 1997; Lande 2009), whereas others supported a decelerating effect (Andersson 1995; Ancel 2000: the quantitative genetics model; Dopazo et al. 2001). The most recent models (Paenke et al. 2007; Borenstein et al. 2006) represent an attempt at unifying these predictions and do so by defining the theoretical conditions under which one or the other effect of learning prevails. In particular, Paenke et al. (2007) argue that the curvature of the fitness landscape predicts when adaptive learning accelerates or decelerates evolution because it determines whether learning confers a larger fitness increase to those phenotypes (as well as underlying genotypes) that are relatively closer to the fitness peak (see also Egas et al. 2004 for a similar argument).

The study by Paenke et al. (2007) assumes a non-evolving learning ability – an approach that is common in the majority of theoretical studies of the Baldwin effect (Hinton and Nowlan 1987; Fontanari and Meir 1990; Ancel 1999; Mayley 1997; Andersson 1995; Ancel 2000: the quantitative genetics model, Dopazo et al. 2001; Borenstein et al. 2006). However, the emerging empirical evidence shows a genetic basis for learning ability such that it is possible to select for higher or lower levels of learning (McGuire and Hirsch 1977; Hirsch and McCauley 1977; Mery and Kawecki 2002; Dukas 2004). Hence theoretical predictions leave the question open as to how adaptive learning influences evolution of innate behaviour if it is allowed to evolve jointly with the innate behaviour. Two exceptions are the studies by Ancel (2000) and Lande (2009), where evolving phenotypic plasticity is modelled as a reaction norm (see also studies in the framework of artificial life/intelligence, e.g., Watson and Wiles 2002; Suzuki and Arita 2004). In other words, learning ability may be interpreted in these studies to be fixed at a very high level such that the most adaptive phenotype is always expressed from within the norm of reactions. The

result of these studies are consistent in that in the initial stage of evolution towards a distant fitness peak, wider norms of reaction are selected and the expression of the optimal phenotype is initially achieved through a plastic response. At the same time, the process of population movement towards the fitness peak is faster in the plastic population than in a population consisting of non-plastic individuals. However, the second stage of this process, i.e., the convergence of the population on the single optimal non-plastic phenotype (given by the fitness peak) is much slower in the plastic population. If learning has a fitness cost, then – in this second stage – it is predicted to be selected against – a process often termed as genetic assimilation in the context of the Baldwin effect (Crispo 2007; Lande 2009).

Studies by Ancel (2000) and Lande (2009) as well as earlier studies assuming non-evolving learning (e.g., Hinton and Nowlan 1987; Fontanari and Meir 1990; Ancel 1999; Mayley 1997) indicate that the rate of evolution should be measured in two stages: (1) when the population evolves towards a distant fitness peak, and (2) when the population is in the vicinity of the fitness peak, i.e., when at least some genotypes in the population express the optimal phenotype innately. Selection may favour different outcomes in these two stages and thus they may concern two different evolutionary processes.

Thus, relevant theoretical tests of the Baldwin effect should explore how various forms of adaptive learning influence evolution (1) towards a distant fitness peak and, separately, (2) in the vicinity of the fitness peak. Empirical evidence for the role of learning in evolution is virtually absent (but see Mery and Kawecki 2004) and requires a model system where genetic variation for both a behavioural trait and the ability to learn are demonstrated.

The framework of this thesis

In this thesis, I investigated whether there is genetic variation for a foraging behaviour of a predatory mite (chapters 2 and 3) and for the ability to modify it by learning (chapter 4). The behaviour in question is the response of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) to volatile compounds that are released by plants in response to plant feeding by its prey. The second part of this thesis contains a review of theoretical tests of the Baldwin effect (i.e., the hypothesis that learning accelerates the rate of evolution; chapter 5), and a theoretical study (chapter 6) wherein I investigate how the predictions of the evolution of innate behaviour change if adaptive learning is allowed to evolve.

Experimental system

The predatory mite *P. persimilis* uses olfactory cues when searching for its prey, the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) that feeds on

parenchyma cells of plant leaves (Lindquist 1998). These cues can be derived from the prey itself, such as odours emanating from faeces or silk that the prey produces abundantly on infested plants (Sabelis and Afman 1983). Another important type of cue includes volatile compounds that are released by plants upon infestation with herbivore; these volatile blends are quantitatively different from blends of uninfested plants (van den Boom et al. 2004) and they are specific to the species of the herbivore (Takabayashi and Dicke 1996). Empirical studies provide ample evidence of the attraction of natural enemies to plant volatiles induced by their prey or host in general (De Moraes et al. 1998; Turlings et al. 1990, 1995; Schnee et al. 2006; Rassman and Turlings 2007; Beyaert et al. 2009), and the attraction of *P. persimilis* to plant volatiles induced by the spider mites in particular (Dicke and Sabelis 1988; de Boer et al. 2004a). Hence, the hypothesis was put forward that the natural enemies (parasitoids or predatory mites such as *P. persimilis*) evolved specific responses to herbivore-induced plant volatiles because such responses improve predator efficiency in prey location under natural settings (and hence fitness, although direct demonstration of fitness benefits under natural settings for *P. persimilis* are not yet documented, and it is scarce for other model systems, see Dicke & Baldwin 2009). The hypothesis is supported by the fact that the composition of the volatile blend is specific to the herbivore species that induced it, and thus provides a signal of the presence of this herbivore. However, under natural settings there is variation in volatile blends that stems from the presence of other herbivores, from the species of the infested plants or from the odours of other uninfested plants (Schröder and Hilker 2008) as well as from abiotic conditions (Holopainen and Gershenson 2010).

Given that *P. persimilis* may improve its foraging success by responding to plant volatiles induced by the feeding of its prey, the presence of variation in the blends of these volatiles under natural settings raises two questions. Firstly, what features of the volatile blend trigger predator attraction, i.e., the predators may respond to specific components present in the blend, alternatively they perceive the entire blend as an entity. The damage caused to the plant by the feeding of spider mites triggers the release of a handful of novel compounds, among which methyl salicylate (MeSa) is common (de Boer et al. 2004; van den Boom et al. 2004). The emission of MeSa is not specific to the infestation by the spider mites; it is also induced by the feeding of other herbivore species (van Poeke et al. 2002; Bukovinszky et al. 2005; Zhu and Park 2005; Snoeren et al. 2010). As such, MeSa may, therefore, serve as a general signal of herbivory. Although the presence of MeSa does not always indicate the presence of spider mites, the reverse is true: feeding by spider mites almost universally triggers the emission of MeSa (for the range of plant species tested to date: Dicke et al. 1990; van den

Boom et al. 2004). Thus, using MeSa as a signal of prey presence may significantly aid *P. persimilis* in the search for prey. In line with this argument, MeSa is one of a few spider-mite induced compounds in lima bean that elicit a positive response of *P. persimilis* when offered alone (de Boer and Dicke 2004a; van Wijk et al., 2008) as well as when offered in mixtures (de Boer and Dicke 2004a). The reverse is true for many other constituents of full blends of volatiles: the presentation of a single constituent often elicits no response from the natural enemies (or herbivores) or it elicits avoidance (van Wijk et al. 2008; Webster et al. 2009). Moreover, by silencing the tomato gene encoding an enzyme crucial for the synthesis of MeSa upon herbivore infestation Ament et al. (2010) obtained the natural blend of volatiles induced by spider mites on tomato with the sole exception of MeSa. The authors subsequently demonstrated that such blend of volatiles no longer attracted *P. persimilis* as the predator did not differentiate between this blend and the blend of uninfested tomato plants. Accordingly, emerging empirical evidence for other model systems supports the hypothesis that natural enemies may respond to only a few specific compounds in the volatile blend, although this response may be dependent on the correct context of volatile presentation (such as, for example, a background of volatiles in specific ratios as in Beyaert et al. 2009; see also Schnee et al. 2006). Thus, we hypothesized that MeSa is the main feature of the blend of volatiles used by *P. persimilis* when searching for prey, although predator responses to this compound may be strengthened by providing an ecologically relevant context of other volatiles.

Secondly, the question arises as to what extent predator responses to relevant plant volatiles are genetically determined given that they can be modified by learning. Learning ability provides *P. persimilis* with one way of updating the responses to relevant compounds encountered in different contexts (i.e., with or without prey; Drukker et al. 2001; de Boer and Dicke 2004b; de Boer et al. 2005; van Wijk et al. 2008). However, predators may have also evolved genetically determined responses to those specific volatile compounds that are consistently and reliably induced by the feeding of prey, particularly if learning comes at a cost. This hypothesis is supported by evidence of genetic variation in the responses of *P. persimilis* to the volatile blends induced by feeding of the prey on the intact plant (Margolies et al. 1997; Jia et al. 2002). Furthermore, *P. persimilis* reared on spider-mite infested cucumber plants is attracted to the odours of spider-mite infested lima bean, although the blend of infested lima bean is novel in that it was never experience before (Shimoda and Dicke 2000, but see Drukker et al. 2001 for evidence to the contrary; however, in this study inexperienced predators were obtained differently: by rearing them in an odourless environment with washed eggs of the prey). The demonstrated innate preferences for the full blend of volatiles from infested plants (lima bean, in this case)

may be partly the result of the underlying innate response to a single compound shared by the blends of many plant species infested by the spider mite – a condition that is indeed fulfilled by MeSa. Van Wijk et al. (2008) found only a moderate innate attraction of *P. persimilis* to MeSa. However, their study, as well as previous studies of preference of *P. persimilis* towards single compounds or their blends (Shimoda and Dicke 2000; Drukker et al. 2001; de Boer and Dicke 2004a,b) commonly measured predator behaviour at the population level, thus in genetically variable populations.

In this thesis I tested whether genetic variation for predator response to MeSa is present in a natural population of *P. persimilis* (i.e., whether predator responses to MeSa have evolutionary potential), and to what extent any genetically fixed responses to MeSa can be modified by experience.

Theoretical framework

I consider phenotypes consisting of two traits: (1) an innate (i.e., genetically determined) behavioural response and (2) a learning ability. I model the innate behavioural response of a phenotype in terms of probabilities of responding to different concentrations of an environmental cue. The distribution of these probabilities is given by a Gaussian function where the mean of a Gaussian function is the evolving variable, while the variance is kept fixed and represents the exploratory range of the phenotype. In biological terms, this model of innate behaviour can be interpreted as the response of a predatory mite to a herbivore-induced plant volatile. The predator responds with certain probabilities, given by a Gaussian function, to a range of concentrations of this volatile encountered in the environment. The mean of the Gaussian function indicates the most frequently chosen concentration of the volatile.

I further assume that the innate responses of a phenotype to a range of concentrations perceived by the phenotype within its exploratory range may be modified by adaptive learning. Adaptive learning is approximated by a function that weights the phenotype's probabilities of responding to different concentrations by the amount of fitness acquired from responding to each of these different concentrations (this is determined by a fitness function). Thus, the phenotype's responses are adjusted such that the fitness of a phenotype is increased. However, the degree of this adaptive change of the innate responses (and the amount of fitness gain due to this change) depends on the level of learning. This level of learning is allowed to evolve jointly with the innate behaviour.

Thus, I express the phenotype as a function of two traits: the innate behaviour and the level of adaptive learning, and assume a fitness function that provides the relationship between phenotype and its fitness. This allows me to construct a fitness landscape that I use to determine the direction and the rate of

phenotypic evolution (Figure 1.1, an example from chapter 6); this is achieved by tracking the position of phenotype on the fitness landscape through time. The theoretical framework used in this thesis is based on the assumption that the evolution of phenotypes proceeds in the direction of increased fitness (as given by the steepest slope from any given position on the fitness landscape). Hence, starting from a given phenotype evolutionary change may proceed either via evolution of the innate response or the level of learning or via changes in both these traits. I use this framework to determine the direction and the rate of phenotypic evolution towards a distant fitness peak in two scenarios: when the initial level of learning is kept fixed and when it is allowed to evolve jointly with the innate response.

Outline of the thesis

In the first two chapters I present the results of experimental tests for genetic variation in the response of *P. persimilis* to the plant volatile MeSa using the so-called iso-female line approach (chapter 2) and a purifying selection within iso-female lines that aimed at setting apart and fixing genotypes distinct with respect to predator response to MeSa (chapter 3). Using the first approach I show that a significant amount of phenotypic variation in predator responses

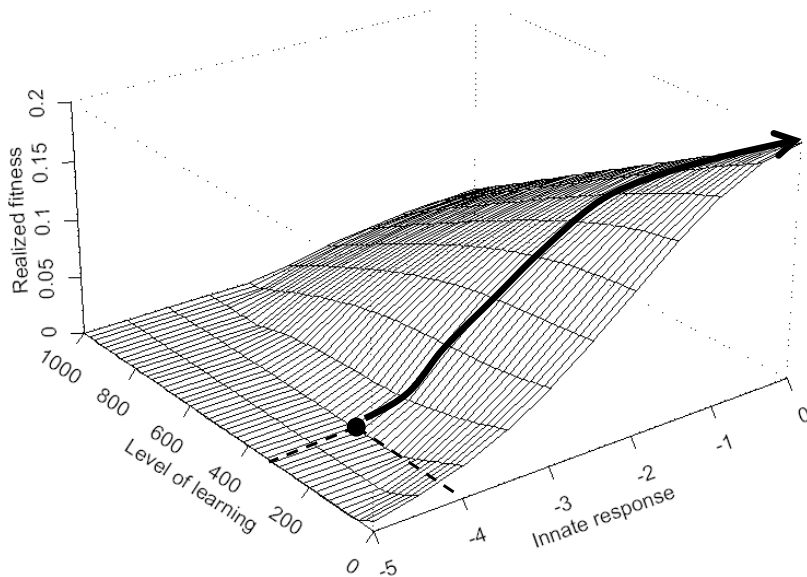


Figure 1.1 – Example of a fitness landscape, i.e., the relationship between fitness (z-axis) and the bi-variate phenotype that consists of an innate response (x-axis) and a level of learning (y-axis). A black dot represents an initial phenotype and the superimposed trajectory shows the evolution of this initial phenotype towards the distant fitness peak for the innate response (i.e., towards the innate response = 0).

to MeSa is explained by variation among (genetically fixed) iso-female lines, thus providing evidence that this behaviour is genetically variable. The amount of variation explained by iso-female lines provides an estimate of the total genetic variation for predator responses to MeSa in this population and indicates that this trait has evolutionary potential, i.e., has the ability to respond to selection (Houle 1992). Moreover, I demonstrate that the addition of background volatiles of uninfested plants changes the predators' responses to MeSa in a manner that depends on physiological state and iso-female line, thus providing evidence that these are, indeed, context-dependent.

Chapter 3 presents experiment where I selected within iso-female lines for genotypes with contrasting responses to MeSa offered in a pure compound. The selection was purifying in the sense that two groups of iso-female lines were established and in one group the lines were propagated via females that showed a preference for MeSa, whereas in the second group the lines were propagated via females that avoided MeSa. Contrary to expectations, I did not obtain two groups of iso-female lines showing preference for MeSa in the treatment group selected to prefer MeSa or avoidance of MeSa in the treatment group selected to avoid MeSa. Instead, there was a shift in the mean response to MeSa in the direction opposite to the selected one. In particular, iso-female lines selected to avoid MeSa shifted their response towards preference for MeSa while the response of the lines selected to prefer MeSa shifted towards avoidance of MeSa. Additionally, I confirm that a significant amount of variation in the responses of *P. persimilis* to MeSa is explained by iso-female lines.

These first two chapters consistently document that there is a significant amount of variation in predator responses to MeSa due to iso-female lines, thus supporting the hypothesis that this behaviour is genetically variable. Future studies will address whether the revealed genetic effects are additive (and thus predict the response to directional selection) or non-additive.

Chapter 4 provides evidence that the genetically determined responses of *P. persimilis* to MeSa can be modified by experience with this compound. This is inferred from a change in the average responses of iso-female lines detected after they were fed or starved in the presence of MeSa. The change in behaviour represented either increased or decreased responsiveness to MeSa in a way that was independent of the nutritional context of this experience (i.e., whether MeSa is experienced in the presence or absence of food), thus suggesting that learned responses of *P. persimilis* are shaped by the amount of MeSa they were exposed to and thus may be based on a non-associative mechanism. Moreover, I found variation among the iso-female lines in the way they responded to the experience, and this result provides support for the hypothesis that the predator's ability to modify responses to MeSa also has a genetic component.

In the final two chapters I review and expand the theory of the Baldwin effect, i.e., on the hypothesis that adaptive learning accelerates the rate of evolution of innate behaviour. In chapter 5, I review theoretical studies of the Baldwin effect that provide contrasting predictions of either accelerating or decelerating effects of learning on evolution. I discuss the dependence of the predictions of these studies on the critical assumptions such as non-evolving learning ability and the effect of learning on phenotype's fitness.

The question of how relaxing the assumption of non-evolving learning changes evolutionary predictions is dealt with in chapter 6. Therein, I model the evolution of an innate behavioural response of a phenotype to an environmental cue where this behaviour can be modified by costly adaptive learning within a preset exploratory range of cue-values. Evolutionary predictions are compared under two scenarios: (1) when innate behaviour evolves towards a distant fitness peak while the learning ability is kept fixed, and (2) when learning ability is allowed to evolve jointly with the innate behaviour. This comparison reveals that allowing adaptive learning to evolve greatly reduces the time to reach the fitness peak when (1) the net effect of learning on phenotypic fitness is large (i.e., learning modifies innate behaviour within a large exploratory range of cue-values), (2) learning has a fitness cost, and (3) the initial level of learning is higher than the evolutionary optimum. Under these conditions there is a critical threshold level of learning above which learning is favoured by selection and evolves towards an optimum level and below which it is selected against. Thus, I find a threshold level of learning above which joint evolution of learning and innate behaviour is accelerated. When the net effect of learning on phenotypic fitness is small (i.e., learning modifies innate behaviour within a small exploratory range of cue-values) evolution of the phenotype proceeds effectively only via changes in the innate behaviour. Together these results allow me to conclude that assuming a form of fixed learning is only justified when the net effect of learning on phenotypic fitness is small.

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2

Response of predatory mites to a herbivore-induced plant volatile: genetic variation for context-dependent behaviour

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Abstract – Plants infested with herbivores release specific volatile compounds that are known to recruit natural enemies. The response of natural enemies to these volatiles may be either learned or genetically determined. We asked whether there is genetic variation in the response of the predatory mite *Phytoseiulus persimilis* to methyl salicylate (MeSa). MeSa is a volatile compound consistently produced by plants being attacked by the two-spotted spider mite, the prey of *P. persimilis*. We predicted that predators express genetically determined responses during long-distance migration where previously learned associations may have less value. Additionally, we asked whether these responses depend on odours from uninfested plants as a background to MeSa. To infer a genetic basis, we analyzed the variation in response to MeSa among iso-female lines of *P. persimilis* using choice-tests that involved either (1) MeSa presented as a single compound, or (2) MeSa with background-odour from uninfested lima bean plants. These tests were conducted for starved and satiated predators, i.e., two physiological states, one that approximates migration and another that mimics local patch exploration. We found variation among iso-female lines in the responses to MeSa, thus showing genetic variation for this behaviour. The variation was more pronounced in the starved predators indicating that *P. persimilis* relies on innate preferences when migrating. Background volatiles of uninfested plants changed the predators' responses to MeSa in a manner that depended on physiological state and iso-female line. Thus, it is possible to select for context-dependent behavioural responses of natural enemies to plant volatiles.

Key words: *Phytoseiulus persimilis*, methyl salicylate, I-tube olfactometer, preference, genetic variation, context dependence

Predatory arthropods are known to respond to herbivore-induced plant volatile chemicals (Dicke and van Loon 2000; Sabelis et al. 2007). This phenomenon prompted the hypothesis that first and third trophic levels conspire against the second: the infested plants attract the natural enemies that can reduce or eliminate herbivore pressure, whereas the predators acquire informa-

tion on the location of its prey (Dicke and Sabelis 1988; Dicke and van Loon 2000; Kessler and Baldwin 2001; but see Allison and Hare 2009). However, for such a system to evolve and function, a number of conditions have to be fulfilled (van der Meijden and Klinkhamer 2000; Janssen et al. 2002). Among them, it requires that the predators evolve behavioural responses to plant-produced volatiles induced by herbivore feeding. These responses may rely on predators learning to associate herbivore-induced plant volatiles with the presence of prey. Alternatively, predators evolved genetically determined preferences for plant volatiles induced by herbivorous prey if in the past generations predators innately responding to such volatiles (i.e., prior to any experience) had higher fitness than those that did not show such behaviour. Fitness benefit would be obtained because these volatiles were reliably coupled with the presence of prey. Therefore we expect genetic bases for predator responses to these plant volatiles that are induced by the feeding of prey, irrespective of the species of infested plant.

We investigated whether there is genetic variation in the response of the predatory mite *Phytoseiulus persimilis* Athias-Henriot to a selected herbivore-induced plant volatile, methyl salicylate (MeSa). MeSa is one of the volatile compounds consistently induced by feeding of the two-spotted spider mite (*Tetranychus urticae* Koch) – the prey of *P. persimilis* – on a variety of plant species (van den Boom et al. 2004). Empirical studies showed that it plays a key role in predator attraction to the volatile blends induced by the spider mite (de Boer and Dicke 2004a; de Boer et al. 2004; van Wijk et al. 2008, Ament et al. 2010). A genetic basis for the responses of *P. persimilis* to blends of volatile compounds has been demonstrated in two studies on selection for responses of satiated predators to the complete blend of volatiles released by spider-mite infested lima bean (Margolies et al. 1997; Jia et al. 2002). However, innate preferences for the full volatile blend of infested bean may be the result of the underlying innate response to a single compound shared by the blends of many plant species infested by the spider mite – a condition that is fulfilled by MeSa. In this study we applied an analysis using iso-female lines (David et al. 2005) to detect genetic variation in response to MeSa among *P. persimilis* lines. A significant difference in the olfactory responses among iso-female lines indicates a genetic basis for this trait, provided that systematic environmental influences are controlled for.

The odour of uninfested plants is a permanent feature of the volatile signal under natural settings. Therefore, we hypothesize that the background odours of uninfested lima bean may affect the predator's perception of MeSa and the strength of response to MeSa. Lima bean leaves heavily infested with spider mites were also used for culturing of the predator population used in this experiment and therefore any pre-conditioning of the predator to the odours

experienced in the culture would be to volatile blend of infested lima bean that it quantitatively different to the blend of uninfested bean (e.g., Dicke et al. 1990). Hence we expected that an enhanced predator response to MeSa presented with the background odours of uninfested lima bean would be a result of altered perception to MeSa when presented with a context rather than due to predator pre-conditioning to specific volatiles. Mechanically damaged plants produce increased amounts of a distinct group of volatiles called green leaf volatiles (GLVs), which are also emitted by intact plants in smaller amounts (Hatanaka 1993; Matsui et al. 2004; van den Boom et al. 2004). Thus, we term the background odours provided by the punched leaf discs used in this experiment as GLVs.

Genetically determined responses to herbivore-induced plant volatiles are particularly relevant, and subject to natural selection, in situations where predators cannot yet have learned the association between the presence of specific volatiles and prey. The predatory mite *P. persimilis* may experience such situations during the migratory phase that follows the exploitation of the previous colony of prey. These phases of the predator life history can be approximated by manipulating the predator's hunger level because food conditions provide the proximate cue for the onset of migratory behaviour (Sabelis and Afman 1994). Satiation prevails during foraging in dense colonies of spider mites and starvation induces take-off to aerial dispersal, followed by exploration of the new landing site (Sabelis and van de Baan 1983; Sabelis et al. 1984; Sabelis and van der Meer 1986; Sabelis and van der Weel 1993; Sabelis and Afman 1994; Pels and Sabelis 1999). During the migratory phase the predators encounter an environment characterized by plant volatiles most likely to be different from those experienced before; the previously learned associations of specific volatiles may be of little value in locating the prey. Therefore, we hypothesize that the innate responses to MeSa depend on the context of the test (satiated vs. starved predators).

We determined the responses of a total of 18 iso-female lines of *P. persimilis* in the olfactory tests where predators were presented with the choice of 1) MeSa in clean air vs. clean air, or 2) MeSa in the background of GLVs vs. GLVs. These tests were performed using either satiated or starved predators. Variable olfactory responses to MeSa among the iso-female lines would indicate genetic variation for this trait in the population under study. In particular, we predicted that this variation is more pronounced in starved predators and we asked to what extent this variation depends on the presence of volatiles of uninfested plants in the background.

Methods and materials

Predatory mites

The base population of *P. persimilis* was maintained in the laboratory (25 °C, 70% humidity, and light:dark = 16:8 conditions) on a diet of two-spotted spider mites (*T. urticae*) on detached leaves of lima bean plants. The culture originated from a sample of predators collected in 2002 at different locations throughout Sicily (Partinico, Scopella, Trappeto, Terrasini, Siculiana, Laghetto, Menfi, Trabia, Alcamo), where they are probably endemic (De Moraes et al. 2004). Iso-female lines were obtained by randomly selecting a number of females from the base population, which from then on were kept separately in Petri dishes. Typically in haplodiploid arthropods such lines are obtained through mating between the virgin female and her sons (oedipal mating). However, *P. persimilis*, just as probably the whole family Phytoseiidae, is pseudo-arrhenotokous, i.e., sons and daughters arise from fertilized eggs and male zygotes become haploid due to paternal genome inactivation and elimination during embryogenesis (Helle et al. 1978; Nelson-Rees et al. 1980). Hence, mating is a prerequisite for producing eggs destined to become females, as well as males. For this reason in this experiment the iso-female lines were established each by a single mated female. There were 18 lines thus established which were subsequently allowed to propagate for seven weeks before the onset of the olfactory tests (thus for at least five generations). All lines were reared in the same climate room (under conditions as described for the base population) to minimize any effect of environment on predatory behaviour. Incidental mixing among the lines was prevented by maintaining each line in a separate rearing enclosed in a plastic container with a small opening covered with gauze (mesh width = 0.07 mm; average adult predator size = 0.5 mm, average predator egg size = 0.2 mm; thus, small enough to prevent immi- and emigration) to allow for airflow. Additionally, each of these containers was surrounded by its own water barrier. Individual iso-female lines were labelled with numbers (1-18) for identification.

