



Differences in learning and memory of host plant features between specialist and generalist phytophagous insects



Daniel H. Tapia^a, Andrea X. Silva^b, Gabriel I. Ballesteros^{c,e}, Christian C. Figueroa^{c,e}, Hermann M. Niemeyer^d, Claudio C. Ramírez^{c,e,*}

^a Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Gran Bretaña 1111, Playa Ancha, Valparaíso, Chile

^b Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

^c Laboratorio de Interacciones Insecto-Planta, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile

^d Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

^e Millennium Nucleus Center in Molecular Ecology and Evolutionary Applications in the Agroecosystems, Chile

ARTICLE INFO

Article history:

Received 21 September 2014

Initial acceptance 5 February 2015

Final acceptance 25 March 2015

Available online 5 June 2015

MS. number: A14-00754R

Keywords:

ecological specialization
host selection
insect–plant relationship
learning
Myzus persicae
retroactive interference

Insects are able to learn from experience acquired in their natal habitat, thereby obtaining adaptive advantages. However, the acquisition of new information could involve defects in retrieving previously learned information (i.e. forgetting), a process known as retroactive interference, which diminishes learning capacities. In this study, we evaluated the learning capacity and the impact of retroactive interference during host searching by ecological specialist and generalist phytophagous insects. We examined whether the generalist aphid, *Myzus persicae* s. str., and the tobacco-specialized subspecies, *Myzus persicae nicotianae* differ in (1) learning capacity, or (2) retroactive interference during host selection, and (3) whether the learning-associated *foraging* gene (*for*) is differentially expressed. Differences in learning capacity and retroactive interference were assessed in bioassays using rearing hosts and alternative hosts followed by choices between or transferences to rearing or alternative hosts. During the pre-alighting phase of host searching, the generalist aphid showed attraction to the alternative host after 12 h of experience, while the specialist showed no attraction to the alternative host regardless of the amount of time on the plant. The retroactive interference experiments showed that when aphids were exposed to an alternative host for different periods, odour attraction to the rearing host persisted in the generalist after 72 h of experience on the alternative host, whereas in the specialist the attraction to the rearing host was lost after 12 h of experience on the alternative host. During the post-alighting phase of host searching, both taxa performed better on their rearing hosts, but in the specialist aphid, a short period on the alternative host reversed this behaviour. In addition, the specialist showed lower levels of gene *for* expression, which could be associated with the differences in learning performance. Herein we present further evidence of differences in learning capacities between a specialist and a generalist aphid, which may influence the process of host searching and evolution of ecological specialization.

© 2015 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Insects are able to learn from environmental cues experienced during their development and immature stages, experiences which could bring adaptive advantages during the adult stage (Faber, Joerges, & Menzel, 1999; Giurfa, 2013). This ability seems to be particularly relevant for phytophagous insects since learning may underlie host specialization (Papaj & Prokopy, 1989), which is one of the most striking features of their interactions with plants

(Schoonhoven, Jermy, & van Loon, 2006). In phytophagous insects, learning is an extensively documented cognitive trait (Bernays & Bright, 2005; Daly & Smith, 2000; Dukas, 2008; Dukas & Bernays, 2000; Egas & Sabelis, 2001; Mery, Belay, So, Sokolowski, & Kawecki, 2007; West & Cunningham, 2002), with host generalist insects making more significant use of learning than host specialists (Bernays, 2001; Bernays, Singer, & Rodrigues, 2004; Levins & MacArthur, 1969). However, learning might also be important for specialists (Steidle & Van Loon, 2003), a prediction needing confirmation. Insect learning relies on cognitive abilities such as acquiring, retaining and processing information, and also on retrieving previously acquired information (Dukas, 2004). It has

* Correspondence: C. C. Ramírez, Laboratorio de Interacciones Insecto-Planta, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

E-mail address: clramirez@utalca.cl (C. C. Ramírez).