Tests of olfactory preference

Behavioural responses of predators to MeSa were tested in a so-called I-tube: a single straight glass tube (length 20 cm, \varnothing 0.5 cm) with a small opening (2 mm) in the middle to introduce the predatory mites (Figure 2.1). This olfactometer was provided with a trap at the end of either arm of the I-tube to collect predators. Opposite the entrance hole, another opening (\varnothing 0.5 cm) was present which was gauze-covered and served as an air-outlet. At the ends of the I-tube there were plastic trap vials (\varnothing 30 mm, height 55 mm) which in turn were connected to jars that either contained a capillary with MeSa or not. The traps were designed as an easy-to-enter-yet-difficult-to-exit vial, and were provided with a

water source (wet cotton wool). After purification by activated carbon filters, the air was flowing at 20 l/h through the jars, the vials and then the arms of the glass tube, leaving the system through the opening in the middle of the I-tube. At release in the middle of the I-tube, a mite finds itself in air streams coming from right and left, i.e., one with MeSa and the other without. Subsequently, it can move left or right in the I-tube and ultimately enters one of the trap vials or remain in the I-tube. After release of the test animals, the entrance hole was sealed with Parafilm®. Pilot experiments showed that the I-tube olfactometer produces results consistent with our knowledge of the responses of *P. persimilis* to the full blend of herbivore-induced plant volatiles (M van Wijk, unpubl.).

Per replicate experiment, a total of 25 adult females were released (except for a few cases where numbers were less than 25, yet larger than 20) sequentially. Visual cues play no role in predator orientation as the predator is blind and orients itself by a means of chemical cues. A previous study using Y-tube set-up showed that there is no effect of possible residues deposited along the path taken by an individual on the choices of the subsequent individuals (Sabelis and van der Baan 1983). After 25 min, the number of mites in each of the two trap vials was counted. The number that remained in the I-tube, was scored as ‘no choice’. For each consecutive replicate of a line a new clean I-tube was used and the side of the arm containing air with MeSa was inter-changed to exclude any unforeseen asymmetries in the experimental set-up. Per line per treatment,

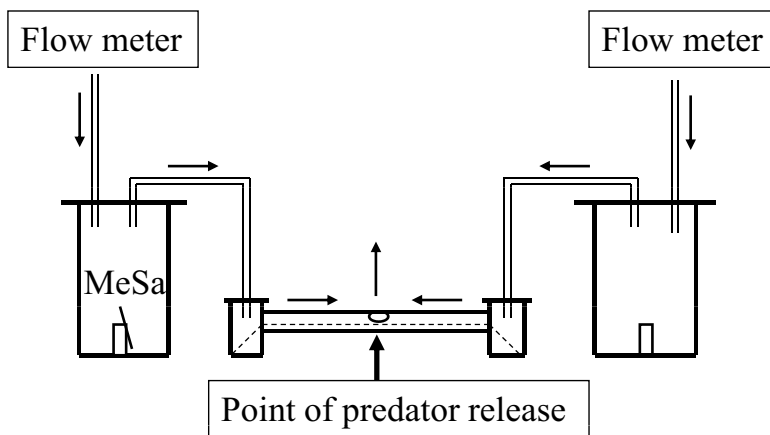


Figure 2.1 – Experimental set-up of the I-tube olfactometer. The diagram depicts a horizontal tube (the glass I-tube) with two openings, where the bigger one serves as an air-outlet. The thick vertical arrow below the I-tube indicates the location of the small opening – the point of release of the predators. The broken line indicates a thin capillary inside the glass tube to provide structure, on each side ending in a metal pin leading down into the trap vial. The trap vials were connected with plastic tubes to jars that either contain a capillary with MeSa or not. The remaining arrows indicate the direction of air-flow. Above the set-up there was a source of dispersed light (not shown).

roughly 120 mites were tested in 4-8 replicate experiments (in one case, three replicates were performed).

Synthetic MeSa (Sigma-Aldrich Fluka, pure; assay $\geq 99\%$) was offered undiluted in a small capillary (9 μl , \varnothing 0.60 mm, Omnilabo) placed in one of the jars connected to the I-tube (Figure 2.1). The air flow was led through the set-up at least two hours before the start of the test. The MeSa evaporation rate was $\pm 30 \mu\text{g/h}$. This evaporation rate was chosen based on preliminary experiments to find a concentration to which the base population exhibited a neutral (50:50) response. Such a set-up allows detection of either increased or decreased responses of the iso-female lines to MeSa with reference to the response of the base population. It is difficult to compare this evaporation rate with previous studies attempting to measure the dose-response relations, because these assessments were based on filter paper as a substrate (de Boer and Dicke 2004b; van Wijk et al. 2008). Unlike filter paper, capillaries generate a constant evaporation rate. It is also difficult to compare this evaporation rate with evaporation from infested Lima bean plants because there is hardly any data available. The only data we are aware of is 0.4-0.8 $\mu\text{g/h}$ estimated by de Boer and Dicke (2004b) from data in Dicke et al. (1999), evaporated from 10 leaves after 3 days of infestation by 50 adult female spider mites per leaf with a flow rate of 30 l/h. It is unclear at what distance from the plant this concentration is measured, and also how such concentrations of MeSa change over the time of infestation.

Experimental design

We assessed olfactory preferences of 18 iso-female lines by subjecting satiated and starved adult female predators to two types of olfactory tests involving choices between either (1) MeSa in clean air vs. clean air, or (2) MeSa with a background of green leaf volatiles (GLVs) vs. green leaf volatiles (GLVs). The background odours of GLVs were provided by discs (\varnothing 1.5 cm) punched from the leaves of 2-weeks-old, uninfested Lima bean plants (*var. Phaseolus lunatus*, Big Lima). New leaf discs were punched for each test from fresh primary leaves, and placed inside the plastic trap vials of the I-tube – one disc in each of the vials – on a ball of wet cotton wool. Airflow was allowed to pass through the I-tube for additional 15 min before the start of the olfactory test to allow the GLVs to reach the middle of the I-tube. The responses of the satiated predators were determined by testing females taken straight from the culture where they were kept in a well fed state. Starved predators were obtained by food-deprivation for 24 h prior to the olfactory tests. During the period of food deprivation mites were kept in a closed Eppendorf vial placed in a climate box at 18 °C, with water provided via a strip of wet filter paper.

The total of 18 olfactory tests performed daily on each of the 18 lines was about the maximal number feasible under the experimental protocol. The main aim of this study was to sample a large number of iso-female lines. Therefore we preferred to test as many lines as possible per day, at the expense of obtaining simultaneous replicates for each line. To minimize effects of the time of day on predatory responses we randomized the order of testing of the 18 lines each day. The number of replicates varied between four and six per hunger level for each olfactory test (i.e., tests with or without GLVs context). The tests were performed within a period of 5 weeks.

Statistical analysis

We aimed to determine (1) whether there is a genetic component in predator response to MeSa and (2) whether this behaviour is dependent on the nutritional status or the environmental context provided by the volatiles of uninfested plants in the background. To address the first question we constructed a mixed-effects model that included the iso-female line effect as the random effect; hence the response variable (i.e., a single data point) was the mean response per replicate per line based on the numbers of individuals that made a choice (hence excluding the no-choice individuals). In this analysis we tested whether the amount of variation explained by the random effect of iso-female line is different from zero. The variation due to iso-female line is a measure of the total amount of variation among tested lines that is due to variation in their genes, provided that the differences among the lines arising from heterogeneity in rearing conditions can be excluded (David et al. 2005). Therefore it comprises the additive genetic variation as well as the non-additive effects of dominance and epistasis. The amount of this variation was estimated using restricted-maximum likelihood method of the SPlus 6.2 software (Pinheiro and Bates 2002; Venables and Ripley 2002) which adjusted for the fixed effects of hunger level (satiated or starved) and GLVs context (MeSa presented alone or MeSa in the background of GLVs). Additionally we tested whether the responses of the lines changed within the period of testing such that there was a decreasing or increasing trend. To this end we also tested the covariate representing the number of days elapsed from the onset of testing the first replicate of a line within a treatment. A significant covariate would indicate that environmental effects, consistent in time, contribute to the differences among the lines. A non-significant covariate, on the other hand, would indicate that no effect of consistent differences among the rearing environment of the lines was detected, thus strengthening the argument that the variation among the lines reflects genetic differences. The number of days elapsed since the onset of testing encompassed a period of 1-2 weeks. Thus, it may have encompassed the responses of more

than one generation of predators (Sabelis 1981). A non-significant covariate would be consistent with a positive correlation between mean values in different generations (a result that supports a genetic basis for preference within iso-female lines, David et al. 2005), although the design of this study did not address this correlation explicitly. The diagnostics plots showed that the assumptions of the mixed effect model were satisfied (the random variables were normally distributed with mean zero and independent for different groups, and the within-group errors were independent and normally distributed with a mean of zero), hence the response variable was not transformed.

To address the second question we tested for the situation where different genotypes respond to MeSa differently, dependent on predator satiation level or volatile presentation (as reflected in two- and three-way interactions between the line effect and the experimental factors). Therefore in the second part of the analysis we also analyzed the probability of an individual choosing MeSa in relation to three explanatory variables: iso-female line (18 lines), hunger level (satiated or starved) and GLVs context (MeSa presented alone or MeSa in the background of GLVs) by fitting a logistic regression model with logit link function and binomial error variance in SPlus 6.2 for Windows. The response variable in this analysis was the response of an individual predator. The relevance of the explanatory variables was assessed based on the comparison of values of deviance and Akaike's Information Criterion for the models that included combinations of one or more of the three explanatory variables (Agresti 1990; Quinn and Keough 2002). We further tested the fit of the regression model with respect to given explanatory variable by comparing this model with an appropriate reduced model (i.e., a model that contains all terms of the complete model but the explanatory variable tested) using the log-likelihood ratio test (Agresti 1990). Additionally, we analyzed the effect of the three explanatory variables on the probability of an individual making a choice (i.e., the probability of individual entering any trap vial in the I-tube).

Results

The first part of this section presents the analysis of the variation detected among the iso-female lines. The second part of this section deals with the analysis of the probability of predators choosing MeSa using the factors iso-female line, hunger level and GLVs context. Finally we discuss the probability of predators making a choice (i.e., the probability of choosing either arm).

Variation among lines

The variation explained by the iso-female line was significantly different from zero ($P = 0.02$; Table 2.1), thus supporting the hypothesis that there is a genetic component in predator responses to MeSa. It is a measure of the total

Table 2.1 – The mixed-effects model of predator responses to MeSa; iso-female line was the random effect and the fixed effects included the hunger level (2 levels: satiated, starved) and GLVs context (2 levels: MeSa presented alone, MeSa in the background of GLVs). The number of days from the onset of testing the line within a given treatment was added as covariate. The significance of fixed effects, their interaction and the significance of the random effect (i.e., the hypothesis that the variance of random effect $\neq 0$) was tested using the likelihood ratio tests (Pinheiro and Bates 2000; Venables and Ripley 2002).

| Effect | | Likelihood ratio (df ₁ , df ₂) | P value | Variance |
|-------------|----------------------------|---|---------|----------|
| Random | Iso-female line | 5.25 (7, 6) | 0.02 | 0.003 |
| | Residual | | | 0.05 |
| Fixed | Hunger level | 6.75 (6, 5) | 0.009 | |
| | GLVs context | 0.05 (6, 5) | 0.81 | |
| | Days from onset of testing | 0.34 (6, 5) | 0.56 | |
| Interaction | Hunger level: GLVs context | 0.03 (7, 6) | 0.86 | |

amount of variation among the tested iso-female lines that is due to genetic variation, provided that the differences among the lines due to heterogeneity in rearing conditions are negligible. We have detected no systematic change in the average responses of the lines within the period of testing ($P = 0.56$; Table 2.1). This result supports the conclusion that the observed differences among the lines were not due to systematic differences among the rearing environment of the lines, but rather reflect genetic variation.

In this part of the analysis the effect of hunger level was found to be significant, but not the GLVs provided as context. However, inspection of the data (Figure 2.2; in each panel the ordering of lines from low to high response results in a different sequence of lines) indicates that there is variation in the responses of different lines dependent on the level of these two factors. This interaction was further explored in the second part of the analysis.

Probability of choosing MeSa

We used the logistic regression method to analyse the probability of predators choosing MeSa in relation to the factors: iso-female line, hunger level and GLVs as context. Comparison of deviance and AIC values throughout all models (that included combinations of one or more of the three effects) revealed that iso-female line, hunger level and GLVs as context, all affected the probability that a predator would choose MeSa (log-likelihood ratio test, $P_{\text{line}} < 0.001$, $P_{\text{hunger}} < 0.001$, $P_{\text{GLVs}} = 0.02$). Figure 2.2 shows the variation among iso-female lines in the proportion of individuals choosing MeSa, categorized by hunger level, when MeSa is presented alone and when it is presented with the background of GLVs. Visual inspection of these figures indicates an interaction between iso-female line, hunger level and GLVs as context. Therefore, we also

compared the deviances and AIC values of the models that included the effects as well as their interaction terms. The final model that best described predator responses included the interaction between the iso-female line and the two fixed effects (log-likelihood ratio tests: $P_{\text{line} \times \text{hunger}} < 0.001$, $P_{\text{line} \times \text{GLVs}} < 0.001$, $P_{\text{hunger} \times \text{GLVs}} = 0.41$, $P_{\text{line} \times \text{hunger} \times \text{GLVs}} < 0.001$). Figure 2.3 exemplifies the three-way interaction by presenting the responses of some of the iso-female lines.

Probability of making a choice

We constructed a logistic regression model describing the probability that a predator will make a choice in the olfaction test (i.e., it will choose either arm). The full model (i.e., line + test + hunger) explained the responses better than the null model (log-likelihood ratio, $P < 0.001$). All main effects were individually significant ($P_{\text{line}} < 0.001$, $P_{\text{test}} < 0.001$, $P_{\text{hunger}} = 0.03$, see Figure 2.4).

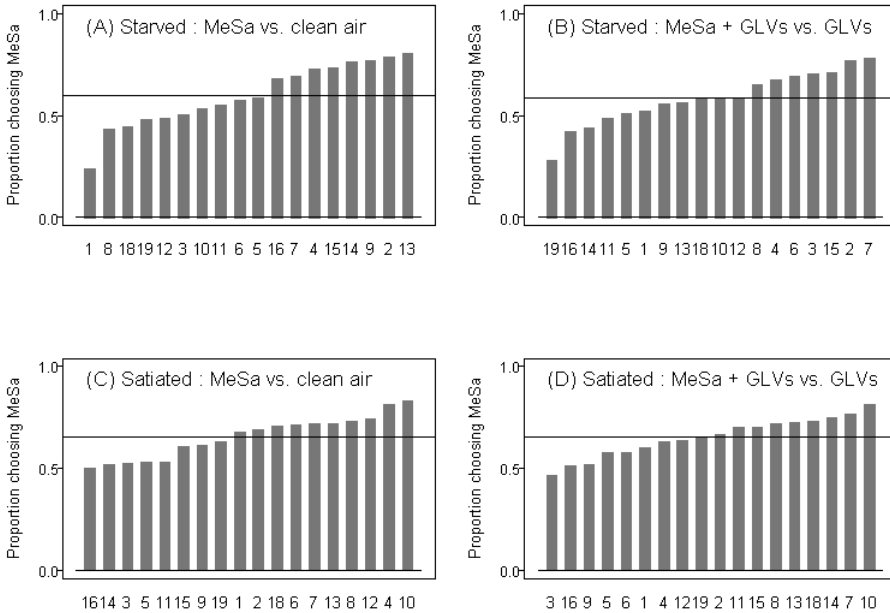


Figure 2.2 – The responses of eighteen iso-female lines of *Phytoseiulus persimilis* by starved predators in the tests (A) MeSa plus clean air vs. clean air, (B) MeSa plus the background of GLVs vs. GLVs, and by satiated predators in the tests (C) MeSa plus clean air vs. clean air, (D) MeSa plus the background of GLVs vs. GLVs. The bars show the proportion of individuals choosing the arm containing MeSa (y-axis) calculated for each line (x-axis). The horizontal lines indicate the average responses obtained by pooling replicates over all iso-female lines within each of the experimental treatments. Note that in each panel the ordering of lines from low to high response results in a different sequence of lines, e.g., line 10 is on the right in (C) and (D) but on the left in (A) and in the middle in (B).

Discussion

In this study we asked whether there is genetic variation in responses of *P. persimilis* to the plant volatile MeSa in a field-collected population. To this end we employed the iso-female line technique where the variation among iso-female lines is interpreted as the variance due to differences in their genes provided the heterogeneity among the rearing environments does not systematically influence the behaviour of individual lines. We showed that the variation in predator responses to MeSa explained by the iso-female line was significantly different from zero. From this result we infer that the studied population harbours genetic variation in olfactory responses to MeSa. There is more variation among the lines when the predators are starved, yet variation is also present in satiated mites. However, it is only when predators are starved that we observe the full range of responses ranging from avoidance to preference. This pattern occurs when predators have a choice of both MeSa presented alone and MeSa against a background of GLVs.

To date, studies that measured the attractiveness of MeSa (or other herbivore-induced plant volatiles) to natural enemies did not control for genetic back-

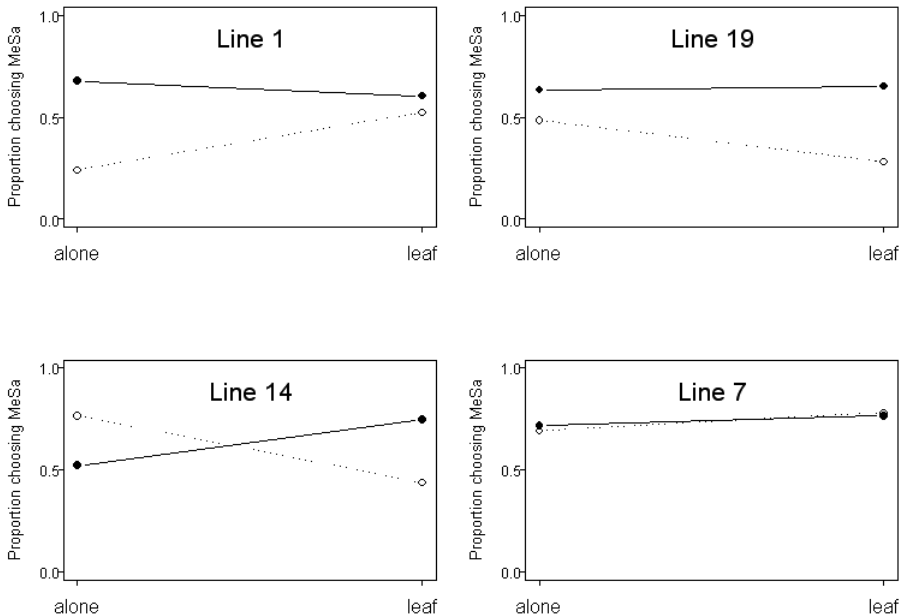


Figure 2.3 – Illustration of the interaction between iso-female line, hunger level and GLVs context in selected iso-female lines; the graphs present the mean proportions of individuals choosing MeSa (values on the y-axis) categorized by the factors. Open circles connected by dashed line refer to the responses of the starved predators, closed squares connected by solid line to the responses of the satiated predators, and the x-axis presents the categorization with respect to GLVs context.

ground (e.g., de Boer and Dicke 2004b; van Wijk et al. 2008). However our results show that predator populations may be genetically variable for the tested responses, and the behaviour of various genotypes may influence the average response measured at the population level. Thus, the average population response may be characterized by a weak attraction or no clear preference if genotypes of extreme innate preferences are tested together and their responses pooled. To illustrate this, we calculated the proportions of individuals choosing MeSa by pooling the replicates over all iso-female lines for each of the experimental treatments. These values (shown in Figure 2.2 as horizontal lines) may be treated as estimates of the responses to MeSa measured at the level of the base population from which the lines originated, under the assumption that different iso-female lines (i.e., genotypes) are represented. Indeed there is very little difference among the experimental treatments.

The inclusion of GLVs in the background of MeSa presentation does not, at first glance, appear to change the overall result that a wider range of responses is expressed by starved predators (compare Figure 2.2a, c and 2.2b, d). However, closer inspection reveals that adding the background of GLVs does

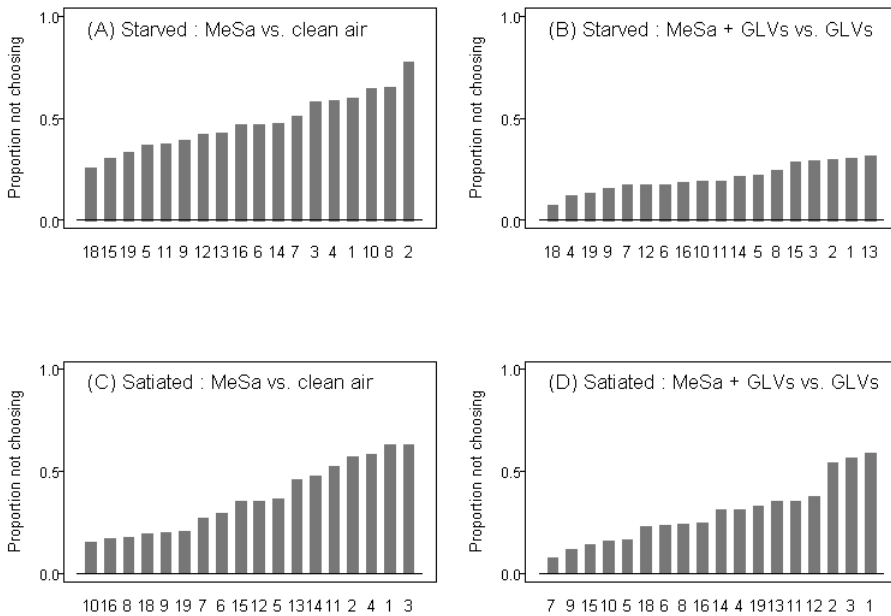


Figure 2.4 – Variation in the mean proportion of no-choice individuals (y-axis) in the 18 iso-female lines (x-axis) of *Phytoseiulus persimilis* categorized by hunger level and GLVs context. No-choice individuals are those that remained in the glass tube for the duration of the test, i.e., did not make a choice. The panels present the proportions observed for starved predators in the tests (A) MeSa plus clean air vs. clean air, (B) MeSa plus the background of GLVs vs. GLVs, and for satiated predators in tests (C) MeSa plus clean air vs. clean air, (D) MeSa plus the background of GLVs vs. GLVs.

change the behaviour of individual lines. We observe a whole range of patterns in the responses of the lines that exemplify a significant three-way interaction between iso-female line, hunger level and GLVs as background (except for line 5 that shows indifferent responses in all tests without exception). In particular, there are iso-female lines characterized by an extreme response to MeSa if the predators are starved and the choice-test involves MeSa in the background of GLVs whereas their responses in all the other treatments are indifferent (lines 19 and 3). The example of these lines may indicate that testing the responses of starved predators to MeSa presented in the background of GLVs are essential conditions for eliciting an innate response of the predators. However, there are also lines characterized by extreme responses to MeSa if the predators are starved and the choice-test involved MeSa presented alone (e.g., lines 1 and 9), and predator responses in all the other treatments are indifferent. The example of these lines may thus indicate that adding GLVs as a background to MeSa confounds the predator's perception of this volatile. The remaining iso-female lines show yet other patterns of interaction between the factors iso-female line, hunger level and GLVs as background (see Figure 2.3 for examples).

We conclude therefore that the responses of *P. persimilis* to MeSa are context-dependent in that they depend on the genetically determined preference of the tested individuals (i.e., their genotypes) as well as on the environmental context in which the volatile is presented. Further research is needed to understand how a biologically relevant single compound cue is perceived and interpreted when presented in a mixture (van Wijk 2007; Shröder and Hilker 2008), a more realistic situation in nature. In our study the addition of a background odour of GLVs increased the proportion of individuals making a choice in olfaction tests (Figure 2.4). Thus, we can conclude that GLVs affected the predator's perception of MeSa. This effect of GLVs on the willingness to choose varied among individual lines (which indicates a genetic basis also in this aspect of behaviour).

Given the genetic basis in the responses to MeSa in this population collected from the field, we conclude that there is opportunity for an evolutionary response on the part of the predators to using MeSa as a plant signal indicating prey presence. The distribution of prey is patchy and unpredictable under natural conditions. Hence, it is expected that *P. persimilis* undergoes long-lasting food deprivation during dispersal (Sabelis and Afman 1994). Our findings may indicate stronger reliance of the predator on genetic predispositions during this migratory phase in which the predator alternate passive aerial dispersal and local exploration for prey patches. Thus, natural selection may be more efficient in shaping the olfactory responses when the predators are starved and therefore probably migrating.

Our results show that the responses of *P. persimilis* to plant volatiles are far from simple. Even in the case of a single plant volatile, we observed genetic variation expressed in a context-dependent manner. Iso-female lines created from a field-collected base population show responses to MeSa ranging from preference to avoidance (although the latter response is much less common). The variation is still present in the population and conflicts with the prediction that preference for environments associated with the presence of MeSa has fitness benefits (i.e., helps locating the prey) and should therefore prevail. This raises the question of the mechanism that maintains the observed genetic variation in the field; the question could be addressed with selection experiments for olfactory responses to single volatile compounds and mixtures. Our results suggest that such experiments will be more successful if conducted on starved predators.

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3

Innate responses of predatory mite *Phytoseiulus persimilis* to a herbivore-induced plant volatile

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Submitted manuscript

Abstract – The responses of the predatory mite *Phytoseiulus persimilis* to herbivore-induced plant volatiles are at least partly genetically determined. Thus, there is potential for the evolution of this behaviour by natural selection. We tested whether distinct predator genotypes with contrasting responses to a specific herbivore-induced plant volatile, i.e., methyl salicylate (MeSa), could be found in a base population collected in the field (Sicily). To this end, we imposed purifying selection on individuals within iso-female lines of *P. persimilis* such that the lines were propagated only via the individual that showed either a preference or avoidance of MeSa. The responses of the lines were characterized as the mean proportion of individuals choosing MeSa when given a choice between MeSa and clean air. Significant variation in predator responses was detected among iso-female lines, thus confirming the presence of a genetic component for this behaviour. Nevertheless, we did not find a significant difference in the response to MeSa between the lines that were selected to avoid MeSa and the lines selected to prefer MeSa. Instead, in the course of selection the lines selected to avoid MeSa shifted their mean response towards a preference for MeSa. An inverse, albeit weaker, shift was detected for the lines selected to prefer MeSa. We discuss the factors that may have caused the apparent lack of a response to selection within iso-female line in this study and propose experimental approaches that address them.

Key words: *Phytoseiulus persimilis*, methyl-salicylate, I-tube olfactometer, genetic variation, preference, avoidance, artificial selection

Plants infested with herbivores release specific volatile compounds that are involved in the attraction of natural enemies of these herbivores (Dicke and Sabelis 1988; Dicke and van Loon 2000; Sabelis et al. 2007). It is well established that the composition of the blends of volatiles depends on the species of herbivore (Sabelis and van de Baan 1983; De Moraes et al. 1998; Du et al. 1998) and the degree of infestation (Du et al. 1998; Maeda and Takabayashi 2001; Nachappa et al. 2006). Therefore, the hypothesis was put forward that herbivore-

induced plant volatiles may serve the natural enemies, such as predatory mites, as a signal of prey presence (Dicke and Sabelis 1988; Dicke and van Loon, 2000; Kessler and Baldwin, 2001; Janssen et al. 2001). However, a single herbivore species may induce qualitatively and quantitatively different volatile blends on different plant species (van den Boom et al. 2004). Moreover, in natural settings the blends of volatiles produced by plants infested with various herbivore species will mix (Shröder and Hilker 2008). These results beg the question whether predators can extract a signal of prey presence from the variation in the blends of herbivore-induced plant volatiles. Adaptive learning may serve to update predator preferences for specific volatiles (or their mixtures) if these are experienced in the presence of suitable prey (Drukker et al. 2000; de Boer and Dicke 2004a; de Boer et al. 2005; Takabayashi et al. 2006). Moreover, a genetically determined response to specific volatiles may evolve by natural selection if these volatiles are consistently associated with the presence of suitable prey and genetic variation in predator response to these volatiles exists (Allison and Hare, 2009).

We studied the responses of inbred iso-female lines established from a population of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) that was collected in the field. In particular, we asked whether it is possible to obtain lines of *P. persimilis* expressing contrasting responses to a specific plant volatile, methyl salicylate (MeSa). MeSa is consistently part of the blend of volatiles induced by feeding of the herbivorous mite *Tetranychus urticae* Koch (Acari: Tetranychidae) – the prey of *P. persimilis* – on a variety of plant species (van den Boom et al. 2004). *Phytoseiulus persimilis* is attracted to a range of concentrations of MeSa presented in pure form (Dicke et al. 1990; de Boer and Dicke 2004b; de Boer et al. 2004; van Wijk et al. 2008). Using gene-silencing techniques, Ament et al. (2010) dramatically reduced the emission of MeSa in tomato plants infested with *T. urticae* without changing the induced emission of the remaining volatiles and found that *P. persimilis* no longer differentiated between the infested and uninfested plants. Together, the evidence supports the hypothesis that the response of *P. persimilis* to MeSa may aid the predator in its search for prey and thus influence its fitness, although evidence for life-time fitness benefits under natural settings is yet to be shown. Furthermore, natural populations of *P. persimilis* harbour genetic variation in their response to MeSa when offered as a pure compound, against a background of volatiles from uninfested plants (Sznajder et al. 2010), as well as genetic variation in the response to the blends of plant volatiles induced by two-spotted spider mites on Lima bean plants (Margolies et al. 1997; Jia et al. 2002). Thus, there is potential for the evolution of predator responses to MeSa through natural selection.

We selected for genotypes with contrasting responses of *P. persimilis* to MeSa when offered as a pure compound. The selection was performed on indi-

viduals within iso-female lines and two groups of iso-female lines were established where in one group the lines were propagated via females that showed a preference for MeSa, whereas in the second group the lines were propagated via females that avoided MeSa. This procedure aimed at setting apart and fixing genotypes distinct with respect to predator response to MeSa in genetically homozygous lines. Lines were thus purified in six rounds of selection, and the response to MeSa was measured after three and six rounds.

Materials and methods

Predators

The base population and the iso-female lines of *P. persimilis* were maintained in a climate room (25 °C, 70% humidity, and light:dark = 16:8) on a diet of *T. urticae* offered on detached leaves of Lima bean plants. Fresh leaves of Lima bean plants infested by two-spotted spider mites were provided on a daily basis to each individual population. The base population originated from a sample of predators collected in 2002 at different locations throughout Sicily (Partinico, Scopella, Trappeto, Terrasini, Siculiana, Laghetto, Menfi, Trabia, Alcamo). Thus, it was presumably genetically variable, although local populations of *P. persimilis* are generally considered to be inbred under natural settings (Helle and Overmeer 1973).

Selection within iso-female line

Previous work (Sznajder et al. 2010) showed that variation among iso-female lines in their mean response to MeSa is greater when the predators are starved. Therefore in this study the selection was conducted on starved predators. These were obtained by food deprivation applied for 24 h prior to a selection test. During the period of food deprivation females were kept in an Eppendorf vial at 18 °C with water provided in a strip of wet filter paper. The vial had a gauze-covered opening to allow for airflow but was otherwise sealed.

Selection criteria were applied to individual females within iso-female lines. The iso-female lines were established by mated females selected from the base population in the first round of selection (a single binary choice-test for MeSa vs. clean air; the details of this choice-test follow in the next section) as illustrated in Figure 3.1. Typically in haplodiploid arthropods such lines are obtained through mating between a virgin female and her sons. However, *P. persimilis* is pseudo-arrhenotokous, i.e., sons and daughters arise from fertilized eggs and male zygotes become haploid due to paternal genome inactivation and elimination during embryogenesis (Helle et al. 1978; Nelson-Rees et al. 1980). Hence, mating is a prerequisite for producing eggs destined to become females as well as males. Therefore, in order to establish iso-female lines we selected

females that had already mated, instead of virgin females, and maintained these lines by brother-sister mating. By doing so we did not control for the genetic contribution of males to the next generation. However, assuming additive genetic variation for the selected trait and random mating, the confounding effect of the genetic contribution via unknown males was expected to be eradicated in the course of the selection rounds.

In the first selection round (Figure 3.1) we established two groups of iso-female lines originating either from (1) the females that showed a preference for MeSa (15 lines, referred to as Plus lines) or (2) the females that showed an avoidance of MeSa (9 lines, referred to as Minus lines). The females selected in the first round are further referred to as founders of the iso-female lines (or founders for shorthand). Each iso-female line was further propagated via females whose responses were consistent with the response of the founder of their line. Propagation of the lines was performed on the basis of single choice-tests (see next section) and the females that made the same choice as the

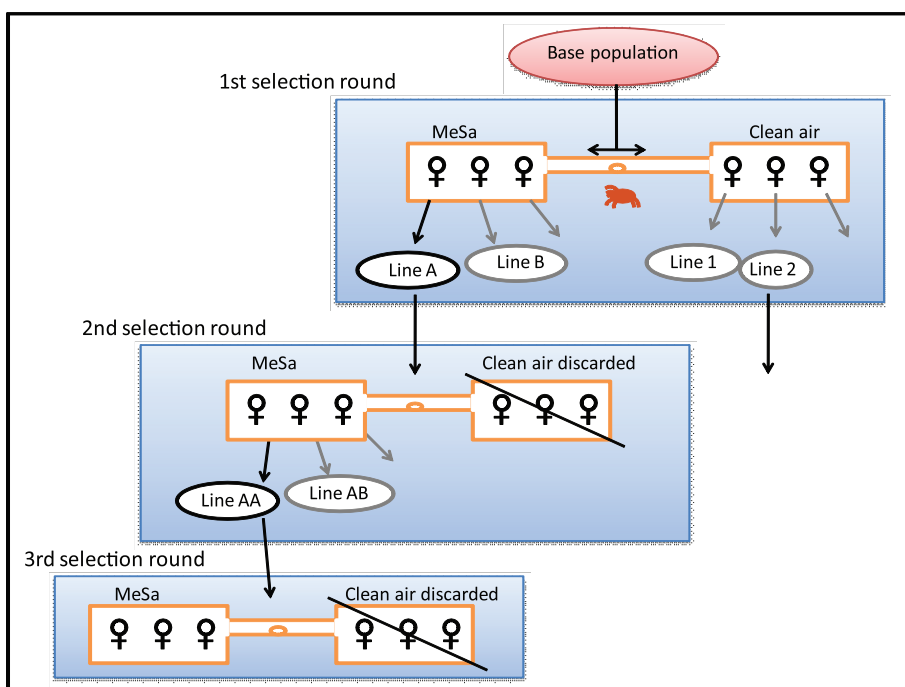


Figure 3.1 – Selection within iso-female lines is illustrated for the special case of the single line A and its descending lines during the first three selection episodes (see *Selection within iso-female line* for details). Note that the label of the iso-female line carries information on relatedness among the lines, i.e., first letter/number indicates the identity of the female selected in the first round of selection (i.e., founder), second letters/numbers indicate the identity of her daughters selected to continue the line by each establishing a new line, and so on.

founder of their line were placed each in a separate Petri dish to establish descendant iso-female lines. We imposed such bottlenecks within each line at intervals of about 2.5 weeks.

In total we imposed six rounds of selection. However, using the above procedure the number of lines would grow exponentially with every selection round (even though some lines failed to establish). Therefore, after three selection rounds we temporarily halted the experiment in order to evaluate the responses of already established lines. The mean responses of these lines were evaluated relative to the mean response of the base population measured at the start of the selection experiment. Selection was continued in Plus lines where the mean proportion of individuals choosing MeSa was not lower than in the base population (i.e., 0.64), and in Minus lines where the mean proportion was not higher than in the base population. Based on this criterion we resumed selection in four Plus lines and in three Minus lines. Thus, after another three rounds of selection we established 19 Plus lines and 14 Minus lines.

Genetic exchange among the iso-female lines was prevented by maintaining each line in a sealed large Petri dish with a small opening covered with gauze (mesh width = 0.07 mm; average adult predator size = 0.5 mm, average predator egg size = 0.2 mm; thus, the mesh width is small enough to prevent immigration and emigration) to allow for airflow. The Petri dish used for rearing was accessed by removing the lid on an alcohol-cleaned surface (to prevent immigration of predators into the opened Petri dish).

Olfactory choice-test

We used a choice-test between MeSa in pure form and clean air to (1) select individual females with respect to their response to MeSa within iso-female lines, and to (2) evaluate the mean responses of these lines after three and six rounds of selection. We used a single choice-test to determine the behaviour of the predator in order to exclude the possibility that the experience with the presentation of MeSa in the experimental set-up influenced its response to MeSa. Individuals that chose to walk towards the clean air source were interpreted as avoiding MeSa, while individuals that chose to walk towards the MeSa source were interpreted as having a preference for MeSa. The responses of the iso-female lines were calculated as the mean proportion of females choosing MeSa (the number of adult females per replicate experiment varied between 17 and 25).

The choice-test was conducted in a so-called I-tube olfactometer: a single straight glass tube (length 20 cm, \varnothing 0.5 cm) with a 2-mm opening in the middle to introduce the predatory mites; Sznajder et al. 2010). This olfactometer was provided with a trap at the end of either arm of the I-tube to collect predators. Opposite the entrance hole, another opening (\varnothing 0.5 cm) was present which was

gauze-covered and served as an air-outlet. At the ends of the I-tube there were plastic trap vials (\varnothing 30 mm, height 55 mm) which in turn were connected to jars that either contained a capillary with MeSa or not. The traps were designed as an easy-to-enter-yet-difficult-to-exit vial, and were provided with a water source (wet cotton wool). After purification by activated carbon filters, air was flowing at 20 l/h through the jars, the vials and the arms of the glass tube, leaving the system through the opening in the middle of the I-tube. At release in the middle of the I-tube, a predatory mite finds itself in air streams coming from right and left, i.e., one with MeSa and the other without. Subsequently, it can move left or right in the I-tube and ultimately it enters one of the trap vials or it stays in the I-tube. After release of the test animals, the entrance hole was sealed with Parafilm®. Pilot experiments showed that the I-tube olfactometer produces results consistent with our knowledge of the responses of *P. persimilis* to herbivore-induced plant volatiles (M van Wijk, unpubl.).

Within a single replicate experiment a group of females was released sequentially into the I-tube, i.e., the individuals were released in the olfactometer one after the other. Visual cues play no role in predator orientation as the predator is blind and orients itself by means of chemical cues. Previous studies using a Y-tube set-up showed that there is no effect of possible residues deposited along the path taken by an individual on the choices of subsequent individuals (Sabelis and van de Baan 1983; van Wijk et al. 2010). After 25 min, the number of mites in each of the two trap vials was counted. The number that remained in the I-tube, was scored as ‘no choice’. For each replicate of a line, a new clean I-tube was used and the side of the arm containing air with MeSa was interchanged to exclude any effects of unforeseen asymmetries in the experimental set-up.

Synthetic MeSa (Sigma-Aldrich Fluka, pure; assay $\geq 99\%$) was offered undiluted in a small capillary (9 μ l, \varnothing 0.60 mm, Omnilabo) placed in one of the jars connected to the I-tube. The air flow through the set-up was started at least 2 h before the start of the test. The MeSa evaporation rate was ± 30 μ g/h. This evaporation rate was chosen based on the responses elicited in the general population. In particular, in order to increase our chances of detecting selection for both an increased and decreased response to MeSa we used the concentration that elicits neutral responses (50:50) in the base population.

Statistical analysis

We hypothesized that the propagation of iso-female lines through individuals that exhibited either preference for MeSa or aversion to MeSa would lead to a shift in the mean responses of those lines towards, respectively, preference for MeSa or aversion to MeSa. Therefore, we asked whether there was a significant

difference in the mean response to MeSa between the Plus lines and the Minus lines after three and (or) six rounds of selection. Moreover, we wished to make inferences about the sources of random variation due to iso-female line. To this end, we constructed a mixed-effects model (Pinheiro and Bates 2000; Venables and Ripley 2002) that included the fixed effects of (1) the selection treatment (two levels: selection for a preference for MeSa, selection for an aversion to MeSa), (2) the time point at which the average responses of the iso-female lines were determined (two levels: after the 3rd round of selection, after the 6th round of selection), and (3) the proportion of no-choice individuals as a covariate. The model assesses the effect of these factors on the proportion of individuals choosing MeSa calculated for each replicate. The random effect consisted of the factor iso-female line nested in the factor founder (where founder was the female selected in the first round of selection, see *Selection*). Such a grouping structure reflected relatedness among the iso-female lines as determined by the first common founder of these lines.

The diagnostics plots for the untransformed response variable showed that the assumptions of the mixed-effects model were satisfied (random variables were normally distributed with mean zero and independent for different groups, and within-group errors were independent and normally distributed around a mean of zero). For this reason the analysis was performed on untransformed data, using SPlus 6.2 software (Pinheiro and Bates 2002). Significance of the fixed effects, their interaction and significance of the random effect was tested using likelihood ratio tests (Pinheiro and Bates 2000; Venables and Ripley 2002).

Results

The behaviour of the base population did not change during the experiment. The mean proportion of individuals choosing MeSa was 0.64 at the start (SE = 0.15, $n = 57$) as well as at the end of the experiment (SE = 0.13, $n = 101$). In contrast, after three rounds of selection the mean responses of the iso-female lines were variable and include preference and aversion in both the Plus and the Minus lines (Figure 3.2a). This variation among the iso-female lines was slightly lower after six rounds of selection (Figure 3.2b). However, the variation in the mean responses of the Minus lines was shifted in the direction opposite to the selected criterion, as compared to the mean responses shown by their ancestral lines after three rounds of selection.

We did not find a significant difference in the mean responses to MeSa between the Plus and Minus lines (likelihood ratio test: $P_{\text{selection}} = 0.09$, Table 3.1). This was the case after three rounds of selection as well as after six rounds of selection (likelihood ratio test: $P_{\text{time-point}} = 0.21$, Table 3.1). However, we found an interaction between the selection and the time-point at which the

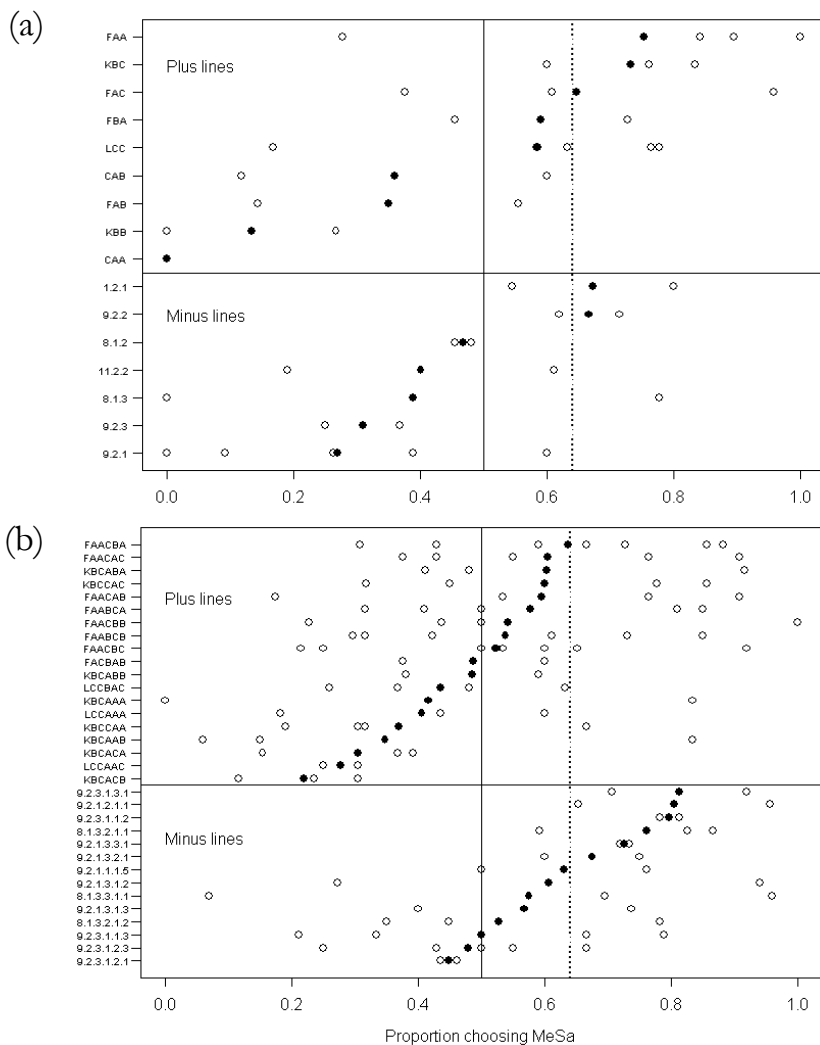


Figure 3.2 – The responses of the iso-female lines propagated via individuals that chose MeSa (labelled with letters) and the lines propagated via individuals that avoided MeSa (labelled with numbers) obtained after (a) three and (b) six rounds of selection. The empty circle represents the proportion of individuals choosing MeSa per replicate ($n = 15\text{--}25$ individuals) calculated excluding the no-choice individuals. The filled circles indicate the mean proportions of individuals choosing MeSa calculated per iso-female line. The dotted vertical line indicates the response of the base population at the start of the selection. Note that not all the lines in (a) were selected past the third selection round (see *Selection within iso-female line* and Figure 3.3). Note also that the relatedness among the lines is indicated by the labels of the lines (see also Figure 3.1). Hence, e.g., the lines FAA, FAC, and FBA share their great grandmother F (i.e., the founder), and the lines FAA and FAC share also their grandmother A.

Table 3.1 – Mixed-effects model of predator response to MeSa in the two-choice test: MeSa versus clean air. The random effect consists of the iso-female line nested in the founder. Fixed effects include the selection (levels: Plus, Minus), the number of selection rounds imposed on the lines (levels: three, six) and the proportion of individuals making no choice added as covariate. The amount of variation explained by grouping with respect to Founder, iso-female line and the residual variance is presented in the form of standard deviations (SD).

| Effect | | Likelihood ratio (df ₁ , df ₂) | SD | P value |
|-------------|---------------------------------|---|--------|---------|
| Random | Founder | (9, 8) | 0.08 | 0.02 |
| | Iso-female line | (9, 8) | 0.0004 | 0.10 |
| | Residual | | 0.29 | |
| Fixed | Selection treatment | (8, 7) | | 0.09 |
| | Time point | (8, 7) | | 0.21 |
| Covariate | Proportion no choice | (8, 7) | | 0.25 |
| Interaction | Selection treatment: Time point | (9, 8) | | 0.02 |

responses of the lines were measured (likelihood ratio test: $P_{\text{selection: time-point}} = 0.02$, Table 3.1). Given the non-significant effect of the selection and the time-point factors, this interaction indicates that there was a significant difference between the Plus and Minus lines at the end of the selection (but not half-way through the selection) and this difference was due to a change in behaviour of the Minus lines but not the Plus lines. Figure 3.2 reveals that the change in the response of the Minus lines was opposite to the direction selected. After six rounds of selection the Minus lines shifted their average responses towards a preference for MeSa, when compared to the responses of their ancestor lines measured after three episodes of selection.

The analysis was performed on data including the responses of the iso-female lines that were not subject to selection past the third round (see *Selection* and *Statistical analysis*). In order to further explore the responses of the lines as a function of the behaviour of their ancestor lines we plotted the mean responses of the lines obtained after six rounds of selection as a function of the behaviour of their ancestor lines that were measured after three rounds of selection. This relationship is presented in Figure 3.3 showing a shift in mean responses of the Minus lines but also an equivalent shift in the responses of the Plus lines (albeit weaker). This result for the Plus lines was not detected in the above analysis using the mixed-effects model presumably due to the greater variation among the Plus lines.

We found a significant effect of founder (likelihood ratio test: $P_{\text{founder}} = 0.02$, Table 3.1), i.e., the grouping with respect to founder (between-founder variation) explained a significant amount of variation in the behaviour of the lines. This confirms that there was genetic variation in predator responses to MeSa in the base population and the founder effect indicates a genetic back-

ground that was ‘frozen’ within the iso-female lines at the start of the selection experiment. Adding the iso-female line effect (nested in the founder effect) did not explain more variation in predator responses to MeSa (likelihood ratio test: $P_{\text{line}} = 0.10$, Table 3.1). The variation explained by the iso-female line effect was smaller (by orders of magnitude) than the variation explained by the founder effect (Table 3.1). The iso-female line effect represents the deviation in the behaviour of individual lines from the mean response of the group of lines that shared a founder (within-founder variation). Thus, the non-significant effect of the iso-female line indicates that there was little within-founder variation among the lines while controlling for the effect of the main factors selection treatment and time point. Moreover, we observed a relatively large amount of residual variance that represents the deviation of the individual replicates from the average response of the iso-female lines (the within-line variability, Table 3.1). Thus,

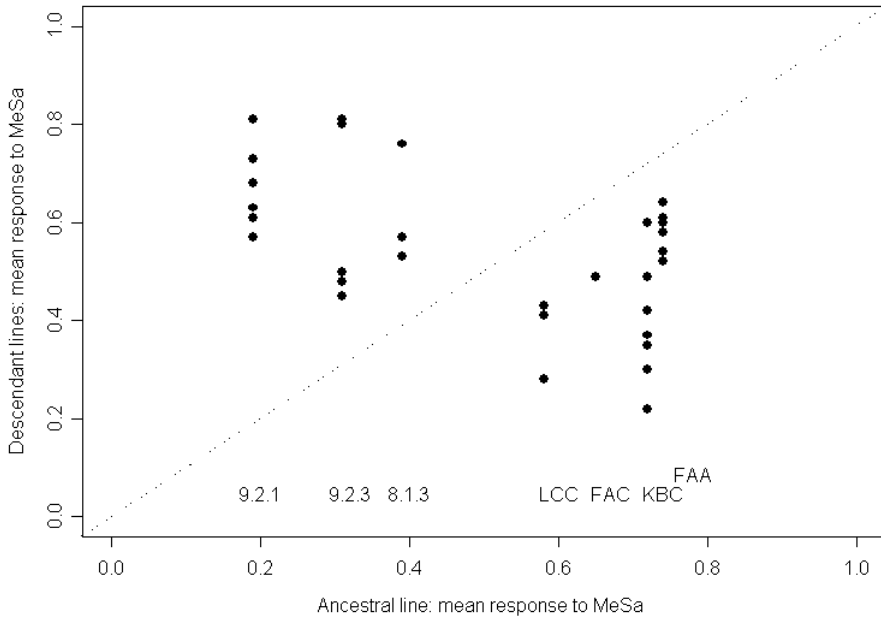


Figure 3.3 – Mean responses of the descendant lines after six rounds of selection (y-axis) as a function of the responses of their ancestral lines after three rounds of selection (x-axis). Only the names of the ancestral lines are shown; letter labels refer to the lines selected for the preference of MeSa (Plus lines) while number labels refer to the lines selected for the aversion of MeSa (Minus lines). The circles represent the mean proportions of individuals choosing MeSa calculated per line (for the responses of individual replicates see Figure 3.2). Successful selection for extreme responses to MeSa would result in a positive correlation between these proportions such that the descendant Plus lines would lie either on or above the dotted diagonal line, while the descendant Minus lines would lie on or below the diagonal line.

there was a substantial environmentally determined component in predator responses to MeSa as measured in the olfactory test used in this study.

The average proportion of individuals not making a choice amounted to 0.16. The covariate representing the proportion of individuals that made no choice in the olfactory test was not significant (likelihood ratio test: $P_{\text{proportion no-choice}} = 0.25$, Table 3.1), i.e., there was no relationship between the mean response to MeSa in a single replicate test and the number of individuals that made no choice in this replicate.

Discussion

We showed that the amount of variation in predator responses to MeSa explained by the founders of the iso-female lines was significantly different from zero, thus confirming that the responses of *P. persimilis* to MeSa have a genetic component (Sznajder et al. 2010). However, we have not found expected differences in the behaviour between the iso-female lines maintained via individuals that chose MeSa (Plus lines) and the lines maintained via the individual that avoided MeSa (Minus lines). Instead, we observed a shift in the mean response to MeSa in the direction opposite to the selection criterion, i.e., the responses of the Minus lines at the end of selection were significantly shifted towards preference for MeSa as compared to the responses of their ancestor lines half way through the selection (Figure 3.3). The corresponding pattern, albeit weaker, was observed for the Plus lines.