been proposed that learning capacities in generalist and specialist insects could be determined by differential defects when retrieving previously learned tasks. New learned environmental cues or external information might interfere with and eventually impede the recall of previously learned similar cues (e.g. host plant volatiles, visual cues), a phenomenon known as retroactive interference (Cheng, 2005; Cheng & Wignall, 2006; Chittka & Thomson, 1997; Frasnelli, Vallortigara, & Rogers, 2010; Gegear & Laverty, 1998; Reaume, Sokolowski, & Mery, 2011; Weiss & Papaj, 2003; Wixted, 2004; Worden, Skemp, & Papaj, 2005). Retroactive interference is a major cause of memory disruption or forgetting and has been verified in several animal taxa, including adult lepidopterans and hymenopterans (Cheng, 2005; Cheng & Wignall, 2006; Chittka & Thomson, 1997; Frasnelli et al., 2010; Gegear & Laverty, 1998; Weiss & Papaj, 2003; Worden et al., 2005) and more recently in *Drosophila* (Reaume et al., 2011).

In an ecological context and in contrast to specialists, generalists are expected to process more information on a larger variety of potential resources (Bernays & Bright, 2001; Bernays et al., 2004; Tosh, Krause, & Ruxton, 2009), switching their attention to different cues and retaining characteristics of those cues in memory for later comparison, thus showing, as compared to specialists, a decreased efficiency of host use. Conversely, specialists are expected to process less information and to show high sensitivity to a few relevant cues, hence showing more efficient responses than generalists. Accordingly, evidence that specialists are more efficient than generalists has found support in most studies addressing the problem (Bernays, 1998, 1999; Bernays & Bright, 2001; Bernays et al., 2004; Dukas, 2004; Egan & Funk, 2006; Farris & Roberts, 2005; Janz & Nylin, 1997; Oppenheim & Gould, 2002; Vargas, Troncoso, Tapia, Olivares-Donoso, & Niemeyer, 2005) (but see Tosh, Powell, & Hardie, 2003; Troncoso, Vargas, Tapia, Olivares-Donoso, & Niemeyer, 2005; Wee & Singer, 2007). However, whether or not there are differences in retroactive interference between generalists and specialists has, to our knowledge, not been studied yet.

Host specialization, a common feature of aphids, is highly dependent on the host selection process (Dixon, 1998; Powell, Tosh, & Hardie, 2006). In fact, aphid species depend on host-plant-specific cues to distinguish between host and nonhost plants (Pettersson, Tjallingii, & Hardie, 2007). Host searching in aphids involves pre- and post-alighting phases, in which different combinations of sensory modalities are used to assess plant suitability (Powell et al., 2006). During the pre-alighting phase, plant suitability is assessed mainly through olfaction of plant volatiles (Niemeyer, 1990; Pickett, Wadhams, Woodcock, & Hardie, 1992), whereas during the post-alighting phase, mainly tactile and gustatory sensory modalities are used and involve a wider range of cues (e.g. plant surface structures, such as trichomes, epicuticular waxes and the wide range of chemicals they contain, and internal plant metabolites; Powell et al., 2006). A question that remains unsolved is how generalist and specialist aphids differ in their ability to learn and forget similar cues on different potential host plants during the pre- and post-alighting phases.

Myzus persicae (Sulzer), one of the most generalist aphid species, is able to feed on more than 400 plant species of over 40 families (Blackman & Eastop, 2000), whereas the subspecies *Myzus persicae nicotianae* (Blackman & Eastop) has been described as an ecological tobacco specialist (Blackman, 1987; Cabrera-Brandt, Fuentes-Contreras, & Figueroa, 2010; Margaritopoulos, Malarky, Tsitsipis, & Blackman, 2007; Olivares-Donoso, Troncoso, Tapia, Aguilera-Olivares, & Niemeyer, 2007). These two aphid taxa, given their close phylogenetic relationship, constitute a suitable system to compare the learning capacities between a specialist and a generalist insect. Hence, in the present work, we evaluated learning