Our experiment aimed at purifying the responses of individual lines and to this end we repeatedly re-established the lines via single mated females that expressed the behaviour in question (i.e., either preference of MeSa or avoidance of MeSa). Therefore, this procedure led to obtaining genetically homozygous lines. Such lines can be further used to study the genetic determination of predator response to MeSa by an analysis of reciprocal crosses between iso-female lines, for example to test whether more extreme or intermediate responses to MeSa can be observed in heterozygous genotypes. Inbreeding in the lines of *P. persimilis* was less likely to be associated with inbreeding depression because in haplodiploids deleterious alleles are immediately expressed in haploid males and therefore can be purged by natural selection (Crozier 1985; Werren 1993; Henter 2003; Charlesworth and Willies 2009; unless these alleles have female-limited effect; Saito 2001; Tien et al., submitted).

Re-establishing the lines via single mated females creates the conditions favourable for random genetic drift. If in this study random genetic drift was stronger than selection within iso-female line, then the genetic differences between and within the Plus and Minus lines would become fixed by chance. In particular, random genetic drift is expected to overcome selection if $N_e s < 1$,

i.e., the product of the effective population size and selection coefficient is less than unity (Hartl and Clark 1989). To illustrate this relationship, suppose that predator response to MeSa is determined by a single gene that has two alleles: an allele for preference of MeSa and an allele for the avoidance of MeSa. Hence, in this study the selection would be effective if selection coefficient was $s > 0.67$ (given that the population is started by a single mated female and N_e in a haplo-diploid system is given by $(9 \cdot N_f \cdot N_m) / (2N_f + 4N_m)$; Hedrick and Parker 1997). In this experiment, in the lines selected for preference for MeSa, the genotypes that showed avoidance of MeSa were eliminated. Hence their fitness was assumed to be $W=0$, and their selection coefficient $s=1$ (in the lines selected for avoidance of MeSa the selection coefficient of the genotype showing preference for MeSa $s=1$). In reality the selection was performed on phenotypes in a single selection test and it cannot be expected that these phenotypes always reflected their genotypes. Therefore the correct genotypes may not have been always selected for, and in such situations the selection coefficient of the out-selected genotypes would have been less than one. However, in this theoretical system, for the selection coefficient to be lower than 0.67 the rate of selecting a phenotype inconsistent with its genotype (e.g., a phenotype that shows preference of MeSa while its genotype would avoid MeSa) must be equal to or more than 0.25 ($s=1-W=1-P_a/P_A$, where P_X is the probability of genotype X contributing to the next generation). This means that in about 75% of selected cases the phenotype reflects its genotype but in 25% of these cases it does not.

The above analysis serves as an illustration only because the details of the genetic architecture of the response to MeSa in *P. persimilis* are not known; in fact our study is among the first (Sznajder et al. 2010) to provide evidence that there is a genetic basis for this behaviour. Therefore the number of loci influencing this trait (and hence the appropriateness of the above model) or the magnitude of the error in selecting wrong genotypes based on their phenotypes are yet unknown. Thus, we cannot fully exclude the possibility that the random genetic drift may have played a role in some of the selection episodes.

It is informative to discuss other factors that may have contributed to the differences in the average responses to MeSa among the Plus and Minus lines in this experiment. In particular, the genetic component for a trait inferred from the differences among iso-female lines (as in this study and in Sznajder et al. 2010) comprises additive as well as non-additive genetic effects. This role of non-additive vs. additive effects in the genetic determination of predator response to MeSa may be explored by analysis of reciprocal crosses between iso-female lines that are different with respect to this trait (e.g., Carton et al. 1992).

For the correct interpretation of a change in the behaviour of iso-female lines due to an experimental treatment the influence of general environmental

effects that pertain to common rearing conditions must be minimised (David et al. 2005). However, in order to explain the results of this study, such general environmental effects would have to influence the Plus lines and Minus lines in a consistently different way. Moreover their effect would have to change between the mid-point (after three rounds of selection) and the end-point (after six rounds of selection) of the experiment. Finally, environmental effects would most likely be also reflected in a shift of the mean response of the base population (as this population was maintained in the same climate room as the selection lines but physically separated). The mean responses to MeSa of the base population, however, did not change as measured between the start and the end of the selection experiment (although fluctuations in the responses of the base population in-between the measurements cannot be ruled out). Based on these results we conclude that general environmental effects may have played only a minor role.

In this study we propagated the lines based on the behaviour of mated females, i.e., for practical considerations the females were allowed to mate in the culture and, thus, prior to the selection test. Therefore, the responses to MeSa of the males were not subject to selection and the genetic contribution of the males to the next generation was not controlled. Assuming additive genetic variance for the selected trait and random mating this effect of uncontrolled genetic contribution of the males should be eradicated in the course of selection rounds. Suppose, however, that mating is non-random such that, for example, females that show preference for MeSa mate more often with males that avoid MeSa. The resulting disassortative mating may lead to responses of the lines fluctuating within the spectrum between preference and aversion as observed in different generations of our selection experiment. Any form of non-random mating would hinder selection and it may possibly be realized with respect to a trait correlated with olfactory responses, rather than with respect to the response to MeSa itself. There is evidence of non-random mating in *P. persimilis*. Virgin females were shown to mate more readily with unrelated mates in no-choice mating experiments (although no such preference for non-kin mates was found in choice experiments; Enigl and Schausberger 2004). Moreover, *P. persimilis* may be able to recognize individuals based on familiarity, as shown for juvenile predators (Schausberger 2005, 2007). A relationship between mating preference and olfactory responses of the partners could provide an explanation for the observed shift in the behaviour of the iso-female lines in our experiment. If such a relationship holds, then selection experiments on the olfactory responses of *P. persimilis* require that the mating is ensured to take place among individuals exhibiting the same olfactory response. Uncovering any such relationship requires that we study the olfactory responses of males as well as females and their mating preferences with respect to this behaviour.

MeSa presented in a pure form without a background of volatiles was a novel experience to the predators in the sense that the predators had not perceived it prior to the experimental test. However, the predators were reared on detached leaves of plants infested with *T. urticae*. Therefore, they had experience with the full blend of plant volatiles released by *T. urticae* – infested plants and this full blend does include MeSa. This experience may influence predator responses in selection, however evidence to date shows that predator responses to pure compounds does not explain subsequent responses to mixtures of these compounds and, conversely, predator attraction to the full volatile blend induced by *T. urticae* is not due to attraction to all individual compounds constituting this blend (van Wijk 2007; van Wijk et al. 2008). There is evidence that experience acquired during rearing on infested plants may influence subsequent predator responses to full volatile blends of these plants when uninfested (Takabayashi and Dicke 1992; but see also van den Boom et al. [2002] for no effect of rearing history on predator responses to full blends). In general, exposing *P. persimilis* to full blends of volatiles (Drukker et al. 2000; de Boer and Dicke 2004a; de Boer et al. 2005; van Wijk 2007) or individual volatile compounds (Sznajder et al. 2010; chapter 4 in this thesis) that are coupled with the presence of *T. urticae* resulted in increased predator preference for these compounds. In line with these results, suppose that prior experience of predators with the full blend of volatiles did increase predator responsiveness to MeSa in the experimental test. In this scenario, predators that showed a preference for MeSa may have acquired this behaviour due to learning. Therefore, purifying selection in this experiment would not change the mean behaviour of the lines established from selecting such individuals (because the learned responses masked the genetically determined behaviour). Alternatively, the selection could further increase the preference of MeSa in those lines because the set-up would effectively select for improved learning of MeSa, provided that there is genetic variation in learning ability in this population. Furthermore, according to this hypothesis, selected predators that avoided MeSa should be interpreted as poor learners presumably revealing their genetically determined response to MeSa. Hence, propagating lines via these individuals would lead to stronger aversion to MeSa exhibited in the descendant lines. As can be seen in Figure 3.3, these predictions are not consistent with the results of this study. Although we cannot exclude the possibility that the experience prior to the selection tests did not influence the predator responses, current knowledge on how experience shapes predator responses to herbivore-induced plant volatiles does not provide a straightforward explanation for the results obtained in this study.

Finally, the role of maternal effects shaping predator responses to MeSa (i.e., non-Mendelian transmission of a trait from mother to offspring) was not

addressed in this study. Maternal effects may influence the magnitude as well as the direction of population responses to selection (Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990). They encompass a diversity of effects deriving from parental influence during rearing (various forms of parental care or the choice of oviposition sites) or cytoplasmic effects pertaining to inheritance of cytoplasmic factors passed on to the egg during oogenesis. To the best of our knowledge there is no parental care in *P. persimilis*, and an effect of oviposition in an environment characterized by different volatile profiles can also be excluded (as all lines were reared on Lima bean leaves infested with *T. urticae*). However, we cannot rule out a cytoplasmic maternal effect.

In conclusion, our results confirm that the responses of *P. persimilis* to MeSa have a genetic component. Future studies should address the response to MeSa (or other herbivore-induced plant volatiles) of males as well as females and thus help explain whether any correlation between predator mating preferences and the response to MeSa may contribute to the outcome of selection.

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4

Genetic variation in learning abilities may mask population-level effects of experience

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Submitted manuscript

Abstract – Effects of learning on individual phenotypes are common, but their role in evolutionary ecology is only starting to be appreciated. In this study we address the question whether there is genetic variation in learning ability and how this affects population-level responses, using iso-female lines of the predatory mite *Phytoseiulus persimilis*. We tested the responses of female predators to methyl salicylate (MeSa) – a volatile compound produced by plants if infested by phytophagous spider mites, the prey of *P. persimilis*. Responses were measured before and after being exposed to MeSa in the presence or absence of food and any change in response to MeSa was taken as an indicator of learning ability. We found variation among isofemale lines in their change in response to MeSa, thus showing genetic variation for learning ability. However, using the base population the same experiments did not show any effect of experience on the response to MeSa. Together, our findings imply that the magnitude of learning as studied in a population as a whole may be underestimated due to genetically determined variation in learning abilities of the individuals constituting the population.

Key words: methyl salicylate, I-tube olfactometer, phenotypic plasticity, *Phytoseiulus persimilis*, preference

In ecological studies it is common practice to study learning behavior by assessing the mean change in response of a group of individuals as a consequence of experience (e.g., Lee and Bernays 1990; Raubenheimer and Tucker 1997; Dukas 1998; Dukas and Bernays 2000; Egas and Sabelis 2001; Mery and Kawecki 2002; Weiss and Papaj 2003). Such groups represent samples from a field population and may therefore harbor genetic variation for the trait under study. In physiological – and sometimes also ecological – studies, learning assays are done using one particular genotype to standardize the genetic background (Dubnau and Tully 1998). This prompts the question whether there is genetic variation in learning behavior and how this affects population-level responses.

If so, it is dangerous to infer learning abilities from experiments on genetically heterogeneous groups from a species. Knowledge on genetic variation for learning is of vital importance to understand population behavior on a longer time scale, because the genetic make-up of the population with respect to this trait may change due to natural selection.

In this article we present results of a study on learning in a predatory mite (*Phytoseiulus persimilis* Athias-Henriot). Its haplodiploid reproductive system allows creating inbred iso-female lines by repeated mother-son or brother-sister-mating. In haplodiploids negative impacts of inbreeding are reduced because deleterious alleles are immediately expressed in the haploid males and therefore these are directly selected against (Crozier 1985; Werren 1993; Henter 2003; Charlesworth and Willies 2009; unless these alleles have female-limited effect; Saito 2001; Tien et al., submitted). In particular, we study variation between such lines in their behavioral response to a relevant odor, as well as the change in response using a learning assay involving either a reward or a penalty. The between-line variation in these responses is then compared with a separate series of responses at the level of the population from which the iso-female lines were obtained. In this way we assessed the consequences of averaging over genetically diverse individuals.

The odor selected for testing behavioral responses of the iso-female lines of *P. persimilis* is methyl salicylate (MeSa). This odor compound is particularly relevant to the foraging behavior of this predatory mite because (1) it is released by many different species of plants in response to the herbivorous spider mite *Tetranychus urticae*, the main prey of this predatory mite (van den Boom et al. 2004), and (2) it is used by the predatory mite as a cue to forage for this prey (de Boer and Dicke 2004). Moreover (3), there is genetic variation in the innate response to MeSa (Sznajder et al. 2010), as well as to the full blend of plant odors containing MeSa (Margolies et al. 1997; Jia et al. 2002).

To test for a change in behavior in response to experience, we associated exposure to MeSa with one day of feeding on eggs of *T. urticae* as a reward, or with one day of food deprivation as a penalty. This protocol was used earlier by Drukker et al. (2000) in experiments to discriminate between different modes of learning (associative learning, sensitization, habituation). Our experiments are not designed to make such distinctions, but merely to detect differences in learning ability among iso-female lines. Any differences in learning ability revealed among the lines indicate the presence of genetic variation (David et al. 2005). We tested five inbred lines established from a single mated female that was selected randomly from the base population (originating from the field thus presumably genetically variable). The genetic variation within the lines was further reduced by repeated brother-sister mating prior to the onset of the experiments.

Therefore, our tests of predator response to experience measured among the lines were conducted against a relatively homogeneous genetic background (as compared to testing iso-female lines each established by different females). This provided a benefit of reducing the confounding effect of variation among the lines in other (genetically determined) traits that may have an influence on animal's behavioural response to experience. The downside of our approach is that the genetic variation in learning ability, from which we aimed to set apart distinct genotypes, might also be reduced (provided genetic variation in learning is present in the studied base population).

Materials and Methods

Predatory mites

The base population of *P. persimilis* was maintained in our laboratory (25 °C, 70% humidity, and continuous light) on a diet of two-spotted spider mites on detached leaves of lima bean. The population was founded from a sample of predators collected in 2002 at different locations throughout Sicily (Partinico, Scopella, Trappeto, Terrasini, Siculiana, Laghetto, Menfi, Trabia, Alcamo). Thus, it was presumably genetically variable, although local populations of *P. persimilis* are generally considered to be inbred under natural settings (Helle and Overmeer 1973). Inbred iso-female lines used in this experiment were obtained by randomly selecting a mated female from the base population. Subsequently five new iso-female lines were established from this line by randomly selecting five mated daughters of this female. Typically in haplodiploid arthropods iso-female lines are obtained through mating between the virgin female and her sons. However, *P. persimilis*, just like most species in the family of Phytoseiidae, is pseudo-arrhenotokous, i.e., sons and daughters arise from fertilized eggs and male zygotes become haploid due to paternal genome inactivation and elimination during embryogenesis (Schulten 1985). Hence, mating is a prerequisite for oviposition. For this reason we selected already mated females, and propagation of the lines occurs through brother-sister mating (full sibs). The bottleneck of randomly singling out one female to continue the line was applied in each of four subsequent generations. The lines were subject to these repeated bottlenecks in order to obtain genetically homogeneous strains that could be compared with the genetically variable base population. After this, each line was reared to large numbers for use in experiments. Thus the inbred lines used in the study originated from the same female (i.e., female A), as indicated by the first letter in the coded names of the lines (e.g., AAFA). Each iso-female line is named with one letter per generation in which the population experienced a bottleneck, with the letter indicating the founding mother. For instance, lines AAFA and AAFC share ancestry up to the third generation, and differ in the

fourth generation with respect to the mother who founded these lines (mothers A and C from grandmother F, etc.).

Tests of olfactory preference

Behavioral responses of the predators to MeSa were not tested in the commonly used Y-tube (Sabelis and van de Baan 1983; Janssen et al. 1997), but in a single straight glass tube (length 20 cm, \varnothing 0.5 cm) with a small opening (2 mm) in the middle to introduce the predatory mites (hereafter referred to as the I-tube; Sznajder et al. 2010). Opposite the entrance hole, another opening (\varnothing 0.5 cm) was present which was gauze-covered and served as an air-outlet. At the ends of the I-tube there were plastic trap vials (\varnothing 30 mm, height 55 mm) to collect predators and these were connected to jars that either contained a capillary with MeSa or not. The traps were designed as an 'easy-to-enter-yet-difficult-to-exit' vial, and were provided with a water source (wet cotton wool). After purification by activated carbon filters, the air was flowing at 20 l/h through the jars, the vials and then through the arms of the glass tube, finally leaving the system through the opening in the middle of the I-tube. At release in the middle of the I-tube, a mite finds itself in air streams coming from right and left, i.e., one with MeSa and the other without. Subsequently, it can move left or right in the I-tube and ultimately enters one of the trap vials or remain in the I-tube. After release of the test animals, the entrance hole was sealed with Parafilm®. Pilot experiments showed that the I-tube olfactometer produces results consistent with our knowledge of the responses of *P. persimilis* to herbivore-induced plant volatiles (M van Wijk, pers. comm.).

Within a single replicate experiment a group of females was released sequentially into the I-tube, i.e., the individuals were released in the olfactometer one after the other. Visual cues play no role in predator orientation as the predator is blind and orients itself by means of chemical cues. Previous studies using a Y-tube set-up showed that there is no effect of possible residues deposited along the path taken by an individual on the choices of subsequent individuals (Sabelis and van de Baan 1983; van Wijk et al. 2010). Hence we considered the choices of individual mites in this set-up as independent measurements.

In total, 20-30 adult females were released per replicate experiment. After 25 minutes, the number of mites in each of the two trap vials was counted. The number that remained in the I-tube, was scored as 'no choice'. For each consecutive replicate of a line, the arm containing air with MeSa was changed to exclude any unforeseen asymmetries in the experimental set-up. Per line per treatment, roughly 120 mites were tested in 4-8 replicate experiments (except for one case, where three replicates were performed).

Synthetic MeSa (Sigma-Aldrich Fluka, pure; assay $\geq 99\%$) was offered undiluted in a small capillary (9 μl , \varnothing 0.60 mm, Omnilabo) placed in one of the jars connected to the I-tube. The air flow was led through the set-up at least 2 h before the start of the test. The MeSa evaporation rate was c. 30 $\mu\text{g}/\text{h}$. This evaporation rate was chosen based on preliminary experiments to find a concentration to which the base population exhibited a neutral (50:50) unconditioned response. Unfortunately, this evaporation rate is hard to compare with previous studies attempting to measure the dose-response relation, because these assessments were based on filter paper as a substrate (de Boer and Dicke 2004). Unlike filter paper, capillaries generate a constant evaporation rate.

Experimental design

We tested for change in response to MeSa in the base population as well as five randomly chosen iso-female lines. Adult females from the base population and the lines were subjected to either of two treatments determining their experience with a reward or a penalty: 24 h feeding on eggs of *T. urticae* (hereafter called FED) in the presence of MeSa and 24 h food deprivation (STARVED) in the presence of MeSa. Shorter exposures are ineffective, according to Drukker et al. (2000). The experiment involved first a test to establish the innate response of well-fed females, and then a conditioned response test with starved or fed females. The conditioned response was tested immediately after the conditioning treatment. Under the FED regime the mites were kept on a wet piece of filter paper containing eggs of *T. urticae* (see below), whereas under the STARVED regime they were kept in the presence of MeSa on wet filter paper without prey eggs. All conditioning took place in vials identical in design to the trap vials of the I-tube using the exact same concentration of MeSa as in the tests.

Adult females used in all experiments were collected directly from the populations. Such samples contain adult individuals of various ages (i.e., very young ovipositing females as well as old females). We tested mixed-age groups of individuals to avoid any confounding effect of age on predator response to the volatile. To minimize any potential effects of the time of day on predator responses, replicates of all tests were randomized with respect to the time of day.

To obtain eggs for conditioning, spider-mite infested bean leaves were put in a 3-l jar with water provided with a droplet of mild detergent. The content of the jar was gently stirred, and then deposited on a pyramid of fine sieves. The eggs were collected on the finest sieve, where they were thoroughly rinsed to remove the detergent and the plant-derived odors. Finally, the eggs were washed off the sieve onto a piece of filter paper. This method of obtaining eggs has been used successfully by Drukker et al. (2000) to obtain neutral innate responses of the predatory mites in a Y-tube olfactometer.

The set-up of these experiments allows us to compare the preference for MeSa before and after experiencing this odor in the presence or absence of food. This set-up does not allow us to discriminate between different kinds of learning, but this is not the aim of our study. Our actual aim is to measure whether there is genetic variation in effects of experience on preference for MeSa and how that affects the response at the population level. In fact, food deprivation alone could cause preference for MeSa to change, and hunger in general is known to be an important motivational factor in animal learning (Dethier 1976). Because our set-up does not control for effects of food deprivation alone, it is possible that changes in preference for MeSa in the STARVED regime are modulated by a hunger effect. This argument does not hold for the FED regime, since all measurements on innate preference are performed on well-fed mites.

Statistical analysis

We asked: (A) whether feeding under exposure to MeSa leads to increased preference for MeSa, and starvation under exposure to MeSa leads to avoidance of MeSa; (B) is there a variation in the response to these two types of conditioning among the inbred iso-female lines? and (C) is there a difference between the responses of the inbred iso-female lines and the base population (that is presumably genetically variable)? To address these hypotheses we constructed mixed-effects logistic regression where the data points consist of the choices of individual mites in the test MeSa versus clean air (see *Tests of olfactory preference* for arguments for treating the responses of individual mites in this experiment as independent). To address the first hypothesis (A) we included two fixed effects: line (six levels - inbred lines 1-5 and the base population) and test (three levels - pre-conditioning, post-fed conditioning and post-starved conditioning). The effect of test contains three levels because there is no reason to expect that the predator response measured before starvation under exposure to MeSa is on average different from predator response measured before feeding under exposure to MeSa. The lines are treated as a fixed effect, although they are in fact a subset of genotypes randomly drawn from the base population. Therefore, the correct interpretation of a significant effect of line in this model is that the differences are found within this specific group of lines (but a different subset of iso-female lines may show a different effect). A random intercept was introduced with observations grouped by replicate (i.e., replicate is a random effect). R software was used for these analyses (package lme4).

Results

Prior to the conditioning treatment all lines (and base) exhibited, on average, avoidance to MeSa (Figure 4.1). There was a significant difference in predator responses between tests such that feeding under exposure to MeSa did indeed

increase preference for MeSa after the conditioning treatment [$P < 0.001$, estimate (SE) = 0.67 (0.11)]. However we found that starvation in MeSa also increased predator preference for MeSa [$P = 0.002$, estimate (SE) = 0.32 (0.10)].

Exploring these unexpected results further we included an interaction term between line and test. This interaction term was highly significant both including [$P < 0.0001$, log-likelihood ratio (df) = 42.5 (10)] and excluding base population as a factor level [$P < 0.0001$, log-likelihood ratio (df) = 40.6 (8)]. Therefore this result supports the hypothesis (B) that there is variation in the response to the conditioning treatment among the iso-female lines.

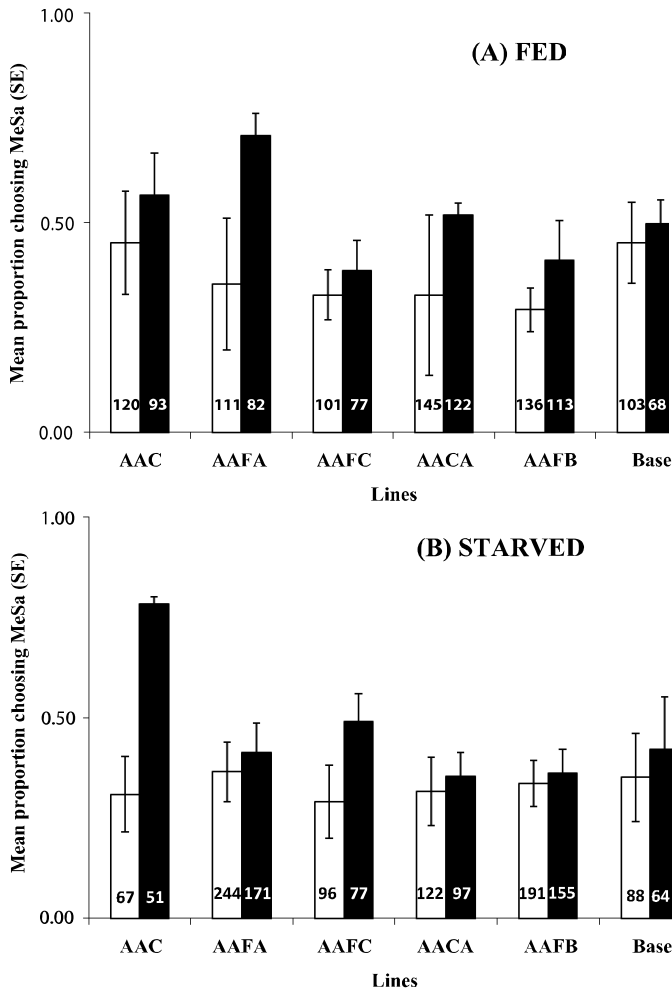


Figure 4.1 – Mean proportion of individuals choosing MeSa in the innate response test (open bars) and the conditioned response test (closed bars), in five iso-female lines and the base population. The conditioning consisted of feeding in the presence of MeSa (A), and food deprivation in the presence of MeSa (B). Numbers superimposed on the bars above the x-axis show the total numbers of individuals per line per treatment.

Next we examine the pairwise tests of the responses to the conditioning treatment within each line. Here, we found that both for feeding under exposure to MeSa and starvation under exposure to MeSa, there is one line (AAFA for feeding and AAC for starvation) whose response after conditioning was significantly different from its response prior to conditioning (for AAFA $P = 0.002$, estimate (SE) = 1.39 (0.44) and $P = 0.001$, estimate (SE) = 1.71 (0.53) for AAC). In both cases these lines show a strong preference for MeSa after conditioning (i.e., after feeding for AAFA and after starvation for AAC; Figure 4.1). Other lines showed similar responses, albeit not as strong (Figure 4.1). In contrast, the response of the base population after the conditioning was not different from its response prior to conditioning ($P = 0.35$, estimate (SE) = 0.9 (0.31) for feeding in MeSa and $P = 0.42$, estimate (SE) = 0.27 (0.33) for starvation in MeSa). This supports hypothesis (C) that there is a difference in the response to experience measured at the level of the lines versus the level of the base population. In particular, the effect of experience on animal behaviour is revealed when studied at the level of inbred iso-female lines but not at the level of the base population.

We conclude that the interaction between the effect of the test and the iso-female line indicates intraspecific genetic variation in learning abilities in *P. persimilis*.