and retroactive interference during the pre and post-alighting phases of host-searching in the aphids *M. persicae sensu stricto* and *M. p. nicotianae*. Aphids were reared on their most common hosts and transferred to alternative hosts; odour preference during the pre-alighting phase was evaluated through olfactometric bioassays and, in a separate experiment, probing behaviour during the post-alighting phase was evaluated through videorecording behaviours on the plant surface. If the generalist aphid is able to process more information on a larger variety of potential resources relative to the specialist aphid, we expected that experience on alternative hosts would not affect the learned preference for or probing efficiency on its rearing host, both during pre- and post-alighting stages (lack of retroactive interference) (see predictions in Fig. 1).

Differences in learning and memory in insects have been associated with differences in the activity of the cGMP-dependent protein kinase (PKG), which is the product of the *foraging* (*for*) gene, also known as *dg2* (Osborne et al., 1997; Thamm & Scheiner, 2014). Natural variation in *for* gene gives rise to different behavioural variants in *Drosophila* flies; variants showing higher learning abilities display stronger retroactive interference (Reaume et al., 2011). However, neither the sequence nor the expression levels of this gene have been associated with learning abilities and retroactive interference. If the level of *for* expression is associated with greater learning abilities and weak retroactive interference, then the expression level of the *for* gene is expected to be higher in the generalist aphid. We were able to test this hypothesis in aphids since the sequence of the *for* gene is found in the genome of the pea aphid, *Acyrtosiphon pisum*. Hence, we assessed retroactive interference through appropriate olfactometric and probing behaviour bioassays and determined expression of the *for* gene in the *Myzus persicae* complex (hereafter *mpfor*) through quantitative reverse transcription PCR (RT-qPCR).

METHODS

Insects and Plants

Aphid individuals were obtained from monoclonal lineages (regularly regenerated from a single parthenogenetic individual) maintained in the laboratory for several generations at 21 ± 2 °C on a 14:10 h light:dark cycle. *Myzus persicae* s. str. lineages were reared on sweet pepper plants, *Capsicum annuum* L. (Solanaceae), and *M. p. nicotianae* lineages were reared on tobacco plants, *Nicotiana tabaccum* L. cv. BY 64 (Solanaceae). These hosts have been described as optimal hosts for these aphid taxa (Olivares-Donoso et al., 2007) and were designated as the rearing host for each taxon, respectively. Using a common rearing host, although possible, could have affected the specialized behaviour, particularly in the case *M. p. nicotianae* lineages specialized on tobacco plants. Therefore, we used thorn apple, *Datura stramonium* L. (Solanaceae), as the alternative host plant for rearing both aphids to test retroactive interference in pre- and post-alighting behaviours. Host transfers were performed within 3 days after the adult alates emerged. All bioassays were carried out at 21 ± 2 °C; 90-day-old plants were used for all behavioural bioassays.

Assessment of Learning Capacity and Retroactive Interference

To identify changes in the original pre-alighting (focusing on odour preferences) and post-alighting (focusing on probing behaviour) phases of host searching by both aphid taxa after an experience on an alternative host, we conducted bioassays with aphids taken from their rearing hosts and transferred to an alternative host. In the case of odour preference bioassays, aphids taken