Discussion

Our study showed that experience with MeSa – a signal of prey presence – does not modify the innate response of *P. persimilis* to this compound if measured as the average response of the base population. Had this been the only experiment to assess effects of experience, we would have concluded that learning plays little role in this behavior. However, we further showed that this apparent lack of an effect of experience can arise when individuals vary in their ability to learn: iso-female lines of *P. persimilis* originating from the base population do show significant effects of experience. In this study we only tested a small subset of all possible iso-female lines (and thus genotypes), which additionally all originated from a single female. This may explain why the innate response tests of the iso-female lines consisted mostly of females avoiding MeSa. Our study demonstrated that genetic variation in learning can be already found within a group of lines established from a single founder sampled from a natural population of *P. persimilis*. A larger subset of iso-female lines from the same population yields innate test responses varying from avoidance to preference (Sznajder et al. 2010). A different (larger) subset of iso-female lines might also show more variable responses to the conditioning treatment used in this experiment. All lines and the base population were reared under identical con-

ditions prior to the experiment and were subsequently subjected to the same treatment, so that the iso-female line is the only factor making a difference. We conclude, therefore, that the observed differences in the response of the iso-female lines to experience have a genetic basis.

Exploring the full array of genotypes determining learning abilities is beyond the scope of this article. In contrast to Drukker et al. (2000), we have not found evidence for associative learning. They tested the same predatory mite species, but from a different origin, and used intact odor blends from infested and uninfested plants, instead of a single odor compound. Such differences in experimental set-up may well explain the contrasting results, but should stimulate more research into mechanisms of learning in this predatory mite.

Although it was not our aim to distinguish different modes of learning, it is relevant to ask which of them may explain the observed changes in behavior. Overall, the change in the behavior of the iso-female lines amounted to an increase in the percentage of individuals choosing MeSa after the predators were fed in MeSa (particularly prominent in line AAFA) as well as starved in MeSa (particularly prominent in line AAC). An associative learning mechanism should result in a decreased preference when starved. Therefore, this mechanism can be ruled out in the tested subset of iso-female lines. This leaves non-associative mechanisms of learning such as, e.g., sensitization or habituation. Sensitization consists of an increased response to a variety of stimuli following an exposure to an intense or noxious, repeated stimulus (Kandel et al., 1993). Therefore this mechanism of learning should result in a significantly increased preference for MeSa when exposed long enough. For instance, the responses of line AAC in the STARVED regime (Figure 4.1B) may indicate sensitization to MeSa because conditioning resulted in significant preference of MeSa in a way that excludes habituation. Habituation result in an indifferent response to MeSa when exposed long enough (Kandel et al. 1993) and it may explain the behavior of the lines AAFC in the STARVED regime and line AACA in the FED regime. For these lines we observed an increase in the percentage of individuals choosing MeSa but of the magnitude that effectively amounts to an indifferent response.

This study provides evidence for the role of genetic factors in learning ability in the predatory mite *P. persimilis*. This in itself may not be surprising; genetic variation in learning and memory is often found when looked for, in many different study organisms (Dukas 2004). However, this finding has two important consequences. First, the ability to learn may simply not be detected if a population of various learning genotypes is examined collectively and their various responses cancel out in the overall effect. This conclusion is relevant for ecological and evolutionary studies of animal behavior that frequently use the effect of experience

manifested in behavioral change as a measure of learning ability. Second, learning ability has attracted attention in evolutionary studies due to its potential to affect the rate of evolutionary change in phenotypic traits (Robinson and Dukas 1999). However, we caution to take into account the fact that learning ability is a trait in itself, subject to natural selection (see, e.g., Mery and Kawecki 2004). The effect of learning on individual phenotypes, and consequently on selection acting on a population of such phenotypes may depend on the amount of variation in learning ability. It is therefore crucial to control for genetic variation in learning ability when studying the effects of experience at the population level.

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5

How adaptive learning affects evolution: reviewing theory on the Baldwin effect

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Submitted manuscript

Abstract – We review models of the Baldwin effect, i.e., the hypothesis that adaptive learning (i.e., learning to improve fitness) accelerates genetic evolution of the phenotype. Numerous theoretical studies scrutinised the hypothesis that a non-evolving ability of adaptive learning accelerates evolution of genetically determined behaviour. However, their results are conflicting in that some studies predict an accelerating effect of learning on evolution, whereas others show a decelerating effect. We begin by describing the arguments underlying the hypothesis on the Baldwin effect and identify the core argument: adaptive learning influences the rate of evolution because it changes relative fitness of phenotypes. Then we analyse the theoretical studies of the Baldwin effect with respect to their model of adaptive learning and discuss how their contrasting results can be explained from differences in (1) the ways in which the effect of adaptive learning on the phenotype is modelled, (2) the assumptions underlying the function used to quantify fitness and (3) the time scale at which the evolutionary rate is measured. We finish by reviewing the specific assumptions used by the theoretical studies of the Baldwin effect and discuss the evolutionary implications for cases where these assumptions do not hold.

Key words: the Baldwin effect, fitness landscape, evolution of phenotype, adaptive learning.

In theory the evolution of plastic phenotypic traits may lead to two extreme outcomes where the trait becomes either genetically fixed (and largely phenotypically invariable) or it is entirely shaped by environmental influences. In-between these extremes lies a spectrum of outcomes where traits contain a genetic component but they are also, to various degrees, modifiable in response to environmental influences. A recurring question in evolutionary biology is how phenotypic plasticity (i.e., the ability to modify phenotype in response to external or internal influences) may influence the outcome and the rate of evolution (Price et al. 2003; West Eberhard 2005; Crispo 2008) by, for example, creating novel selectable forms that are entirely environmentally induced when

there is not any genetic basis for such a variant (as in populations that colonize a novel environment). This question is particularly relevant in the face of the growing body of evidence that various forms of plasticity (such as the ability to learn) have a genetic basis (Mery and Kawecki 2002; Dukas 2004) and thus may evolve jointly with the genetically determined phenotype.

In this article we focus on the hypothesis that adaptive learning (i.e., learning that improves fitness) facilitates evolution of the genetic basis for phenotypic traits. This hypothesis has its origins in the arguments put forward by Mark Baldwin (1896, 1902), a contemporary of Charles Darwin. These arguments concern a population that finds itself in a new environment, and thus, presumably, does not contain a genetic basis for the complete phenotype that would be optimal in this new environment (i.e., the phenotype that achieves the highest possible fitness). Baldwin argues that adaptive plasticity allows sub-optimal individuals to acquire higher fitness. Hence, learning improves the survival of the population of such individuals and thus it facilitates that the genetic evolution may proceed. Moreover, he observes that under these conditions there is direct selection for the ability to learn adaptively and, simultaneously, indirect selection for any heritable variation carried by the plastic individuals favoured by direct selection. The central argument of Baldwin is that selection for the ability to acquire a fitter phenotype through learning may coincide with the genetic basis for the fitter phenotype (i.e., these indirectly selected genes provide a basis for a fitter phenotype). If this condition is fulfilled, then the selection for improved learning facilitates adaptive evolution of the genetic basis for the trait. Therefore, adaptive learning is predicted to accelerate evolution.

This hypothesis of Mark Baldwin, known in the literature as the Baldwin effect (Simpson 1953) has spurred numerous theoretical studies whose general approach is to measure the rate of evolution of a genetically determined trait, given different levels of a non-evolving ability to learn adaptively. Their results are ambiguous; some studies provide evidence for an accelerating effect of adaptive learning on evolution (Hinton & Nowlan 1987; Fontanari and Meir 1990; Mayley 1997; Ancel 2000 – the norm of reactions models; Lande 2009), yet others show a decelerating effect of learning on genetic evolution (Papaj 1994; Anderson 1995; Ancel 2000 – the quantitative genetic model; Dopazo et al. 2001; Borenstein et al. 2006).

In this article we analyse the theoretical studies of the Baldwin effect with the aim of explaining how learning yields these two contrasting effects. In order to do so, we analyse how – in these studies – learning influences the relationship between different phenotypes and fitness and thereby influences the evolutionary response to selection. In fact, it is one of the underlying assumptions of the

Baldwin effect that learning changes relative fitness differences among phenotypes by conferring a larger fitness increase to those phenotypes (as well as underlying genotypes) that are already relatively closer to the fitness peak (the selection for the ability to acquire fitter phenotype through learning coincides with the genetic basis for the fitter phenotype). Moreover, we discuss the predictions of the theoretical studies in two distinct evolutionary stages that are characterized by different evolutionary end-points derived from the Baldwin effect. The Baldwin effect concerns the evolution of a phenotypic trait towards a single and distant fitness peak; this process is initially realized through the selection of plastic phenotypes but it is finalized when these plastic phenotypes are substituted by a genetically determined and optimal phenotype (presumably because learning has a fitness cost, Baldwin 1896, Simpson 1953). Hence, the theoretical studies of the Baldwin effect generally estimate the amount of time (in generations) needed for the completion of this entire process, but they also allow to separately analyse (1) the number of generations until the first genetically determined and optimal phenotype appears in the plastic population, and (2) the number of generations until this genetically determined optimal phenotype replaces the plastic phenotypes, (which represents the general idea of staging the Baldwin effect, as first proposed by Simpson 1953). These two evolutionary stages may have different time scales and evolutionary dynamics. Therefore, it is reasonable to derive conclusions about the effect of learning on evolution separately for these two evolutionary stages. In the next section, we begin by analysing the results of the theoretical studies of the Baldwin effect, grouped with respect to the concepts of adaptive learning (or adaptive plasticity in general) and the assumed fitness function. We compare the evolutionary rates in the two stages of the Baldwin effect (as defined above) obtained in these studies, whenever these rates are available.

Models of the Baldwin effect: The concept of adaptive learning and the choice of fitness function

The hypothesis on the Baldwin effect states that evolution of an innate (i.e., genetically determined) trait proceeds faster in populations that harbour plastic individuals, than in populations that harbour none of such individuals. Therefore, the general approach in published studies of the Baldwin effect is to measure the rate of evolution of an innate trait given different levels of a non-evolving ability to learn. However, these studies vary with respect to assumed fitness functions; the fitness landscapes they describe range from a single-peak ‘needle-in-haystack’ type to a single-peak landscape with a gradual slope, or a rugged landscape that contains many fitness peaks of varying heights. Moreover, although they all model adaptive learning (i.e., learning leads to a change of phenotype in the direction of increased fitness) the reviewed studies

of the Baldwin effect use different methods and assumptions to achieve such an effect of plasticity in their model systems.

In their seminal model reviving interest in the Baldwin effect, Hinton and Nowlan (1987) track the changes in the frequency of the alleles associated with fitness pay-off. The increase in the frequency of the allele associated with superior fitness is taken as a yardstick of adaptive evolution. In particular, genotypes are modelled as byte strings that consist of a number of loci. These loci can contain one of two types of alleles and the genotype that is completely homogeneous with respect to one particular type of the allele is taken to be the optimal one (i.e., confers the highest fitness). The assumed fitness landscape, therefore, is of the unimodal 'needle-in-haystack' type. Adaptive learning is introduced by another allele which is not fixed, but can be switched to the type that confers higher fitness based on a learning algorithm, and individuals are allowed to search for the correct setting of these alleles in a number of trials during their lifetime. The individuals that learn the optimal phenotype are preferentially selected for mating (where the probability of being selected for mating increases with decreasing the number of trials the individuals need to learn the optimal phenotype) and thus have more offspring.

The model shows that such learning dramatically speeds up evolution in the population of individuals capable of learning, a result corroborated by Fontanari and Meir (1990) who analyse evolution on the same fitness landscape, using the same learning protocol but assuming asexual reproduction. In fact, the population lacking an allele for learning (i.e., the 'unspecified' alleles that get fixed by learning) cannot find this evolutionary end-point. The explanation for these results is that at least some individuals harbour the set of fixed alleles that is not too different from the optimal one and hence they have a higher chance of finding the correct setting of all 'unspecified' alleles by learning within the time specified for learning. In other words, thanks to learning these genotypes (that are already closer to the fitness peak) gain a relatively higher fitness than do the plastic genotypes with fewer correct setting of alleles. These findings are also consistent with the argument of Baldwin that learning confers higher fitness gain to those genotypes that are already closer to the fitness peak and thus accelerates evolution of the genetic basis for the optimal phenotype. Nevertheless, the other observation of the model by Hinton and Nowlan is that 'unspecified' alleles are not entirely out-selected and remain in the population, indicating different evolutionary dynamics once the population evolves to the vicinity of the fitness peak. However, this result may also be attributed to the fact that learning in this model has no fitness cost.

The study by Mayley (1997) provides evidence that the cost of learning plays a critical role in the interplay between learning and genetic evolution. It also examines in more detail the relationship between the complexity of a fitness landscape and the effect of learning on evolution. In particular, the author compares the movement of a plastic population on unimodal and rugged (i.e., many fitness peaks of varying height) fitness landscapes. In his model, a genetically determined phenotype, represented by a point on the fitness landscape, is considered to evolve if it moves in the direction of the fitness peak. Mayley finds that there is no evolution on a unimodal fitness landscape if learning is cost-free because the optimal phenotype is acquired entirely by learning. Adaptive evolution on a unimodal fitness landscape is only possible, when there is a cost of learning. Yet, on a rugged fitness landscape the population evolves irrespective of the cost of learning. Mayley's results demonstrate that learning is more likely to facilitate evolution on a rugged fitness landscape, i.e., where there is more than one fitness peak and/or, initially, learning allows the phenotypes to reach only the local fitness peaks but not the global fitness peak (i.e., modifies the phenotype such that it has the highest possible fitness). Moreover, in both the unimodal and rugged fitness landscapes the cost of learning is critical for the convergence of the population on the single optimal genotype, i.e., the genotype whose fitness cannot be improved by learning.

Borenstein et al. (2006) constructed a rugged fitness landscape characterized by a number of local fitness peaks of steadily increasing heights and one global fitness peak. In their model the population continues evolving towards the global optimum by crossing the intermediate fitness valleys and converging on local fitness peaks. The authors measure the rate of evolution as the time it takes the population to reach the global fitness peak and they approximate adaptive learning through the application of an algorithm which allows a learning genotype to repetitively explore the fitness landscape and to modify its phenotype according to the detected fitness gains. This learning process stops when continuation of sampling and learning cannot secure further fitness gains (i.e., the genotype has found the local fitness peak). As a consequence of this learning process all genotypes of the population acquire the same fitness, determined by the local fitness peak, because they all are equally capable of learning. This way of modelling the phenotypic effect of learning is more akin to the way learning is modelled in a series of models due to Hinton & Nowlan, (1987) and Mayley (1997). One feature characteristic to this approach is that genotypes capable of learning can sample potentially large areas of the fitness landscape and modify their phenotypes accordingly. In the model of Borenstein et al. the learning process effectively smoothes the fitness landscape, i.e., it reduces fitness

differences among genotypes. Model simulations carried out by Borenstein et al. confirm that such an effect of learning is associated with slower evolution on a unimodal fitness landscape. However, on a rugged fitness landscape the learning process results in faster evolution because the reduced fitness differences among genotypes help the population to cross fitness valleys, thereby allowing evolution towards the global fitness peak. At the same time, a population of individuals that cannot learn may never be able to cross the fitness valley and find the global optimum. These results prompt Borenstein et al. to conclude that the complexity of the fitness landscape, i.e., the presence of multiple fitness peaks and fitness valleys, determines whether the effect of learning on evolution is accelerating or decelerating.

A separate class of models using the quantitative genetics framework to measure the rate of phenotypic evolution assumes a unimodal fitness landscape (i.e., containing a single fitness peak), given by a Gaussian function (Anderson 1995; Ancel 2000 – quantitative genetics model). These studies introduce an adaptive effect of learning by an increase in the selection variance. Thus the learning process modelled is equivalent to (a small) adaptive shift of the genetically determined trait value of all sub-optimal individuals. This combination of the fitness function and the way of modelling learning results in decreased phenotypic variance and decreased fitness differences among different phenotypes. Moreover, this evolutionary scenario approximates the second stage of the Baldwin effect: the stabilizing selection acting on the population in the vicinity of the fitness peak. Characteristically, these two studies show that learning extends the time required for convergence of the population on the optimal genotype as compared to the evolution in a population with individuals that cannot learn, thus supporting a decelerating effect of learning on evolution.

The same conclusion is drawn by Papaj (1994) in a model that measures the time required for the population to evolve a genetically determined, optimal phenotype (i.e., a genotype that has a highest possible fitness without any learning). This study also assumes a unimodal fitness landscape that is provided by a negative quadratic function (shape of inverted parabola). And, as a consequence of adaptive learning, different phenotypes eventually converge on the single fitness peak. Thus, in this study learning also effectively decreases the phenotypic variance and fitness differences among the phenotypes.

Another class of studies involves modelling adaptive plasticity as a norm of reactions. Ancel (1999, 2000), in her norm of reaction model, explicitly addresses the rates of evolution in the two stages of the Baldwin effect, while varying the degree of plasticity reflected in the width of the norm of reaction. The

mid-point of the norm of reactions represents the genetically determined trait value (i.e., the innate trait) while the phenotype with highest fitness within this range (based on the fitness function) represents the phenotype acquired through learning. Thus, all phenotypes are able to express the optimal phenotype if the norms of reaction of these phenotypes are wide enough to contain the fitness peak (as might be the case when the population is already in the vicinity of this fitness peak), even though there is variation in the innate value in such a population.

On the other hand, setting the initial width of norms of reactions such that they do not contain the optimum, models a scenario where a population evolves towards a distant fitness peak. Ancel (2000) examines how this plasticity affects the rate of evolution in two types of unimodal fitness landscapes: (1) a spiked landscape where a single genotype scores the highest fitness and all the other genotypes score the same flat fitness (also referred to as the 'needle-in-the-haystack' landscape, as in Hinton & Nowlan 1987), and (2) a Gaussian fitness function. The novel aspect of Ancel's model is that the width of the norm of reaction is allowed to evolve such that the upper and lower bounds of the norm of reactions may shift from one generation the next. For the two settings of the fitness function, Ancel shows that costly adaptive plasticity generally accelerates the first stage of the Baldwin effect, i.e., it shortens the time required for the first optimal genotype to emerge in the population (Ancel 2000). This effect is associated with the initial selection for the wider norms of reactions (Ancel 1999). In contrast, plasticity decelerates the second stage of the Baldwin effect, i.e., it extends the time between the emergence of the optimal genotype and population convergence on this genotype because the wide norm of reaction effectively allow all individuals to learn the optimal phenotype (Ancel 2000).

These results of Ancel provide further evidence that adaptive learning accelerates evolution in the initial stages of the Baldwin effect, i.e., evolution towards a distant fitness peak. However, the decelerating effect of learning prevails in the second and final stage of the Baldwin effect. The results of Ancel obtained for the two stages of the Baldwin effect are corroborated by the study of Lande (2009) where plasticity is also modelled as a reaction norm evolving under the Gaussian fitness landscape. These two studies are a notable exception in the theory of the Baldwin effect by allowing phenotypic plasticity to evolve jointly with the innate trait (see also studies in the framework of artificial life/intelligence, e.g., Watson and Wiles 2002, Suzuki and Arita 2004).

Thus, the theoretical studies indicate that the effect of learning on evolution is not constant as the population evolves on a fitness landscape towards a

distant fitness peak. Therefore it is reasonable to conduct a comparative analysis of the theoretical studies of the Baldwin effect on the studies that measure evolution within the same evolutionary stage (and at the same time scale). In fact, any long-term measure of evolutionary rate (such as, e.g., the time until a first genetically determined optimal phenotype appears in a population) is a net effect of the evolutionary responses occurring at each generation during evolution towards an evolutionary end-point. It is informative, therefore, to analyse how learning may influence this short-term rate of evolution occurring from one generation to the next. This is the approach used in the recent model by Paenke et al. (2007) and in the next section of this article we analyse the approach and results of this model.

Adaptive learning and the response to selection

Paenke et al. (2007) analyse how a population's response to directional selection changes with improved adaptive learning (or some forms of developmental noise). To this end, the authors analyse how the relationship between phenotype and fitness changes as adaptive learning is improved. In particular, the authors compare the rate of evolution of the innate trait at two different and fixed levels of plasticity and analytically demonstrate that improved adaptive plasticity strengthens the response to selection (and thus accelerates evolution) when it magnifies fitness differences among phenotypes: this is reflected in the steeper relationship between phenotype and fitness. Conversely, improved adaptive plasticity weakens the response to selection (and thus decelerates evolution) when it reduces fitness differences among phenotypes: this is reflected in the lower slope of the function relating phenotype and fitness.

By assuming a non-evolving learning ability, the authors entirely focus on the evolution of the innate trait (although in this model the evolution of adaptive learning may also be incorporated, thus introducing a second axis for the evolution of the phenotype). This allows them to derive a correspondence between their general result as presented above and specific properties of the fitness function (evaluated only in the direction of innate trait) reflected in the shape of the fitness function. In particular, the authors predict that learning magnifies fitness differences among phenotypes when the fitness landscape (evaluated in the direction of the innate trait) is convex. Conversely, adaptive learning reduced fitness differences among phenotypes when this fitness landscape is concave. The authors extend this analysis by assuming various specific functions for the innate phenotype and non-evolving adaptive plasticity, such as used in Ance (2000) or Andersson (1995) to demonstrate that there exists a fitness landscape on which adaptive learning, as it is modelled, accelerates evolution.

The predictions of Paenke et al. (2007) are derived under the assumptions that the selection is directional (i.e., fitness consistently increases with the value of the innate trait) and non-evolving learning equally modifies different phenotypes (the authors point out that a form of learning that is dependent on the distance of the innate phenotype from the fitness peak may lead to novel predictions). Other assumptions of this framework include the assumption that there are no non-additive or dominance effects shaping the expression of the phenotype, or that there is no genetic covariance between the innate trait and adaptive plasticity.

The approach in the study of Paenke et al. (2007) provides an elegant demonstration of how adaptive learning influences the short-term rate of evolution, i.e., the response to selection measured from one generation to the next when learning is kept fixed. However, allowing the evolution of adaptive learning ability may change the long-term dynamics if the curvature of the fitness landscape is not overall uniform (which is assumed in the model of Paenke et al. 2007).

In summary, the results of Paenke et al. (2007) allow for the conclusion that the effect of adaptive learning on evolution depends on the shape of the fitness function as well as the model of adaptive learning. In particular, adaptive change due to learning that is large relative to the distance of the innate trait from the fitness peak (such that optimal (or nearly optimal) phenotype can always be learned), is more likely to decelerate evolution of the innate trait irrespective of the curvature of the fitness landscape. This theoretical possibility may be unlikely, however, given that in a population adapted to an old environment (that is distant from the new fitness peak as argued in the Baldwin effect), low levels of plasticity are expected (Lande 2009), particularly if plasticity has a fitness cost. There may be selection to maintain high levels of plasticity in a population if there are frequent changes of environment (Stephens 1991). However, in such a theoretical situation the environment (and thus a fitness landscape) is dynamical, while the theoretical studies of the Baldwin effect generally assume a constant environment (and thus a constant fitness landscape).

Conclusions

The effect of adaptive learning on evolution of genetically determined traits is the subject of a long-standing debate and the theoretical treatments of this question provide contrasting results. Here, we discussed how these contrasting results can be partly explained from the different ways in which the theoretical studies measure the evolutionary rate. The traditional end-point of the Baldwin effect is the complete convergence of a population on an initially distant fitness peak associated with reduction in the level of adaptive learning. Adaptive learn-

ing is considered to accelerate evolution if it helps to reach this end-point faster. This measure however may fail to adequately describe the effect of learning on evolution if this effect is not constant but changes as the population evolves on a fitness landscape (particularly, a rugged fitness landscape). A measure of short-term evolutionary change as occurring from one generation to the next may be better suited to detect the variable effect of learning on evolution. The recent study by Paenke et al. (2007) provides such a framework where such a measure is employed to demonstrate how learning influences fitness differences among different innate phenotypes, thus either accelerating or decelerating the evolution of the innate phenotype. By relating the effect of learning on fitness differences among phenotypes to the shape of the fitness function (that determines these fitness differences) the authors demonstrate how theoretical predictions of the Baldwin effect depend on the choice of fitness functions. However, our analysis of this and other theoretical studies of the Baldwin effect indicates that the model of adaptive learning (i.e., how learning is modelled to change the innate phenotype) also matters to the theoretical predictions.

By definition adaptive learning modifies the phenotype so as to increase its fitness. However, adaptive learning may be characterised with respect to how much it modifies the innate phenotype given the distance of this innate phenotype from a fitness peak. In other words, the magnitude of the phenotypic modification due to learning can be modelled as either a small or a large step in phenotype space, depending on the size of the exploratory range attributed to the individuals. In particular, simulation models (Hinton & Nowlan 1987; Mayley 1987; Borenstein et al. 2006) employ learning which allows the genotype to sample large areas of a fitness landscape in search of a local fitness peak. In this process, phenotypes are allowed to experience many learning trials during their lifetime (as in Hinton & Nowlan 1987; Fontanari and Meir 1990) or adaptive search is repeated until phenotypic fitness can no longer be improved (Mayley 1997; Borenstein et al. 2007). Therefore, the optimal phenotype can be learned by all phenotypes. In contrast, in another class of models (Anderson 1995; Ancel 2000 – quantitative genetic model) adaptive learning effectively involves a relatively small (with respect to the distance of the innate trait from the fitness peak) adaptive shift of the innate trait in the direction of increased fitness. This phenotypic modification is assumed to be fixed (i.e., the magnitude of the shift cannot be increased/decreased in response to the fitness gains determined by the fitness function) and the resulting learned phenotype may not be very different from the genetically determined one. This distinction between the two ways of approximating adaptive learning, based on the potential of learning to modify the phenotype, is relevant because each of the two modes of learning has distinct consequences for the fitness of individual pheno-

types. This may be particularly relevant in the case of evolution on a rugged fitness landscape. Adaptive learning that has a large potential to modify the phenotype is exemplified by unconstrained adaptive search of the fittest options on the fitness landscape. We argue that the effect of such learning on evolution is less likely to depend on the local curvature of the fitness slope because it allows genotypes to sample distant areas of the fitness landscape. On the other hand, adaptive learning modelled as a small shift of the phenotype is much less likely to allow the population to cross fitness valleys and find a global fitness peak.

Current theory is based on the assumption that learning is a fixed trait, and concentrates on tracking evolution of the genetically determined component of a phenotype. This assumption is challenged by the empirical evidence showing that adaptive learning can be successfully subjected to artificial selection (e.g., Mery and Kawecki 2002). It remains to be shown how the current theoretical predictions change if adaptive learning is allowed to evolve jointly with the genetically determined trait value. Moreover, although not considered in the theory on the Baldwin effect, the mechanism of learning may not always be adaptive (as in the case of non-associative mechanisms of learning) and may give rise to entirely different evolutionary dynamics.