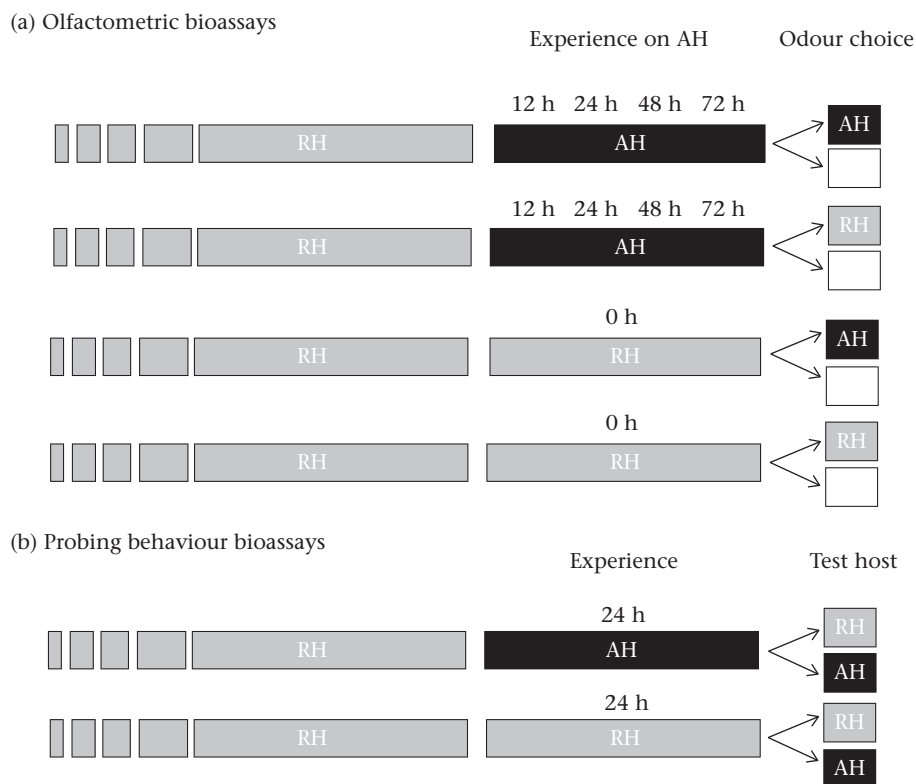


Figure 1. Schematic representation of the experiments performed to study learning capacity and retroactive interference in *Myzus* spp. during: (a) pre-alighting behaviour through olfactometric bioassays and (b) post-alighting behaviour through probing behaviour bioassays. These experiments were performed similarly for both *Myzus persicae* s. str. and *Myzus persicae nicotianae*. Rearing hosts (RH) were sweet pepper for *M. persicae* s. str. and tobacco for *M. persicae nicotianae*; the alternative host (AH) was thorn apple for both aphid taxa. White boxes indicate control arms of the olfactometer.

from their rearing host were transferred for 0, 12, 24, 48 and 72 h to an alternative host (Fig. 1a). These experiments allowed us to evaluate the time needed to learn or forget volatile cues, and also to determine the time frame for the experience treatments during the probing behaviour bioassays (Fig. 1b), which were performed with a different set of aphids.

Thorn apple was chosen as the common alternative host for both aphid taxa. Note, however, that although different rearing plant species may exert differential pre-imaginal effects (for examples in other phytophagous insects, see: Moreau, Rahme, Benrey, & Thiery, 2008; Wu, Shen, An, Huang, & Zhang, 2011), the rearing host species (sweet pepper and tobacco) were chosen to ensure that both aphids were in optimal physiological condition at the time of the experiments. In addition, given the high performance of *M. p. nicotianae* on sweet pepper under laboratory conditions, comparable to that on its natural optimal host tobacco, we also used sweet pepper as an alternative host for *M. p. nicotianae*, thus allowing us to explore its suitability as a host that affects learning in this aphid taxon.

Learning Capacity and Retroactive Interference during Pre-alighting Behaviour (Olfactometric Bioassays)

We used the rearing host as the stimulus in the olfactometer bioassays to assess whether the attraction mediated by its volatiles is retained after exposure to an alternative host for different periods. Odour choice experiments involved exposure to the rearing host or alternative host as the odour cue against pure air. For all experiments, a four-arm Plexiglas olfactometer designed by Pettersson (1970) was used. Two adjacent arms were connected with Teflon tubing to a glass bell-jar containing a test plant

(stimulus arms); the other two arms were connected either to a bell-jar with another plant or to an empty bell-jar (control arms). For dispersion of volatiles from the bell-jars, air previously purified by charcoal filters was sucked through a hole in the centre of the olfactometer with a resulting flow of 200 ml/min. Thus, the arena of the olfactometer consisted of a flat surface with four arm zones (two stimulus and two control zones) and a central zone. The olfactometer was surrounded by a cylinder of white paper (height = 15 cm) to avoid external visual stimuli. The focal individual was gently introduced into the arena through the central hole using a fine paintbrush, and its behaviour monitored for 10 min after 2 min of acclimatization. Time spent in each arm of the olfactometer was registered using The Observer software v.3.0 (Noldus Information Technology, Wageningen, The Netherlands); insects were discarded if they showed no reaction within 7 min of being placed in the olfactometer. After each experiment, tubing, bell-jars and olfactometers were washed sequentially with distilled water and ethanol and then oven-dried. To avoid bias, connections between the arms of the olfactometer and the stimulus sources were periodically alternated and lighting was provided from above; to avoid pseudoreplication, individuals were tested only once (Ramírez, Fuentes-Contreras, Rodríguez, & Niemeyer, 2000). Ten replicates were performed for each treatment.

Learning Capacity and Retroactive Interference during Post-alighting Behaviour (Probing Behaviour Bioassays)

Host transfers on the leaf surface (from an alternative host or a rearing host to either a rearing host or an alternative host) were combined into the following treatments: (1) specialist with experience on alternative host, tested on rearing host; (2) specialist with

experience on alternative host, tested on alternative host; (3) specialist with experience on rearing host (without experience on alternative host) tested on rearing host; (4) specialist with experience on rearing host (without experience on alternative host) tested on alternative host; (5) generalist with experience on alternative host, tested on rearing host; (6) generalist with experience on alternative host, tested on alternative host (7) generalist with experience on rearing host (without experience on alternative host) tested on rearing host, and (8) generalist with experience on rearing host (without experience on alternative host, tested on alternative host). Thirty replicates were performed for each treatment. Given that behavioural changes due to learning of new host cues during the pre-alighting phase of host searching were apparent after 24 h of experience and did not change significantly afterwards, this was the time frame chosen for experience treatments in these bioassays.

Probing behaviour of alate individuals of both taxa with different rearing history was studied through video recordings using a digital video camera (Sony DCR-HC62). Recordings started after gently placing aphids with a fine paintbrush on the first mature leaf counted from the top to the bottom of the plant, and lasted until either the aphid flew away from the plant, remained inactive for more than 15 min, started a long-duration probe (probe lasting more than 5 min), or until a pre-set observation period (30 min) was achieved. We used the position of the rostrum, antennae and body as external indicators of probing by the aphid on the plant (Troncoso et al., 2005; Vargas et al., 2005). The following behavioural patterns could be discerned: antennal and rostrum activities, wing displays during take-off from the plant, movement on the leaf, and abandoning the plant by walking. In relation to these patterns, we evaluated the following variables: (1) time to the first probe, (2) time spent probing before a long-duration probe, (3) time to long-duration probe, (4) number of probes before the aphid performed a long-duration probe, (5) proportion of individuals that performed a long-duration probe, (6) proportion of individuals that flew away from the plant, and (7) time to take-off from the plant. The video recordings were later analysed using The Observer software (Noldus, 1995).