The Baldwin effect concerns the evolution towards a distant fitness peak; hence the assumption of the fitness landscape, where the innate trait is under directional selection and evolves towards a distant optimum whose position in the landscape is constant in time. It remains to be explored how adaptive learning influences evolution of the genetic basis for phenotypic traits on a dynamic fitness landscape because, for example, the environment changes frequently or the optimal phenotype depends on the frequency of other phenotypes in the population.

Theory has shown that the cost of learning plays a crucial role in the evolutionary dynamics of traits modified by learning. Experimental evidence for costs of learning are only beginning to emerge (Mery and Kawecki 2003; or the cost of phenotypic plasticity see Auld et al. 2010), yet they are essential to motivate biologically realistic cost functions in the theoretical models of joint evolution of learning and innate behaviour. Any cost of learning determines the evolution of learning and, therefore, it will play a particularly relevant role in any model of joint evolution of adaptive learning and innate behaviour. Another common assumption awaiting empirical scrutiny is that all genotypes are equally capable of learning. This, however, need not be the case and theoretical predictions may change entirely if the level of learning is variable for different genotypes (for example, if there is a correlation between the genetically determined trait value and the level of learning as discussed in Mery and Kawecki 2004).

To date, empirical evidence for a role of learning in evolution is virtually absent (but see Mery and Kawecki 2004). An empirical approach requires a model system where genetic variation for both a behavioural trait and the ability to learn are demonstrated, and where the level of learning and the innate value of the behavioural trait can both be quantified as separate traits.

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6

Does an evolving ability to learn promote or impede evolutionary change?

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Abstract – Adaptive learning changes the fitness of a genotype and either speeds up or impedes evolution of innate (*sensu* genetically determined) behaviour according to models that assume different, fixed levels of learning. Here, we model the rate of evolution when the costly and adaptive ability to learn evolves. We consider the behavioural response of a phenotype to an environmental cue, assume a relation between the cue-value and fitness, and explore the effect of fixed vs. evolving levels of adaptive learning within a preset exploratory range of cue-values. Away from the response that confers maximum fitness, learning accelerates evolution of the innate response but close to this fitness peak it is the (fixed) level of learning which determines whether evolution is accelerated or decelerated. If learning is allowed to evolve and the exploratory range is large enough, learning evolves either to zero or towards an optimum, depending on its initial level and the cost. If this level is beyond the optimum, then the number of generations to reach the fitness peak of the innate behaviour is greatly reduced. Thus, there is a threshold level of learning above which joint evolution of learning and innate behaviour is accelerated.

Key words: Baldwin effect, fitness landscape, evolution of phenotype, adaptive learning, evolution of learning.

Evolution proceeds by changes in the genetic make-up, but natural selection acts on phenotypic differences that have only a partial genetic basis. Thus, phenotypic variation masks genetic variation, thereby possibly slowing down evolutionary change (e.g., Falconer 1981). For example, otherwise less fit individuals effectively mask fitness differences in a genetically variable population, if they exhibit adaptive learning, i.e., the ability of an individual to modify behaviour so as to promote fitness. However, a contemporary of Darwin – Mark Baldwin (1896) – came to the opposite hypothesis: adaptive learning accelerates evolution of genetically determined (further referred to as *innate*) traits. To date, this hypothesis, also known as the Baldwin effect, was addressed main-

ly in theoretical studies (Hinton and Nowlan 1987; Fontanari and Meir 1990; Papaj 1994; Anderson 1995; Mayley 1997; Ancel 1999, 2000; Dopazo et al. 2001; Borenstein et al. 2006; Paenke et al. 2007; but see Mery and Kawecki 2004 for an experimental approach). It proposes that selection promotes individuals capable of acquiring a fitter phenotype by learning when no genetic predisposition for such a phenotype, as yet, exists. The Baldwin effect predicts that learning confers a larger fitness increase to those phenotypes (as well as underlying genotypes) that are relatively closer to the fitness peak (Baldwin 1896). Therefore, selection will act to improve learning and indirectly also create selection for innate behaviour. According to Baldwin, in a non-plastic population selection of fitter genotypes proceeds slower because there is no learning that confers the additional fitness benefits to genotypes that are already closer to the fitness peak.

Current theoretical models establish whether the accelerating or the decelerating effect of adaptive learning dominates the evolutionary process. The general approach is to measure the rate of evolution of an innate behaviour given different levels of a non-evolving ability to learn. However, the predictions of these models did not lead to a consensus: some lent support for an accelerating effect of adaptive learning on evolution (Hinton and Nowlan 1987; Fontanari and Meir 1990; Ancel 1999, 2000: the norm of reactions model; Mayley 1997), whereas others supported a decelerating effect (Andersson 1995; Ancel 2000: the quantitative genetics model, Dopazo et al. 2001). The most recent models (Paenke et al. 2007; Borenstein et al. 2006) represent attempts at unifying these different predictions and do so by defining the theoretical conditions under which one or the other effect of learning prevails. In particular, Paenke et al. (2007) argue that the curvature of the fitness landscape predicts when non-evolving and cost-free learning accelerates or decelerates evolution (see also Egas et al. [2004] for a similar argument). In their model they assume that adaptive learning generates a small shift in the expression of a genotype. They show that if already fitter genotypes gain more fitness due to such an effect of learning, then fitness differences among neighbouring genotypes will be magnified. This will result in stronger selection and thus faster evolution of the innate behaviour. Conversely, if less fit genotypes gain more fitness from learning, then the fitness differences among the neighbouring genotypes will be reduced. The result will be weaker selection and slower evolution of the innate behaviour. It should be noted that the results of Paenke et al. (2007) consider evolution of innate trait only, while adaptive learning is kept fixed.

Neither Paenke et al. (2007) nor earlier studies addressed the possibility of the evolution of learning ability itself. A notable exception are the models by Ancel (2000) and Lande (2009) which consider the evolution of reaction norm

(i.e., a range of phenotypes that a genotype is able to produce) and not the evolution of learning ability. In the model by Ancel adaptive learning is set at a maximum level by assuming that plastic individuals always choose the fittest phenotype from their norm of reactions. Ancel shows that such adaptive plasticity accelerates evolution in the initial stages of the Baldwin effect, i.e., evolution towards a distant fitness peak. This effect is associated with the initial selection for the wider norms of reactions. However, the decelerating effect of learning prevails in the second and final stage of the Baldwin effect. The results of Ancel are corroborated by the study of Lande (2009) where plasticity is also modelled as a reaction norm evolving under the Gaussian fitness landscape. These two studies are a notable exception in the theory of the Baldwin effect by allowing phenotypic plasticity to evolve jointly with the innate trait (see also studies in the framework of artificial life/intelligence, e.g., Watson and Wiles 2002, Suzuki and Arita 2004).

However, learning abilities are known to vary among individuals and there is growing empirical evidence for a genetic basis so that lower or higher levels of learning can be selected for (McGuire and Hirsch 1977; Mery and Kawecki 2002; Dukas 2004). In fact, it was already Mark Baldwin whose verbal argument was based on the assumption that the innate behaviour as well as the ability to learn are subject to selection (Baldwin 1902).

In the model presented here we simulate evolution on a fitness landscape to examine whether allowing adaptive learning to evolve accelerates or decelerates evolution towards the fitness peak of a genetically determined behaviour and compare this with the scenario in which the ability to learn is fixed.

The model

We consider a population under selection on a continuous phenotypic trait such as an innate (i.e., genetically determined) behavioural response to a concentration of a continuous environmental cue (e.g., an olfactory signal). The response of an innate phenotype to any specific concentration is given by a probability density function I that is a Gaussian function with a fixed, non-evolving variance σ_I^2 and an evolving mean μ_I :

$$I(\mu_I, \sigma_I, c) = \frac{1}{\sigma_I \sqrt{2\pi}} \exp \left[-0.5 \left(\frac{c - \mu_I}{\sigma_I} \right)^2 \right].$$

Thus μ_I represents the innate behavioural response that is evolving in this model. Here c is the concentration of the environmental cue, and we interpret the variance σ_I^2 as the measure of the width of the exploratory range of the

organism (which could in principle also evolve, but this is ignored here). The mean and variance determine the range of concentrations that an organism with these phenotypes can explore and consequently learn about. The variance σ_I^2 can also be thought of as the reaction norm, but note that this norm has no assigned hard boundaries (the soft boundaries being determined by the variance of the distribution function). Given that the variance is not evolving in our system, for ease of presentation we suppress variate σ_I when discussing $I(\mu_I, c)$. In the absence of learning the function $I(\mu_I, c)$ is the probability density function for a phenotype's response to differing levels of concentration of the environmental cue (that the phenotype perceives within its exploratory range). In biological terms, this model of the innate behaviour can be interpreted as the genetically determined response of a predatory mite to a cue of prey presence whose value is continuous. For example many predatory mites use herbivore-induced plant volatiles to locate their herbivorous prey; our model simulates a biological situation where a predatory mite uses a concentration of a single plant volatile to locate prey (as suggested by the study of, e.g., de Boer and Dicke 2004). The predatory mite responds with certain probabilities, given by a Gaussian function, to a range of concentrations of this volatile encountered in the environment. The mean of the Gaussian function, μ_I , indicates the concentration of the volatile that the predator chooses most frequently.

The fitness reward obtained in a given value of the concentration c is given by the function F . In our model fitness function $F(c)$ is also Gaussian with a variance σ_F^2 and a mean μ_F :

$$F(c) = \frac{1}{\sigma_F \sqrt{2\pi}} \exp \left[-0.5 \left(\frac{c - \mu_F}{\sigma_F} \right)^2 \right].$$

The value of μ_F is set to zero and the variance σ_F^2 is set to a fixed value (see Appendix 6.2). Again for ease of presentation we suppress these two variables as they are fixed. The mean of this Gaussian function μ_F indicates the concentration that provides the highest fitness reward.

The measure of adaptive learning is provided by a non-negative value, n . The learning function L is chosen to be an exponential function with the exponent given by the product of n , a parameter defining the level of adaptive learning, and F , the fitness pay-off from the response to a concentration of the environmental cue:

$$L(n, c) = \exp[nF(c)].$$

We represent learning as a multiplicative adjustment to the organism's innate response, $I(\mu_I, c)$. Therefore, the probability of response of a phenotype with a given μ_I and σ_I^2 to concentrations lying in the range $[c_1, c_2]$ is:

$$\int_{[c_1, c_2]} I(\mu_I, c) L(n, c) / N(\mu_I, n) \, dc,$$

where $N(\mu_I, n) = \int I(\mu_I, c) L(n, c) \, dc$ is the normalizing constant (introduced to ensure that the integral of this response function is equal to one). As shown in Figure 6.1, this procedure introduces the modification that leads to the organism having a higher fitness with learning than without. This learning function is borrowed from theoretical models of the behavioural response of ants to scent trails (Millonas 1992) where the parameter n reflects how closely an ant follows the scent gradient. This function provides three main advantages. Firstly, it allows us to model adaptive learning, i.e., learning that modifies the innate response so as to gain fitness. Secondly, it allows us to vary the level of adaptive learning by varying the value of parameter n . In particular, the larger the value of the parameter n , the more precisely the innate probability of response is adjusted to follow the fitness function. Thirdly, we can allow evolution of learning ability by making n an evolving variable. Hence both the innate response represented by μ_I and the learning ability represented by n are allowed to evolve in this model. These advantages are not all shared by other systems that model the learning effect. Such systems include modelling an exploratory range within which individuals always chooses the single most fitness-rewarding behaviour (e.g., Ancel 1999, 2000), or modelling a constant phenotypic shift in the direction of increased fitness (e.g., Paenke et al. 2007). Such approximations either do not contain an evolving variable quantifying the level of learning or model the exploratory range rather than adaptive learning.

We calculate the phenotype's realized fitness $W(\mu_I, n)$ as the sum of fitness pay-offs obtained from all concentrations sampled by this phenotype with the probabilities given by the 'learned' probability density function:

$$W(\mu_I, n) = \int [I(\mu_I, c) L(c, n) / N(\mu_I, n)] F(c) \, dc - C(n).$$

$N(\mu_I, n) = \int I(\mu_I, c) L(c, n) \, dc$ is the normalizing constant, c = concentration, and $C(n)$ is the cost of learning given by the function

$$C(n) = n * \kappa,$$

where κ is the cost coefficient. This function assumes that there is a cost of learning and memory, which is 'paid' in terms of energy spent on maintaining

cognitive structures and/or processes (Dukas 2004; Mery and Kawecki 2003). Hence, we subtract the cost which is dependent on the level of learning from the phenotype's fitness. The value of the cost is a product of the level of learning and a cost coefficient κ . In absence of empirical evidence we set the cost coefficient κ equal to either zero or some arbitrary fixed value (see Appendix 6.2).

We assume that evolution of this phenotype proceeds in the direction of maximum increase in fitness which is given by the gradient vector at this point in the fitness landscape (Lande 1979; Rice 2004). The gradient vector indicates the direction and the rate of evolution, assuming that (1) genetic variances of the evolving traits remain constant in time and are of comparable magnitude, that (2) there is no genetic correlation between the two evolving traits, and that (3) genetic variation of these traits is additive. Given these assumptions, we let the population be represented by a single phenotype on the fitness landscape. To trace the evolution of such a phenotype that consists of two traits, we calculate the partial derivatives of realized fitness $W(\mu_I, n)$ with respect to the mean of the innate behaviour μ_I , and the measure of the learning ability n , to obtain the gradient vector:

$$\nabla W = (\partial W / \partial \mu_I, \partial W / \partial n).$$

At any point on the fitness landscape, the direction and the magnitude of the gradient vector provide the direction and the rate of evolution of the phenotype. The partial derivative $\partial W / \partial \mu_I$ describes the rate of evolution of the innate behaviour (i.e., the rate of change in the value of the innate response represented by μ_I) holding the level of learning n fixed. While the partial derivative $\partial W / \partial n$ describes the rate of evolution of the level of learning holding the innate behaviour fixed. Together these properties allow us to determine (1) the direction and the rate of evolution of the innate response when the response and learning ability jointly evolve, (2) the rate of evolution of the innate response given different, yet fixed, levels of learning, and (3) the rate of evolution in the absence of learning when the innate response completely determines the behaviour. Comparing (2) and (3) is what occurs in the models of the Baldwin effect to determine whether adaptive learning accelerates or decelerates evolutionary change (Papaj 1994; Hinton and Nowlan 1987; Fontanari and Meir 1990; Mayley 1997; Andersson 1995; Ancel 1999, 2000, Dopazo et al. 2001; Borenstein et al. 2006). We expand these models by comparing the predictions generated for (2) and (3) with those for (1).

The gradient vector points in the direction of the fastest rate of increase in fitness, given a marginal change in the genetic bases of the two traits underlying

the phenotype. Therefore it can be interpreted as a measure of the short-term evolution from one generation to the next (i.e., when a mutation results in a small phenotypic change). We wish to also infer the effect of learning on long-term evolution. To this end we calculate the time (in generations) required for a phenotype to evolve up to a fitness peak (i.e., the optimal value) by re-calculating the gradient vector at each generation. However, as evolution slows down dramatically in the vicinity of the fitness peak, for this practical reason we proceeded with the calculations up to an arbitrarily small distance from the fitness peak (the value of which is to be found in Appendix 6.2). We therefore compare the rate of short- and long-term evolution given either a fixed or an evolving learning ability.

Noting that $I(\mu_I, c)L(c, n)/N(\mu_I, n)$ is a probability density function we interpret

$$E[X] = \int [I(\mu_I, c)L(c, n)/N(\mu_I, n)] X(c) dc,$$

as the expected value of the function $X(c)$. Given the specific functions for I , L and F assumed in this study, we derive the following expressions for the components of the gradient vector:

$$\begin{aligned} \partial W / \partial \mu_I &= E[F * (1/I) * \partial I / \partial \mu_I] - E[F] * E[(1/I) * \partial I / \partial \mu_I] \\ &= \text{covariance}[F, (1/I) * \partial I / \partial \mu_I], \end{aligned}$$

and

$$\begin{aligned} \partial W / \partial n &= E[F^2] - E[F] * E[F] - \kappa \\ &= \text{variance}[F] - \kappa. \end{aligned}$$

The derivation of these results is provided in Appendix 6.1. Thus in this study the rate of evolution in the innate response ($\partial W / \partial \mu_I$) depends on the covariance between fitness and a function determined by the innate probability function and its first derivative, whereas the rate of evolution of learning ability ($\partial W / \partial n$) depends on the variance in fitness as experienced by a phenotype (an individual receives many pay-offs by exploiting the range of concentration over its lifetime, i.e., the range of fitness pay-offs weighted by the probability distribution IL/N of this phenotype) and the value of the cost coefficient κ .

Correspondence with the Paenke et al. model

Paenke et al. (2007) focus on the evolution of the innate trait at different fixed levels of learning. In particular, the authors examine fitness differences among

phenotypes that differ with respect to the innate trait value, at two different fixed levels of learning. These fitness differences are reflected in the slope of the fitness landscape evaluated along the axis of the innate trait (the larger the slope, the larger are the fitness differences among phenotypes). In this respect their model is similar to ours. In their model, as in our study, the slope of the fitness landscape in the direction of the innate trait is given by the component of the gradient vector $\partial W/\partial \mu_I$, while the sign of the mixed derivative $\partial^2 W/\partial n \partial \mu_I$ indicates whether increasing the level of learning results in a steeper or milder slope of the fitness landscape in the direction of the innate trait (i.e., whether improving learning increases or decreases fitness differences among phenotypes with different innate traits). Paenke et al. derive a correspondence between this general result and the shape of the fitness function (evaluated only in the direction of innate trait). In particular the authors predict that learning magnifies fitness differences among phenotypes when the fitness landscape is convex, and conversely adaptive learning reduces fitness differences among phenotypes when this fitness landscape is concave.

It is important to stress however that in the model of Paenke et al., the evolution of the phenotype effectively proceeds on a one-dimensional fitness landscape because the level of learning remains constant. In our model both the innate trait and the level of learning are allowed to evolve, and as such the fitness landscape is two dimensional. This allows us to explore evolution in the fitness landscape along three paths: (1) parallel to the axis of the innate behaviour, holding the level of learning fixed, (2) parallel to the axis of the learning ability, holding innate behaviour constant and (3) along the path of evolution of phenotype, i.e., when both the level of learning and the innate behaviour jointly evolve. In particular we examine how the short-term evolution from one generation to the next (as explored in Paenke et al. 2007) translates to changes in the long-term rate of evolution on a more complex fitness landscape when both the innate trait and learning evolve.

Results

We follow evolution of a phenotype on a fitness landscape in two parameter settings concerning the exploratory range. These settings are determined by the ratio of σ_I^2 (i.e., the exploratory range of a phenotype) to σ_E^2 (i.e., the range of all the environmental options). Firstly, we assume a small variance ratio (i.e., small exploratory range), meaning that an individual phenotype can sample, and thus learn, a range of environmental options (i.e., concentrations) much smaller than the full range σ_E^2 (Figure 6.1A). This setting corresponds to the effect of learning being modelled as a small phenotypic shift in the direction of increased fitness, as in Anderson (1995), Ancel (2000) and Paenke et al. (2007).

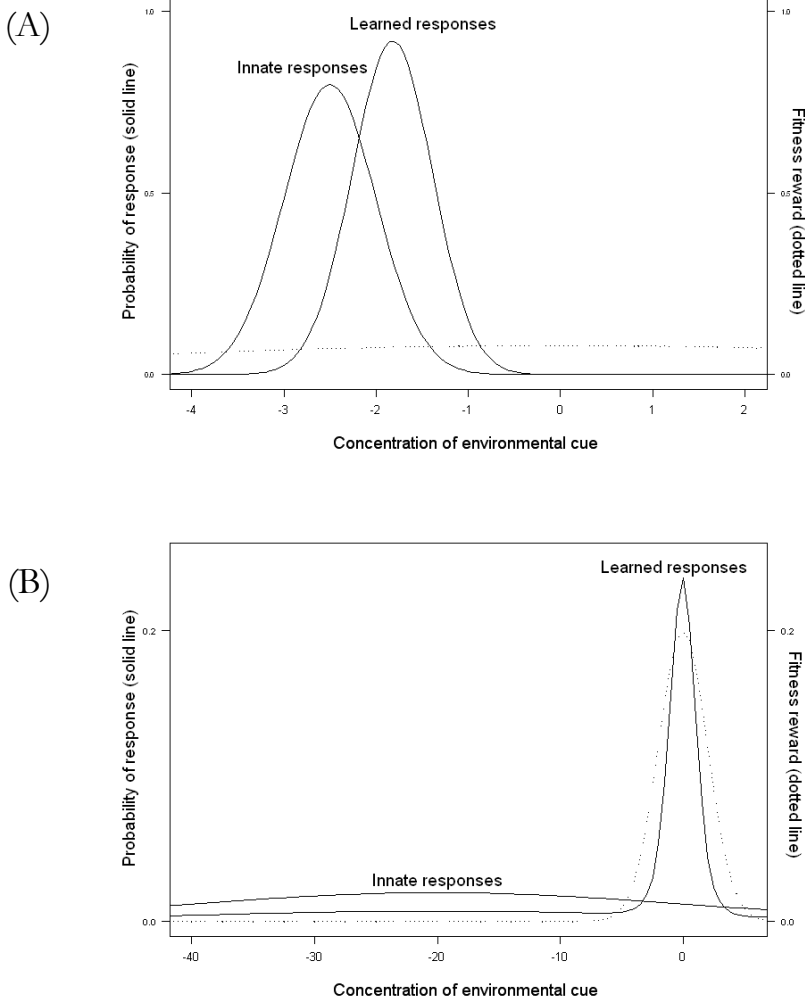
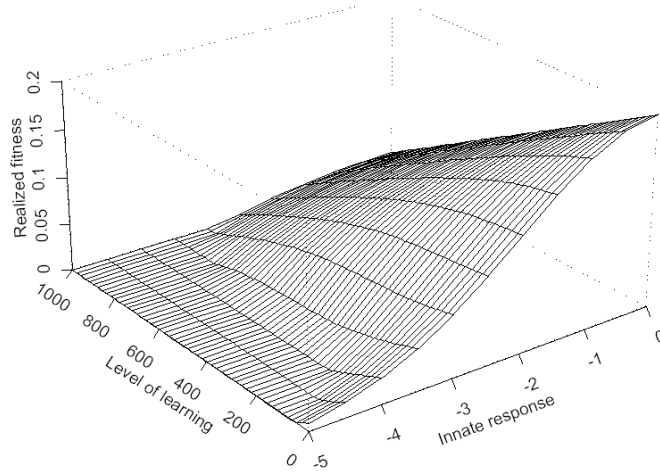


Figure 6.1 An example of innate and learned responses of a phenotype (solid lines) to a range of concentrations of an environmental cue (x-axis). The probabilities of the innate response (y-axis on the left hand-side) are provided by a normal distribution (response $\sim N(\mu_I, \sigma_I^2)$). Responding to any given concentration of the environmental cue provides a fitness pay-off that is determined by the assumed fitness function (fitness pay-off $\sim N(\mu_F, \sigma_F^2)$, dotted line, y-axis on the right-hand side). The phenotype can explore the range of environmental cues and thus adaptively modify its innate responses within either (A) a small exploratory range σ_I^2 or (B) a large exploratory range σ_I^2 . The width of the exploratory range is defined as either small or large relative to the variance of the fitness function σ_F^2 . Adaptive learning weights the innate probability of response to a given concentration by the fitness pay-off obtained from choosing this concentration dependent on the level of adaptive learning n ; in the shown examples (A) $n = 500$, and (B) $n = 20$.

Secondly, we assume that σ_I^2 is large as compared to σ_F^2 (large exploratory range), meaning that phenotypes sample, and thus learn, a relatively wide range of environmental options (Figure 6.1B). This setting corresponds to the effect of learning being modelled as a large phenotypic shift in the direction of increased fitness, as in Hinton and Nowlan (1987), Fontanari and Meir (1990), Borenstein et al. (2006). This correspondence exists in terms of the net effect

(A)



(B)

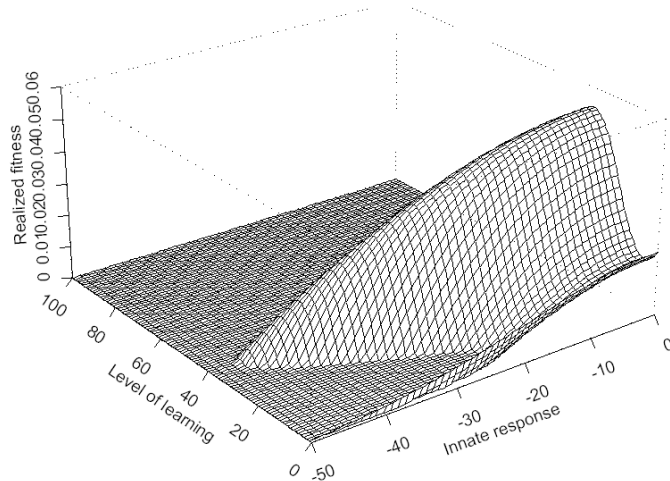


Figure 6.2 The fitness landscape that illustrates the relationship between phenotype and its realized fitness W (z-axis); the phenotype samples and thus learns within either (A) a small exploratory range or (B) a large exploratory range. The phenotype is determined by the innate response (indicated by the mean of a normal distribution μ_I on x-axis) and the level of learning n (y-axis). Note that some phenotypes have zero fitness because for these phenotypes the cost of learning outweighs its benefits. The cost coefficient κ used to calculate this cost is set to equal half the maximum variance in fitness F as found among all phenotypes (see Appendix 6.2).

of learning on phenotypic fitness and not in terms of the mathematical operations used to model learning. The two analysed settings for the exploratory range put different constraints on the potential of adaptive learning to modify innate behaviour and treat the exploratory range as an aspect of behaviour that is separate from learning ability (see Appendix 6.2 for the exact values of the parameters used in these two settings).