Analysis of *mpfor* Gene Expression

We examined transcriptional levels of the *foraging* gene in *M. persicae* s. str. (*mpfor* gene) and *M. p. nicotianae* (*mpnfor* gene) using RT-qPCR. Three biological replicates per taxon were used, using three alate aphids in each replicate. We included two additional samples from apterous morphs of *M. persicae* s. str. corresponding to different aphid lineages (G and N genotypes), as calibrators for the genetic analyses (Silva, Jander, Samaniego, Ramsey, & Figueroa, 2012). Aphids taken from their optimal rearing hosts (see above) were quickly frozen in liquid nitrogen and stored at -70°C until RNA isolation. Total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen, Cat no. 74904) for each treatment (i.e. winged *M. persicae* s. str., winged *M. p. nicotianae* and two apterous *M. persicae* s. str. clones), with a resulting range of 50–150 ng/ μl of RNA (Nanodrop ND-1000, NanoDrop Technologies, Wilmington, DE, U.S.A.). Genomic DNA was removed with DNA-freeTM kit (Ambion) and reverse transcription was performed with the AffinityScript QPCR cDNA Synthesis kit (Agilent) using 0.45 μg of total RNA. Primers for the amplification of *for* gene in *M. persicae* s. str. and *M. p. nicotianae* were designed from available sequences in databases, using the FastPCR (v.5.4.30) and AmplifX (v.1.3.7) packages, and checked in NCBI/Primer-BLAST (forward: ACTGGACGAGATACGCCAGATA; reverse: AGTGGCCAA-GACTTGTAGATCGGA). After studying its dynamic range, the cDNA was diluted to 1:10 taking 2 μl for PCR reactions (12.5 μl final

volume). Each PCR reaction mixture contained 2.5 μM of each primer, 6.25 μl of SYBR Green PCR Master Mix (Applied Biosystems) and 0.375 μl of Rox dye (dilution 1:500). Negative controls were included for detecting cross-contamination, and all PCR reactions were carried out in triplicate in a Mx3000P QPCR System (Stratagene) under the following cycling conditions: 10 min at 95°C , followed by 50 cycles of 15 s at 95°C , 30 s at 58°C and 15 s at 72°C . A dissociation curve was included immediately after each PCR using a ramp of $55\text{--}95^{\circ}\text{C}$, to confirm the absence of nonspecific amplifications.

The relative expression ratio was computed by relative quantification using the comparative Ct method (Applied Biosystems User Bulletin No. 2 P/N 4303859, 1997) (Silva et al., 2012) and the two best reference genes were selected with the NormFinder algorithm (Legeai et al., 2010). Among *glyceraldehyde-3-phosphate dehydrogenase GADPH* (DW011095), *cyclophilin-10-like* (EC388830), *actin* (EE262754), *ribosomal protein LPO* (DW011949) and *ribosomal protein L7* (DW361765), NormFinder identified *GADPH* (for primers details see Silva et al., 2012) as the most stable expressed house-keeping gene followed by *cyclophilin-10-like*. We used only the data obtained using *GADPH* as the reference gene, since both reference genes showed similar results. The \log_2 of relative expression ratio per gene was calculated and plotted for each biological replicate, using the quantification cycle of either *M. p. nicotianae* or apterous *M. persicae* s. str. (G and N genotypes) as calibrators. In the first case, for each biological replicate, we performed a *t* test between the \log_2 -relative expression and 1 (which was considered as the reference value for no significant change). In the second case, we performed an ANOVA with taxa (two levels) as a factor nested in genotypes (G and N) and relative expression as the response variable.

Sequencing of PCR Products and BLAST Analysis

To explore potential structural and functional differences, we obtained the sequence of the *foraging* gene for both taxa. Total RNA from 53-day-old alate individuals of each aphid taxon was extracted separately (1.3 $\mu\text{g}/\mu\text{l}$ and 2.0 $\mu\text{g}/\mu\text{l}$ for *M. persicae* s. str. and *M. p. nicotianae*, respectively) using the RNeasy Plant Mini kit (QIAGEN, Valencia, CA, U.S.A.) according to the instructions of the manufacturer. Complementary DNA synthesis was performed using 2 μg of DNase-treated total RNA of *M. persicae* s. str. and of *M. p. nicotianae*, using the ThermoScriptTM RT-PCR System (Invitrogen) in a total volume of 22 μl . RT-PCR was performed with 1 μl of cDNA (972.96 and 974.35 $\mu\text{g}/\mu\text{l}$ for *M. persicae* s. str. and *M. p. nicotianae*, respectively) as template for the amplification of *mpfor*. Specific primers for *mpfor* (forward: 5' AGTACCGACTTCGCTTTCAC 3'; reverse: 5' GCAAGATAGGAGGAGTTAGG 3') were designed based on the predicted sequences from the aphid *Acyrtosiphon pisum* recovered from Aphidbase (Legeai et al., 2010) (Gbrowse accession numbers: *for* orthologous ACYPI008877-RA, ID = XM 001952056), using the software Primer Premiere v.5.0.1 (PREMIER Biosoft International, Palo Alto, CA, U.S.A.). The PCR for *mpfor* was performed in a total volume of 25 μl containing 10 mM dNTPs, 2.0 mM Mg^{2+} , 10 μM of each primer, 1 μl of template cDNA and 0.50 U of Pfu Ultra II Fusion HS DNA polymerase (Stratagene) in IX polymerase chain reaction buffer. Amplification of *mpfor* was performed at 95°C for 2 min, followed by 27 cycles at 95°C for 20 s, 56°C (annealing temperature) for 20 s and 72°C for 1.5 min, with a final extension step at 72°C for 10 min.