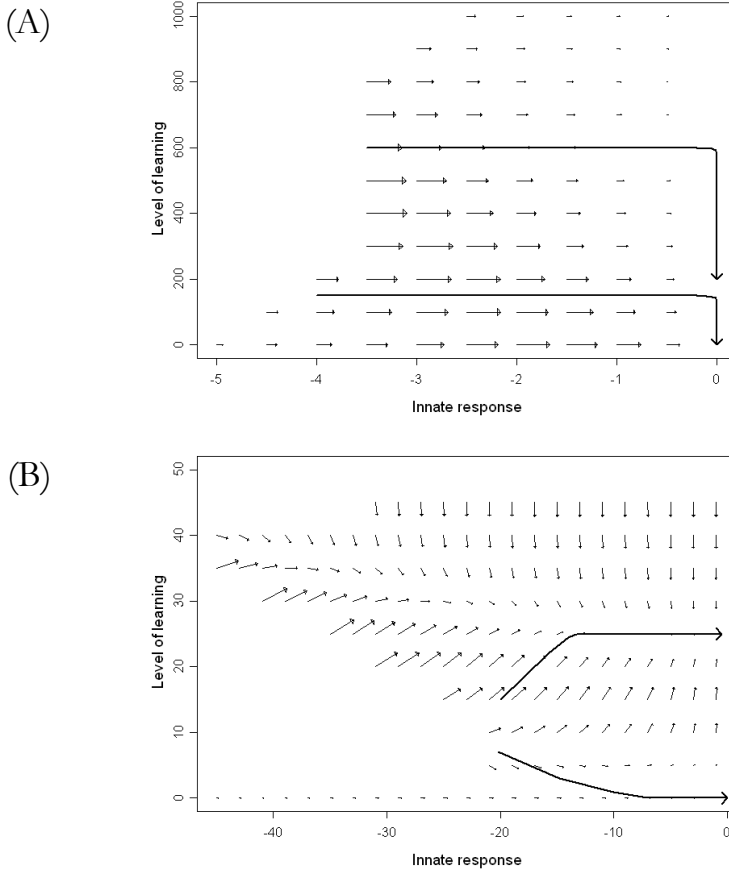


Figure 6.3 The instantaneous rate of phenotype evolution illustrated by the gradient vector obtained for different phenotypes on the fitness landscape (as shown in Figure 6.2). The phenotypes can sample and thus learn about the environment within either (A) a small exploratory range, or (B) a large exploratory range. The gradient vector points in the direction of phenotype evolution, i.e., in the direction of the fastest increase in phenotypic fitness given a marginal change in the genetic bases of the two traits underlying the phenotype. Note that for some phenotypes there is no gradient vector shown; these phenotypes have zero fitness (compare with Figure 6.2). We trace the instantaneous evolution starting from two different initial phenotypes to illustrate the predictions of the long-term evolution towards the fitness peak when both the innate response and the level of learning evolve.

For any point on the fitness landscape (Figure 6.2A for small exploratory range, Figure 6.2B for large exploratory range) we evaluate the instantaneous rate of phenotype evolution provided by the gradient vector at different, but fixed levels of learning (Figure 6.3A for small exploratory range, Figure 6.3B for large exploratory range). The rate of evolution of the innate behaviour is extracted from the gradient vector by taking the length of its horizontal component (Figure 6.4A for small exploratory range, Figure 6.4B for large exploratory range). To assess whether learning accelerates or decelerates evolution, we compare predictions for when the level of learning $n = 0$ and when $n > 0$ (as in, e.g., Anderson 1995; Ancel 2000; Papaj 1997). Away from the fitness

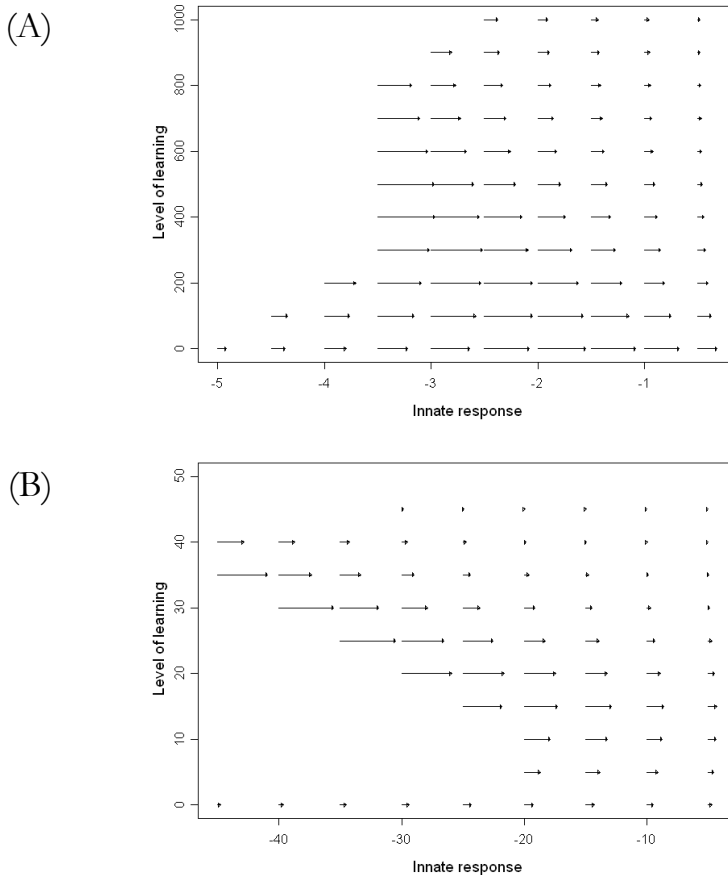


Figure 6.4 The instantaneous rate of evolution of the innate response holding the level of learning fixed extracted from the length of the horizontal component of the gradient vector (as shown in Figure 6.3). The phenotypes can sample and thus learn about the environment within either (A) a small exploratory range, or (B) a large exploratory range. For some phenotypes there is no vector shown; these phenotypes have zero fitness (compare with Figure 6.2).

peak for the innate behaviour (Figure 6.2) any fixed level of learning (n) accelerates evolution of the innate behaviour (as compared to non-learning) irrespective of whether the exploratory range is small (Figure 6.4A) or large (Figure 6.4B). Close to the fitness peak for the innate behaviour, it decelerates this process when the exploratory range is small (Figure 6.4A). However, when the exploratory range is large, an increase in the fixed level of learning first leads to accelerated evolution of the innate behaviour up to a point where any further increase in the level of learning leads to decelerated evolution (Figure 6.4B).

To assess what happens to the evolution of the innate behaviour if we allow the level of learning to evolve, we can deduce how the level of learning changes from Figure 6.3 (see superimposed arrows). For a small exploratory range (Figure 6.3A) it remains rather constant until innate behaviour has evolved to its fitness peak where there will be selection against learning. For a large explorato-

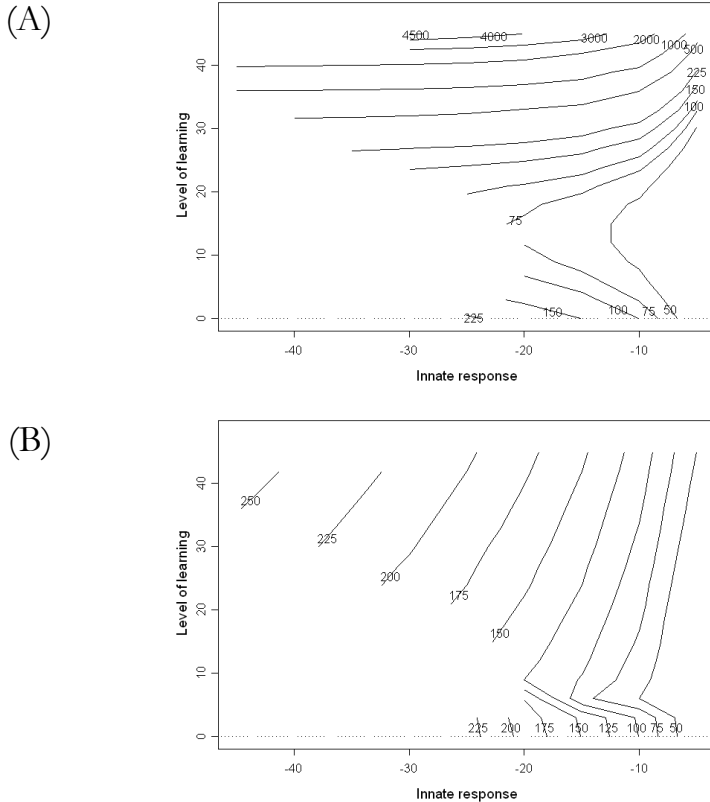


Figure 6.5 The time required for a phenotype to evolve up to a fitness peak from an initial combination of the innate response (x-axis) and the level of learning n (y-axis); the contour lines indicate the initial phenotypes that require the same number of generation to reach the fitness peak. The initial level of learning is either (A) kept fixed or (B) allowed to evolve jointly with the innate behaviour.

ry range (Figure 6.3B) the level of learning will evolve depending on its initial level, either to zero or towards an optimum. Thus, there is a critical threshold for the level of learning below which there is selection against learning and above which selection optimizes the level of learning.

Given these outcomes for the evolution in the level of learning, we can now assess how they influence the time for the innate behaviour to reach the fitness peak. For a small exploratory range there will be little impact because learning hardly changes evolution. For a large exploratory range, however, there will be an impact due to the bi-stable outcome of evolution in the level of learning, as shown by comparing Figure 6.5A (fixed level of learning) and 6.5B (evolving level of learning). Starting from a given phenotype, the number of generations for the innate behaviour to reach the fitness peak is reduced by orders of magnitude, provided the initial level of learning is higher than the optimal level of learning (Figure 6.6). If it is smaller, evolving levels of learning increase the number of generations to reach the fitness peak (though by less than one order of magnitude).

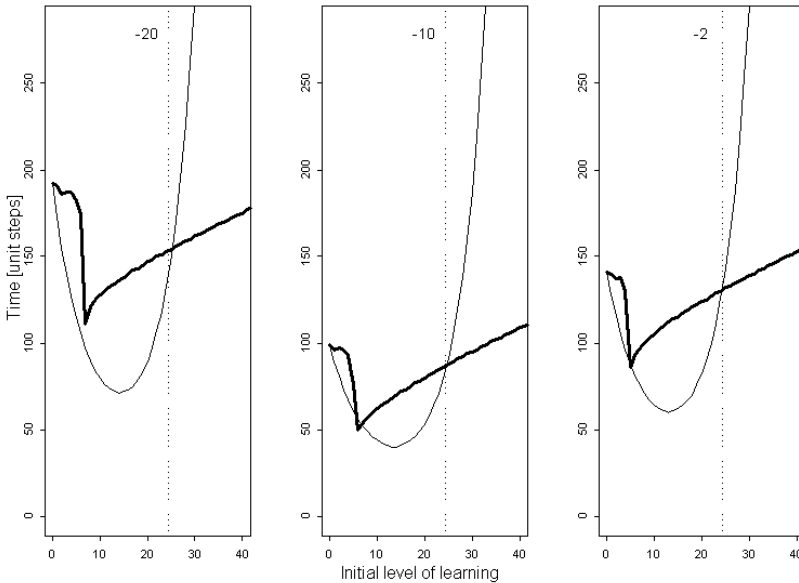


Figure 6.6 The time required for a phenotype to evolve up to a fitness peak shown as a function of its initial level of learning n and exemplified for three cases of the initial innate response (indicated by the means of the normal distribution $\mu_I = -20$, $\mu_I = -10$ and $\mu_I = -2$, shown at the top of each panel). Adaptive learning modifies the innate response within a large exploratory range and is either kept fixed at the initial level (thin solid line) or allowed to evolve jointly with the innate behaviour (thick solid line). The dotted vertical line indicates the optimal level of learning (i.e., the value of n at which $\partial W/\partial n = 0$ given that $\mu_I = 0$).

In conclusion, the evolutionary predictions for fixed and evolving learning are at odds especially when the initial level of learning assumed in the analysis is beyond the evolutionary optimum. In addition, the evolutionary predictions are not very different when adaptive learning provides a small fitness return (relative to the cost of learning), i.e., when the initial level of learning is low or when adaptive learning is constrained by a small exploratory range.

The evolution of costly learning

We show that given a certain cost of learning, the evolution of learning from zero is not possible although a medium level of learning is associated with higher fitness in the setting of large variance ratio; and depending on the initial level, adaptive learning evolves either to an optimal level or to zero. Here we explain how this bi-stable outcome of evolution depends on the cost of learning.

In this model we assume that the cost of learning increases linearly with the level of learning n , at the rate determined by the magnitude of the cost coefficient. The analytical result of our model states that the rate of instantaneous evolution of learning $\partial W/\partial n$ equals the variance in fitness F (as evaluated for a given phenotype) minus the cost coefficient κ (see Appendix 6.1). Thus a phenotype experiences selection against learning if the cost coefficient $\kappa >$ variance in fitness F evaluated for this phenotype (i.e., $\partial W/\partial n$ has a negative sign). Conversely, a phenotype experiences selection for improved learning if the cost coefficient $\kappa <$ variance in fitness F as evaluated for this phenotype (i.e., $\partial W/\partial n$ has a positive sign). In the setting of a large variance ratio the maximum variance in F , that determines the value of the cost coefficient κ (see Appendix 6.1), is found for a phenotype with $n \neq 0$. Therefore for phenotypes where $n = 0$ (or it is very low), the cost coefficient $\kappa >$ variance in fitness F and these phenotypes experience selection against learning starting from zero level.

We obtain conditions that favour the evolution of learning from the zero level by setting the value of the cost coefficient κ to be less than the maximum variance in F as found among the non-plastic phenotypes, i.e., where $n = 0$ (effectively this means a lower cost of learning). We illustrate this in the setting of a large variance ratio by re-calculating the gradient vectors for the situation where the cost coefficient κ is equal to half the maximum variance found among the non-plastic phenotypes (Figure 6.7A). The evolution of learning from the zero level is prevented when the initial phenotype starts far from the fitness peak as the cost of learning in this neighbourhood still outweighs its benefits. This phenomenon, however, is reversed near the fitness peak. Moreover some learning is retained by phenotypes at the optimal innate response as their exploratory range is wide in comparison with the narrow range of available environmental options (as determined by σ_I^2 of the fitness function F). We con-

clude that, as long as there is a non-zero cost of learning, there exists a critical threshold for the level of learning below which there is selection against learning and above which selection optimizes the level of learning. This threshold moves down with increasing costs of learning. The upper bound to the cost is

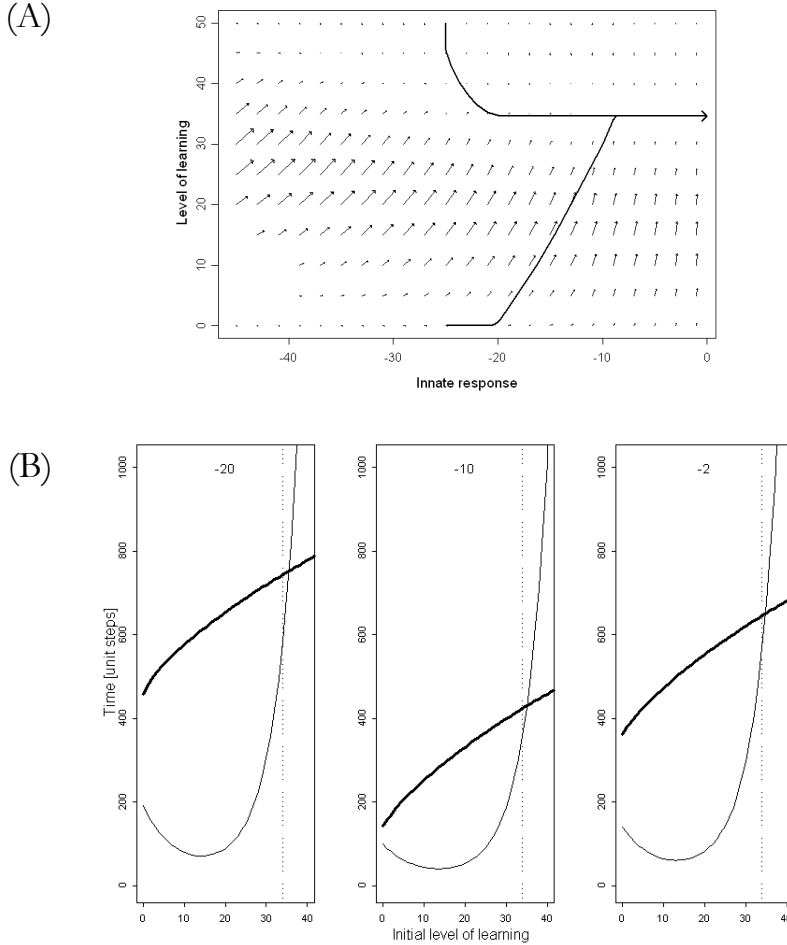


Figure 6.7 The direction and rate of phenotype evolution when the cost of learning is equal to half the maximum variance in fitness as found among non-plastic phenotypes; (A) the instantaneous rate of phenotype evolution illustrated by the gradient vector and (B) the time required for a phenotype to evolve up to a fitness peak shown as a function of its initial level of learning n exemplified for three cases of the initial innate response (indicated by the means of the normal distribution $\mu_I = -20$, $\mu_I = -10$ and $\mu_I = -2$, shown at the top of each panel). Phenotypes learn within a large exploratory range and the level of adaptive learning is either kept fixed at the initial level (thin solid line) or allowed to evolve jointly with the innate behaviour (thick solid line). The dotted vertical line indicates the optimal level of learning (i.e., the value of n at which $\partial W/\partial n = 0$ given that $\mu_I = 0$).

given by the value of the maximum variance in fitness found among all phenotypes such that if the cost coefficient $\kappa \geq$ this maximum variance then improved learning is always selected against.

Next, we ask how the cost of learning may, indirectly, influence the evolution of innate behaviour. In our model varying the value of the cost coefficient κ does not influence the rate of instantaneous evolution of the innate behaviour evaluated at a given level of learning n , due to the assumption that the cost of learning is subtracted from phenotypic fitness and hence it is dropped from $\partial W/\partial \mu_I$. However, the cost coefficient κ may influence the long-term evolution of innate behaviour by influencing the rate of evolution of adaptive learning. In the extreme case of cost-free adaptive learning the phenotype evolves almost exclusively towards improved learning until the level of learning is reached at which further evolution in both the level of learning and innate behaviour is so slow as to be negligible. Thus if there is a non-zero cost of learning and the cost coefficient κ is less than the maximum variance in fitness F found among all possible phenotypes, then we find an optimal level of learning $n \neq 0$. If the initial level of learning is higher than this optimum then the long-term evolution of the innate behaviour is greatly accelerated provided that learning is allowed to evolve (compare thin and thick lines in Figure 6.7B). However, levels of learning below the optimum decelerate evolution of the innate behaviour.

Discussion

In this article, we construct a general model and provide it with specific functions to test the Baldwin effect, i.e., the hypothesis that adaptive learning accelerates the rate of evolution of innate (i.e., genetically determined) behaviour. Our model allows for the evolution of innate behaviour given different but fixed levels of adaptive learning (i.e., level of learning, n , where learning occurs within a predefined (small or large) exploratory range) but also when adaptive learning is an evolving trait in itself. We find that fixed levels of learning decelerates evolution of the innate behaviour (as compared to non-learning) when this behaviour is close to the response that confers highest fitness. However, away from this fitness peak learning accelerates this process. Both results are in agreement with Ancel (2000) and Lande (2009). We further show that allowing levels of learning to evolve greatly reduces the time to reach the fitness peak (i.e., by orders of magnitude), given that (1) learning is realized within a sufficiently large exploratory range, (2) learning has a fitness cost, and (3) the initial level of learning is higher than the evolutionary optimum. We therefore predict that making the level of learning an evolvable trait matters to how learning affects the evolution of the innate behaviour and the number of generations needed to reach the fitness peak, but only if the exploratory range is sufficiently large. If the width of the

exploratory range is too small, evolution of the phenotype proceeds effectively only via changes in the innate behaviour (Figure 6.3A). Thus, assuming a form of fixed learning (as in, e.g., Fontanari and Meir 1990; Andersson 1995; Ancel 2000: the quantitative genetics model; Dopazo et al. 2001; Paenke et al. 2007; Borenstein et al. 2006) is only justified when the net effect of learning on phenotypic fitness is small. Moreover, we predict that if the exploratory range is sufficiently large and learning is costly, there is a critical threshold level of learning above which learning is favoured by selection and evolves towards an optimum level and below which it is selected against (Figure 6.3B). If the initial level of learning is sub-optimal, evolution of learning causes an increase in the number of generations to reach the fitness peak (although by less than an order of magnitude) as compared to the situation where learning is fixed.

Given these predictions we now ask whether they are robust against modifications of the specific functions chosen for (1) the innate behaviour function $I(\mu_I, \sigma_I, c)$, (2) the learning function $L(n, c)$ and (3) the learning cost function $C(n)$. Moreover, (4) we may ask whether the predictions are robust to making the exploratory range an evolutionary variable in addition to the level of learning (n).

The innate behaviour function $I(\mu_I, \sigma_I, c)$ in our model is given by a normal distribution. The mean of the normal distribution is regarded as the evolving innate behaviour and the variance of the normal distribution provides soft boundaries of the exploratory range within which the innate responses to a range of environmental cues may occur. An alternative function might be that the innate behaviour is realized within fixed, hard boundaries. This function, however, would not yield qualitatively different results, because it only modifies the exploratory range, but not its presence.

The learning function $L(n, c)$ applied in our model allows for modification of the innate probability distribution in a manner that is adaptive independent of the form of the innate probability distribution and the shape of the fitness function. Moreover, irrespective of its shape, any function employed to model adaptive learning must result in an adaptive shift of the innate behaviour, and therefore, have the same fitness consequences as the learning function used in our model. An alternative way of modelling adaptive learning (rather than modelling the norm of reactions) might be that a constant amount is added to the value of the innate trait. This process results in a fixed and adaptive shift of the phenotype provided that the fitness landscape is increasing (i.e., the fitness slope is always positive). Other learning functions may be applied which model a plastic shift that is adaptive within either a predefined or an unconstrained exploratory range. However, in terms of the net effect of learning on phenotypic fitness these different modes of learning correspond to settings of our model, where the exploratory range is extremely small or sufficiently large.

The learning cost function $C(n)$ in our model depends linearly on the level of learning. An alternative might be that the cost of learning increase exponentially with the level of learning. This alternative cost function would, however, generate only qualitative changes in the predictions. It would further hinder the evolution of initially low levels of learning and possibly lower the critical threshold level of learning above which learning is favoured by selection and below which it is selected against.

The exploratory range of a phenotype is kept fixed in our model but it may be allowed to evolve jointly with the level of learning and the innate behaviour. Our model predicts that if the initial conditions include a large exploratory range and low level of costly learning (particularly when such phenotype starts far from the fitness peak) then adaptive learning is selected against. The large exploratory range will itself be selected against, if it comes at a cost. Selection decreasing the exploratory range, however, will lead to conditions that further hinder the evolution of adaptive learning (Figure 6.3A).

The results of our model, therefore, point to a paradox where high levels of evolving adaptive learning accelerate the evolution of phenotype, but the evolution of learning from an initially low level critically depends on the cost of learning. Elucidating the relationship between the level of adaptive learning and its cost will, therefore, contribute to the theoretical predictions of the effect of learning on the evolution of phenotype. Moreover, under natural settings the assumption of an environment that is constant in time may not be satisfied or fitness benefits of adaptive learning may depend on the density/frequency of other learning individuals in a population. These aspects require both empirical and theoretical scrutiny.

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Appendix 6.1

Gradient vector

The gradient vector on the fitness landscape is given by the partial derivatives of phenotypic fitness W with respect to the two traits: (1) the innate preference represented by the variable μ_I , and (2) the level of learning represented by the variable n . Therefore

$$\nabla W = \left(\frac{\partial W}{\partial \mu_I}, \frac{\partial W}{\partial n} \right)$$

(a) The partial derivative of W with respect to variable μ_I is given by:

$$\left(\frac{\partial W}{\partial \mu_I} \right) = \frac{\partial}{\partial \mu_I} \left(\int \frac{IL}{N} F dc \right) \cdot \kappa * n = \int L F \frac{\partial}{\partial \mu_I} \left(\frac{I}{N} \right) dc,$$

where the realized fitness of a phenotype is calculated as the sum of obtained fitness rewards from all the sampled concentrations of the environmental cue denoted by c .

Given that

$$\frac{\partial}{\partial \mu_I} \left(\frac{I}{N} \right) = \frac{N \frac{\partial I}{\partial \mu_I} - I \frac{\partial N}{\partial \mu_I}}{N^2}, \text{ and that in this model}$$

$$\frac{\partial I}{\partial \mu_I} = I \frac{(c - \mu_I)}{\sigma_I^2}, \text{ and}$$

$$\frac{\partial N}{\partial \mu_I} = \frac{\partial}{\partial \mu_I} \int IL dc = \int \frac{\partial}{\partial \mu_I} IL dc = \int \frac{\partial I}{\partial \mu_I} L dc,$$

the partial derivative of W with the respect to μ_I is given by

$$\begin{aligned} \frac{\partial W}{\partial \mu_I} &= \int \frac{L(c)F(c) \left[I(c) \left(\frac{c - \mu_I}{\sigma_I^2} \right) \int I(c')L(c')dc' - I(c) \int I(c')L(c') \left(\frac{c' - \mu_I}{\sigma_I^2} \right) dc' \right]}{N^2} dc \\ &= \int \frac{LFI}{N} \left(\frac{c - \mu_I}{\sigma_I^2} \right) dc \int \frac{IL}{N} dc' - \int \frac{LFI}{N} dc \int \frac{IL}{N} \left(\frac{c' - \mu_I}{\sigma_I^2} \right) dc' \\ &= E \left[F \left(\frac{c - \mu_I}{\sigma_I^2} \right) \right] - E[F] E \left[\frac{c - \mu_I}{\sigma_I^2} \right]. \end{aligned}$$

The final result can be re-written to allow any form of the function I for genetically determined preference to be inserted:

$$\frac{\partial W}{\partial \mu_I} = E \left[F \left(\frac{1}{I} \frac{\partial I}{\partial \mu_I} \right) \right] - E[F] E \left[\frac{1}{I} \frac{\partial I}{\partial \mu_I} \right] = \text{covariance} \left(F, \frac{1}{I} \frac{\partial I}{\partial \mu_I} \right).$$

(b) Partial derivative of W with respect to variable n is given by:

$$\left(\frac{\partial W}{\partial n} \right) = \int FI \frac{\partial}{\partial n} \left(\frac{L}{N} \right) dc - \frac{\partial}{\partial n} (\kappa * n).$$

Given that

$$\frac{\partial}{\partial n} \left(\frac{L}{N} \right) = \frac{N \frac{\partial L}{\partial n} - L \frac{\partial N}{\partial n}}{N^2}, \text{ and}$$

$$\frac{\partial L}{\partial n} = e^{nF} F,$$

$$\frac{\partial N}{\partial n} = \frac{\partial}{\partial n} \int IL \, dc = \int \frac{\partial L}{\partial n} I \, dc = \int F e^{nF} I \, dc = \int FLI \, dc, \text{ and}$$

$$\frac{\partial}{\partial n} (\kappa * n) = \kappa,$$

the partial derivative of W with respect to variable n is given by

$$\begin{aligned} \frac{\partial}{\partial n} W &= \int \frac{FI \left[\int IL \, dc' L F - L \int FLI \, dc' \right]}{N^2} \, dc - \kappa \\ &= \int \frac{F(c)I(c) \left[L(c) \left(F(c) \int I(c')L(c') \, dc' - L(c) \int F(c')L(c')I(c') \, dc' \right) \right]}{N^2} \, dc - \kappa \\ &= \int \frac{F^2 IL}{N} \, dc \int \frac{IL}{N} \, dc' - \int \frac{FIL}{N} \, dc \int \frac{FIL}{N} \, dc' - \kappa \\ &= E[F^2] - E[F]E[F] - \kappa = \text{variance}(F) - \kappa. \end{aligned}$$

Appendix 6.2

Parameter settings

We followed evolution of a phenotype on a fitness landscape in two parameter settings that were determined by the ratio of σ_I^2 (i.e., the exploratory range of phenotype) to σ_F^2 (i.e., the range of the environmental options). In particular, throughout the model we assumed that the fitness function given by the normal distribution was characterized by variance $\sigma_F^2 = 2$ and the mean value $\mu_F = 0$. For setting a small variance ratio, the variance of the innate behaviour function was taken as $\sigma_I^2 = 0.2$; thus the exploratory range of the phenotype was small compared to the full range of environmental options σ_F^2 . Conversely, for setting a large variance ratio the variance of the innate probability distribution was taken as $\sigma_I^2 = 20$. Thus, the exploratory range of the phenotype was large compared to the full range of environmental options σ_F^2 .