Purified PCR products of *M. persicae* s. str. and *M. p. nicotianae* were sequenced by Genytec (Genética y Tecnología Ltda, Santiago, Chile). Sequences were obtained from chromatograms using the software Phred (Ewing, Hillier, Wendl, & Green, 1998); poly-T tails and low-quality extremes were removed from the analysis.

Assembly was performed with the software CAP3 (Huang & Madan, 1999) using the default parameters (40 bp minimum overlap, 80% minimum identity). BLASTX was used for sequence annotation (Altschul, Gish, Miller, Myers, & Lipman, 1990). Multiple alignments were achieved using CLUSTALW (Thompson, Higgins, & Gibson, 1994) and orthologous was obtained with BLASTX from NCBI database.

Statistical Analysis

In the olfactometric bioassays, we compared the total time spent in stimulus and control zones using a Student's *t* test for paired data. In probing behaviour bioassays, we analysed behavioural variables using a three-way MANOVA on ranked data, since data were not normally distributed (Conover & Iman, 1985). Factors were specialization (the two taxa), experience (with or without experience in alternative host) and host plant (rearing host or alternative host). LSD post hoc analyses were used to test specific a priori hypotheses. Comparisons between proportions were performed with a chi-square contingency test for several proportions, followed by a Z test for proportions with Yates correction for continuity (Zar, 1996). We used a simple Student's *t* test for unpaired data to identify differences in the relative expression of the *for* gene among biological replicates. Nested ANOVA was used to compare relative expression among taxa including two genotypes per taxa.

RESULTS

Learning Capacity and Retroactive Interference during the Pre-alighting Behaviour

In the olfactometric experiments designed to assess the effect of experience on an alternative host, the generalist *M. persicae* s. str. showed attraction to the alternative host after 12 h of experience

on it, but it showed no attraction towards the alternative host without previous experience with this plant (Fig. 2a). In contrast, *M. p. nicotianae* showed no attraction to the alternative host regardless of the time spent on it (Fig. 2b). The experiments designed to evaluate retroactive interference showed that when exposed to thorn apple as an alternative host for different periods, odour attraction to the rearing host persisted in *M. persicae* s. str. even after 72 h of experience on the alternative host (Fig. 2c), whereas in *M. p. nicotianae*, attraction to the rearing host was lost after 12 h of experience on the alternative host (Fig. 2d). When sweet pepper was used as the alternative host instead of thorn apple, *M. p. nicotianae* showed attraction to the alternative host after 24 h of experience (Fig. 3a), but attraction to the rearing host was also lost after 12 h (Fig. 3b).