In order to infer the effect of learning on long-term evolution we should calculate the time (in generations) required for a phenotype to evolve up to a fitness peak (given by $\mu_F = 0$) by re-calculating the gradient vector each generation. However, because evolution slows down dramatically in the vicinity of the fitness peak, this would take excessive computing time and for this practical reason we proceeded with the calculations up to an arbitrarily small distance from the fitness peak. Thus, we calculated the time for an initial phenotype to evolve up to $\mu_I = -4$ in the setting of large variance ratio, and the time to $\mu_I = -0.5$ in the setting of small variance ratio (as illustrated in Figure 6.5). One exception concerns the Figures 6 and 7 where the long-term evolution from the initial innate response $\mu_I = -2$ was calculated as time to reach $\mu_I = -0.5$.

The value of the cost coefficient used in the learning function L was set to half the maximum variance in fitness F (i.e., the range of fitness pay-offs available to a phenotype given its learned probability distribution) found among all phenotypes from the assumed phenotype space. If the cost coefficient were equal to the maximal variance, all learning phenotypes would be selected against (as their realized fitness would be zero). As the cost coefficient was equal to half the maximal variance, for some phenotypes the cost of learning was larger than their realized fitness. In that case, the fitness of such phenotypes is set to zero.

7

Summary

This thesis deals with the evolution of behavioural traits that have both a genetic basis and can be modified; the modification I have examined concerns an adaptive (learnt) response to environmental influences. In theory, the evolution of such traits may lead to two extreme outcomes, one where the trait becomes genetically fixed (and phenotypically invariable), and the other where it is entirely shaped by environmental influences. Between these extremes lies a spectrum of traits containing a genetic component but also, to differing degrees, a modifiable component. A recurring question in evolutionary biology is how phenotypic plasticity (i.e., the ability to modify a phenotype in response to external or internal influences) may influence the rate of evolution of a trait that itself has a genetic basis. To address this question we require a model system in which there is (a) a genetic variation for both the genetically determined behavioural trait and the ability to learn, and (b) in which the level of learning and the innate value of the behavioural trait represent separate quantifiable traits. Theoretical predictions may also be obtained by tracking the evolution of such a behavioural trait in a theoretical model system.

The first part of this thesis contains the experimental tests for the presence of genetic variation in the behaviour of the predatory mite, *Phytoseiulus persimilis*, towards specific plant odours. These odours are blends of volatile compounds that plants emit when infested by a herbivorous mite that represents prey to the predatory mite. They are referred to as HIPV, which is shorthand for herbivore-induced plant volatiles. The composition of the blend of volatiles is specific to the combination of plant species and herbivore. Therefore, it has been proposed that natural enemies of the herbivores may use HIPV as signals of prey presence and thereby locate their prey. This hypothesis is supported by the well-documented attraction of predatory mites to HIPV. However, blends of volatiles are known to vary between plant species, and to vary with differing size and duration of infestation. Moreover, in a natural setting different plant species co-occur and may be infested by multiple species of herbivores. This begs the question, how predators extract a signal of prey presence from the variation in the blends of

HIPV. Adaptive learning may serve to update and reinforce predator preferences for specific volatiles (or their mixtures) after experiencing these in the presence of suitable prey. Moreover if specific HIPV are consistently coupled with the presence of prey, then genetically determined predator preferences for these HIPV may evolve by natural selection. However, existing evidence for genetically determined responses to HIPV in *P. persimilis* is contradictory. Some studies demonstrate a preference of the predators to a specific HIPV even if they have no prior experience with these plant volatiles. In contrast, other studies report that prior experience with a particular HIPV may be necessary to trigger the preference for that volatile. However these studies measure predator behaviour at the population level, and thus, in genetically variable populations. To date there is little evidence for genotypes differing with respect to preference for a particular HIPV in a natural population, although a genetic basis for the responses of *P. persimilis* to full blends of volatile compounds induced by the prey has been demonstrated.

In part I of this thesis I investigated the genetic basis for the response of *P. persimilis* to a volatile compound induced by the feeding of a herbivorous mite, *Tetranychus urticae*, its prey. Evidence to date indicates that this compound, methyl salicylate (MeSa), is a volatile consistently present in the blend induced by *T. urticae* when feeding on a variety of plant species. In the first two chapters I have presented tests of the genetic basis for the response of *P. persimilis* to MeSa. To this end I have used two approaches: the iso-female line approach (Chapter 2), and purifying selection withing isofemale lines (Chapter 3).

In Chapter 2 I have shown that there is variation in predator responses to MeSa among genetically homogenous lines (the iso-female lines) of the predator, and thus confirming that there is a genetic component in this behaviour. Moreover I have demonstrated that predator responses to MeSa are context-dependent in that they are modified both by the physiological state of the predators (i.e., whether they are well fed or starved), and by the background odours of volatiles released by uninfested plants. These results indicate that this behaviour has evolutionary potential, i.e., it has the ability to respond to selection. Moreover they suggest that the responses of this predator to MeSa may depend on an ecologically relevant context of other volatiles (i.e., volatiles associated with the presence of the prey).

In Chapter 3, I selected within iso-female lines for genotypes with contrasting responses of *P. persimilis* to MeSa offered as a pure compound. The selection was purifying in the sense that two groups of iso-female lines were established and in one group the lines were propagated via females that showed a preference for MeSa, whereas in the second group the lines were propagated via females that avoided MeSa. I hypothesized that the propagation of iso-female lines through individuals that exhibited either preference for MeSa or aversion

to MeSa would lead to a shift in the mean responses of those lines towards, respectively, preference for MeSa or aversion to MeSa. Contrary to these predictions, I observed a shift in the mean response to MeSa in the direction opposite to the selection criterion. At the same time this study confirmed that there is a genetic component in the predator responses to MeSa (reflected in the variation among the genetically homogeneous iso-female lines used in the selection). One possible explanation for this result is a correlation between predator mating preferences and the response to MeSa. In previous studies, more often than not, the focus has been on the response of females to HIPV. Our results caution that in order to understand the evolution of this behaviour, future studies should address the response to HIPV of males as well as females.

Finally, in Chapter 4 I used the iso-female line approach to study whether genetically determined predator responses to MeSa can be modified in response to experience. The ability to learn has been demonstrated for this predator only at the population level. By analysing the responses of different iso-female lines both prior to and after an experience with MeSa, I have demonstrated that there are different genotypes with respect to learning ability. Consequently this predator trait may evolve jointly with the genetically determined preference for MeSa (or other HIPVs).

In the second part of this thesis I explored theoretical predictions for the evolution of behavioural traits modified by adaptive learning, i.e., the learning to improve fitness. In particular, I addressed the hypothesis that adaptive learning accelerates the rate of evolution of a genetically determined trait. This hypothesis is referred to in the literature as the Baldwin effect. Theoretical studies of this effect (reviewed in the Chapter 5) predict that adaptive learning may either accelerate evolution of the genetically determined behaviour or it may slow down the evolution. Adaptive learning changes the rate of evolution by modifying the fitness of different phenotypes. In particular when adaptive learning increases the fitness differences among different phenotypes then the response to selection is stronger, and thus evolution speeds up. In contrast when adaptive learning decreases the fitness differences, then the response to selection is weakened, and evolution slows down. There are two factors that determine how adaptive learning affects the fitness differences among different phenotypes: (a) the shape of the fitness landscape, and (b) the potential of adaptive learning to modify the genetically determined phenotype (i.e., whether the net effect of learning on phenotypic fitness is large or small). These predictions concern the effect of learning on the rate of evolutionary change from one generation to the next. However, allowing learning to evolve causes a change in the long-term dynamics on complex fitness landscapes (such as a rugged landscape) and thus the extrapolation from local evolutionary changes (one generation to the next) to longer time frames is not possible.

To date most studies that have examined the Baldwin effect assumed a fixed (non-evolving) level of learning, and tracked the rate of evolution of the innate (i.e., genetically determined) trait. In Chapter 6 I expanded this theory by tracking the rate of evolution when both the innate trait and the level of learning are allowed to evolve. Comparing these approaches I determined that allowing adaptive learning to evolve greatly reduces the number of generations required to reach the fitness peak when (1) the net effect of learning on phenotypic fitness is large (in my model this is reflected in learning within a large exploratory range), (2) learning has a fitness cost, and (3) the initial level of learning is higher than the evolutionary optimum. In addition I have found that there exists a critical level of learning above which learning is favoured by selection and evolves towards an optimum level and below which learning is selected against. Moreover above this threshold the joint evolution of learning and innate behaviour is accelerated. In contrast, when the net effect of learning on phenotypic fitness is small (i.e., learning within a small exploratory range), then the evolution of the phenotype proceeds effectively only via changes in the innate behaviour.

Together the theoretical results of my research point to the following conclusions. High levels of adaptive and evolving learning have the potential to accelerate the evolution of an innate trait towards a distant fitness peak (i.e., the evolution of a population that colonizes a novel environment). However when there is a nonzero cost to learning, there exists a threshold below which learning is selected against. Previous theoretical studies of phenotypic plasticity often predict low levels of plasticity in a population adapted to an old and constant environment. Therefore there is no reason to assume a priori high levels of learning when we model evolution in a novel environment. In this setting the evolution of learning critically depends on the cost of learning. In particular, if the cost of learning is too high then the potential for learning to accelerate the evolution of the innate trait is never attained. In contrast, in a constantly varying environment there exist selective pressures maintaining high levels of plasticity.

In conclusion the wealth of theory of the Baldwin effect confirms that adaptive learning may play a positive role in the evolution of innate traits. Relevant questions include: how to estimate the increase in fitness due to adaptive learning, what is the cost of learning in terms of fitness and how adaptive learning influences evolution in a varying environment. Most importantly, these questions await empirical evidence that currently is virtually absent. Such an empirical approach requires a model system where there is genetic variation for both a behavioural trait and the ability to learn. The experimental studies presented in this thesis concerning the response of predatory mite *P. persimilis* to a herbivore-induced plant volatile indicate that both these requirements are satisfied for this system.

S

Samenvatting

Dit proefschrift gaat over de evolutie van gedragskenmerken die zowel een genetische basis hebben als veranderbaar zijn. De verandering die ik heb onderzocht is een adaptieve (aangeleerde) respons op omgevingsinvloeden. De evolutie van zulke kenmerken kan, theoretisch gezien, leiden tot twee extremen, namelijk enerzijds genetische fixatie (en fenotypische invariabiliteit) van het kenmerk, en anderzijds een situatie waarin het kenmerk geheel wordt gevormd door omgevingsinvloeden. Tussen deze extremen ligt een veelheid aan kenmerken die een genetische component bevatten maar ook, in meer of mindere mate, een veranderbare component. Binnen de evolutionaire biologie bestaat de terugkerende vraag hoe fenotypische plasticiteit (d.w.z. de mogelijkheid om een fenotype te veranderen als respons op externe of interne invloeden) van invloed zou kunnen zijn op de evolutiesnelheid van een kenmerk dat op zichzelf een genetische basis heeft. Voor het beantwoorden van deze vraag is een model nodig met a) genetische variatie voor zowel het genetisch vastgelegde gedragskenmerk als leervermogen en b) waarin de mate van het leren en de waarde van het aangeboren gedragskenmerk afzonderlijk kwantificeerbaar zijn. Voorspellingen zouden ook verkregen kunnen worden door de evolutie van een dergelijk gedragskenmerk te bestuderen aan de hand van een theoretisch model.

Het eerste gedeelte van dit proefschrift omvat experimenteel onderzoek naar de aanwezigheid van genetische variatie in het gedrag van de roofmijt *Phytoseiulus persimilis* met betrekking tot specifieke plantengeuren. Deze geuren zijn mengsels van vluchtige stoffen, herbivoor-geïnduceerde plantengeurstoffen (HIPVs) genoemd. Deze stoffen worden door een plant afgegeven na aantasting door herbivore mijten, die een prooi vormen voor de roofmijten. De samenstelling van het mengsel van plantengeurstoffen is specifiek voor de plantensoort – herbivoor combinatie. Het is daarom aannemelijk dat natuurlijke vijanden van herbivoren HIPVs kunnen gebruiken als signaal voor de aanwezigheid van prooi en zo de locatie van prooi kunnen detecteren. Deze hypothese wordt ondersteund door het goed gedocumenteerde gegeven dat roofmijten worden aangetrokken door HIPVs. Het is echter bekend dat de samenstelling van plantengeurstoffen varieert tussen plantensoorten en ook varieert met verschillende grootte en duur van de aantasting. Bovendien komen in natuurlijke situaties meerder plantensoorten naast elkaar voor en vinden besmettingen met

meerdere soorten herbivoren plaats. Dit roept de vraag op hoe roofmijten uit de variatie in samenstellingen van HIPVs een signaal voor de aanwezigheid van prooi kunnen afleiden. Adaptief leergedrag zou kunnen dienen om de predatorvoorkeur voor specifieke plantengeurstoffen (of een mix daarvan) bij te houden en te versterken, na eerdere ervaringen van deze vluchtige stoffen in de aanwezigheid van geschikte prooi. Als daarnaast ook specifieke HIPVs sterk gekoppeld zijn aan de aanwezigheid van prooi, dan zouden genetisch bepaalde predatorvoorkeuren voor deze HIPVs kunnen evolueren door natuurlijke selectie. Echter, eerdere bevindingen over een genetisch bepaalde respons op HIPVs in *P. persimilis* spreken elkaar tegen. In sommige onderzoeken is een predatorvoorkeur voor specifieke HIPVs aangetoond, zelfs zonder eerdere ervaring met deze plantengeurstoffen. Andere onderzoeken daarentegen lieten zien dat eerdere ervaring met een bepaalde HIPV noodzakelijk zou kunnen zijn voor de totstandkoming van de voorkeur voor die plantengeurstof. Echter, in deze onderzoeken is gekeken naar predatorgedrag op populatie niveau en dus in genetisch variabele populaties. Er is tot op heden nog weinig bekend over verschillen in genotype met betrekking tot de voorkeur voor een bepaalde HIPV in een natuurlijke populatie, hoewel er is aangetoond dat de respons van *P. persimilis* op prooi-geïnduceerde samenstellingen van vluchtige stoffen een genetische basis heeft.

In het eerste gedeelte van dit proefschrift heb ik onderzocht of er een genetische basis is voor de respons van *P. persimilis* op een vluchtige stof die geïnduceerd wordt door vraat van een prooi, de herbivore mijt *Tetranychus urticae*. Er is eerder aangetoond dat deze vluchtige stof, methylsalicylzuur (MeSa), in ruime mate aanwezig is in de samenstelling van vluchtige stoffen die geïnduceerd wordt door *T. urticae* bij vraat op verschillende soorten planten. In de eerste twee hoofdstukken heb ik de genetische basis voor de respons van *P. persimilis* op MeSa aan de hand van experimenten laten zien. Hierbij heb ik twee manieren van aanpak gebruikt: isofemale lijnen (hoofdstuk 2) en selectie binnen isofemale lijnen (hoofdstuk 3).

In hoofdstuk 2 heb ik aangetoond dat er variatie bestaat in predatorresponsen op MeSa tussen genetisch homogene predatorlijnen (de isofemale lijnen), hiermee wordt bevestigd dat dit gedrag een genetische component heeft. Bovendien heb ik laten zien dat predatorresponsen op MeSa afhankelijk van de context zijn, in zoverre dat de responsen veranderd worden door zowel de fysiologische staat van de predatoren (d.w.z. het wel of niet goed doorvoed zijn) als door de achtergrondgeuren van vluchtige stoffen die door niet-aangetaste planten worden uitgescheiden. Deze resultaten wijzen erop dat dit gedrag zou kunnen evolueren, d.w.z. dat het de mogelijkheid heeft om op selectie te reageren. Bovendien suggereren de resultaten dat de respons van deze predator op

MeSa, afhankelijk zou kunnen zijn van een ecologisch relevante context van andere vluchtige stoffen (d.w.z. vluchtige stoffen die geassocieerd zijn met de aanwezigheid van prooi).

In hoofdstuk 3 heb ik selectie binnen isofemale lijnen voor genotypen met contrasterende predatorresponsen op MeSa uitgevoerd. Twee groepen van isofemale lijnen werden verkregen (*purifying selection*), een met lijnen op basis van vrouwtjes met een voorkeur voor MeSa, en de ander met lijnen op basis van vrouwtjes die MeSa bleken te vermijden. Mijn verwachting was dat vermeerdering van isofemale lijnen op basis van individuele vrouwtjes met een voorkeur voor dan wel afkeer van MeSa, zou leiden tot een verschuiving van de gemiddelde reactie van de groepen lijnen in de richting van, respectievelijk, voorkeur voor MeSa en afkeer van MeSa. Het bleek, echter, dat er een verschuiving van de gemiddelde respons op MeSa optreedt, in de richting tegengesteld aan het selectie criterium. Tegelijkertijd bevestigt dit onderzoek dat de predatorrespons op MeSa een genetische component heeft (tot uiting gebracht in de variatie tussen de genetisch homogene isofemale lijnen die in de selectie gebruikt zijn). Een mogelijke verklaring voor dit resultaat is dat er een correlatie is tussen paarvoorkeuren van de predator en de respons op MeSa. Eerder onderzoek is grotendeels gericht geweest op de respons van vrouwtjes op HIPVs. Onze resultaten wijzen erop dat, om de evolutie van dit gedrag te kunnen begrijpen, toekomstig onderzoek zich dienen te richten op de respons op HIPVs van zowel mannetjes als vrouwtjes.

Tot besluit heb ik in hoofdstuk 4 de isofemale aanpak gebruikt om te onderzoeken of genetisch bepaalde predatorresponsen op MeSa gemodificeerd kunnen worden in reactie op ervaring. Leervermogen is bij deze predator alleen op populatie niveau aangetoond. Met het analyseren van de responsen van verschillende isofemale lijnen, zowel voor als na een ervaring met MeSa, heb ik aangetoond dat er verschillende genotypen zijn in ten opzichte van leervermogen. Dit predatorkenmerk zou dus samen kunnen evolueren met de genetisch bepaalde voorkeur voor MeSa (of andere HIPVs).

In het tweede gedeelte van dit proefschrift heb ik theoretische voorspellingen verkend voor evolutie van gedragskenmerken die veranderd zijn door adaptief leren, d.w.z. leren waarmee fitness verbeterd wordt. Ik heb in het bijzonder gericht op de hypothese dat adaptief leren de evolutiesnelheid van een genetisch bepaald kenmerk verhoogt. Deze hypothese wordt in de literatuur het Baldwin effect genoemd. Theoretisch onderzoek naar dit effect (besproken in hoofdstuk 5) voorspelt dat adaptief leren ofwel de evolutie van genetisch bepaald gedrag zou kunnen versnellen, ofwel de evolutie zou kunnen vertragen. Adaptief leren verandert de evolutiesnelheid door de fitness van verschillende fenotypen te wijzigen. In het bijzonder, wanneer adaptief leren het fitnessverschil tussen ver-

schillende fenotypen verhoogt dan is de respons op selectie sterker, en dus wordt de evolutie versneld. Hier tegenover staat dat wanneer adaptief leren de fitnessverschillen verlaagt, de respons op selectie verzwakt wordt en de evolutie zo vertraagt. Er zijn twee factoren die bepalen hoe adaptief leren de fitnessverschillen tussen verschillende fenotypen kan beïnvloeden: (a) de topografie van het fitness landschap, en (b) adaptief leren zou genetisch bepaalde fenotypen kunnen veranderen (d.w.z. de grootte van het netto effect van leren op de fenotypische fitness). Deze voorspellingen gaan over de invloed van leren op de snelheid van evolutionaire verandering tussen opeenvolgende generaties. Echter, als leren aan evolutie onderhevig is, dan veroorzaakt dit een verandering in de lange termijn dynamiek bij complexe fitness landschappen (zoals een golvend landschap), daarom is de extrapolatie van plaatselijke evolutionaire veranderingen (tussen opeenvolgende generaties) naar langere tijdspannes niet mogelijk.

Tot op heden is in het meeste onderzoek naar het Baldwin effect de aanname gedaan van een gefixeerd (niet evoluerend) leerniveau, waarbij de snelheid van evolutie van een aangeboren (d.w.z. genetisch bepaald) kenmerk werd gevolgd. In hoofdstuk 6 heb ik deze theorie uitgebreid door de snelheid van evolutie te volgen, wanneer zowel het aangeboren kenmerk als het leerniveau kan evolueren. Door deze manieren van aanpak te vergelijken heb ik kunnen vaststellen dat het laten evolueren van adaptief leren tot gevolg heeft dat het aantal generaties dat nodig is om een fitness piek te bereiken sterk gereduceerd wordt als (1) het netto effect van leren op fenotypische fitness groot is (in mijn model komt dit tot uiting in het leren binnen een groot exploratief bereik), (2) leren ten koste gaat van de fitness, en (3) het initiële leerniveau hoger is dan het evolutionaire optimum. Daarnaast heb ik gevonden dat er een kritiek leerniveau is waarboven leren een selectievoordeel oplevert en naar een optimaal niveau evolueert, en dat onder dit niveau leren selectief nadelig is. Tevens wordt boven deze drempel de gezamenlijke evolutie van leren en aangeboren gedrag versneld. Wanneer het netto effect van leren op fenotypische fitness daarentegen klein is (d.w.z. leren binnen een klein exploratief bereik) dan verloopt de evolutie van het fenotype in feite alleen door veranderingen in aangeboren gedrag.

Alles bijeengenomen duiden de theoretische resultaten van mijn onderzoek op de volgende conclusies. Hoge niveaus van adaptief en evoluerend leren hebben de mogelijkheid om de evolutie van een aangeboren kenmerk te versnellen, in de richting van een ver verwijderde fitness piek (d.w.z. de evolutie van een populatie die een nieuwe omgeving koloniseert). Wanneer er echter kosten verbonden zijn aan leren, dan bestaat er een drempelwaarde waaronder selectie op zal treden tegen leren. In eerder theoretisch onderzoek naar fenotypische plasticiteit zijn vaak lage niveaus van plasticiteit voorspeld in een populatie die aangepast is aan een oude en constante omgeving. Daarom is er geen reden om

a priori uit te gaan van hoge leerniveaus bij het modelleren van evolutie in een nieuwe omgeving. In deze omstandigheid is de evolutie van leergedrag kritisch afhankelijk van de kosten van leergedrag. Vooral als de kosten van leergedrag te hoog zijn, dan zal de mogelijkheid dat leergedrag evolutie van aangeboren kenmerken versnelt nooit bereikt worden. Daarentegen zullen er in een constant variërende omgeving selectiedrukken bestaan die hoge mate van plasticiteit in stand houden.

Ter conclusie bevestigt de overvloed aan theorie over het Baldwin effect dat adaptief leren een positieve rol zou kunnen spelen in de evolutie van aangeboren kenmerken. Relevante vragen hierbij zijn: hoe kan de verhoging in fitness als gevolg van adaptief leergedrag geschat worden, wat zijn de kosten van leren uitgedrukt in fitness en hoe kan adaptief leergedrag evolutie in een veranderende omgeving beïnvloeden. Het belangrijkste is dat een empirisch antwoord op deze vragen tot op heden nagenoeg ontbreekt. Een dergelijke empirische aanpak vereist een model dat genetische variatie voor zowel een gedragskenmerk als voor leervermogen bevat. De experimenten die in dit proefschrift zijn beschreven, betreffende de respons van de roofmijt *P. persimilis* op een herbivoor-geïnduceerde plantengeurstof, laten zien dat hierbij aan beide voorwaarden voldaan is.



About the author



Beata Sznajder studied in the Jagiellonian University in Cracow, Poland. She chose evolutionary ecology as her specialization and received her MSc degree in 1999. In her thesis she tested whether the ability of the collared flycatcher to mount immune response is reflected in the degree of fluctuating asymmetry that the bird's body exhibits. The experiments for her MSc project were conducted in the Swedish island of Gotland where Beata worked as a field assistant for a project on the costs of reproduction and immunocompetence in hole-nesting birds. Following her graduation she was involved in projects in evolutionary ecology of passerine birds and parasitic wasps conducted in the Netherlands Institute of Ecology in Heteren, Netherlands. In 2002 she began her PhD project under the supervision of Prof. Maurice W. Sabelis and Dr Martijn Egas of the Population Biology Section in the Institute for Diversity and Ecosystems Dynamics of the University of Amsterdam. Within her PhD project she also stayed for three months in the group of Prof. Junji Takabayashi in the Center for Ecological Research of Kyoto University. The results of her PhD research are contained in this book.

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