Learning Capacity and Retroactive Interference during the Post-alighting Behaviour

In the experiments designed to assess the effect of experience on an alternative host on post-alighting behaviours, three-way MANOVA verified that aphid diet breadth (generalist and specialist) and experience on the alternative host affected the aphid's post-alighting behaviour, and revealed significant interactions of experience on alternative host*host plant*specialization and on alternative host*host plant, and a marginal, but nonsignificant, interaction of specialization*host plant (Table 1). Post hoc comparisons revealed that experience on an alternative host affected leaf surface behaviour of both taxa differently (Table 2). When an experimental aphid had no previous experience on an alternative host, individuals of both taxa initiated probing sooner, spent less time probing before a long-duration probe, reached a long-duration probe sooner, and performed fewer probes before a long-duration probe on the rearing host than on the alternative host. In contrast, when experimental aphids had

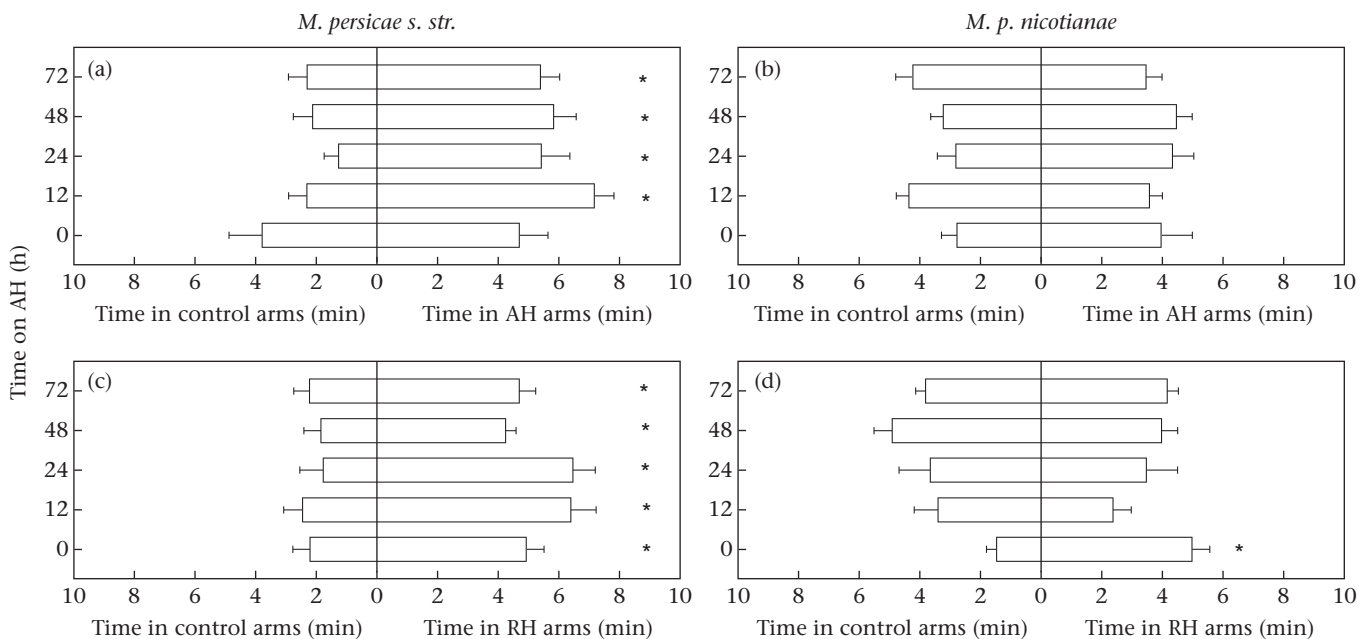


Figure 2. Test of the ability of *Myzus* spp. to learn new hosts and their propensity to forget old hosts (retroactive interference): olfactory responses (time on treatment arms; mean \pm SE) of *Myzus persicae* s. str. (a, c) and *Myzus persicae nicotianae* (b, d) after different durations of experience on an alternative host (AH). Rearing hosts (RH) were sweet pepper for *M. persicae* s. str. and tobacco for *M. persicae nicotianae*; the alternative host was thorn apple for both aphid taxa. Plants were absent in the control arms of the olfactometer. Data were compared by a Student's *t* test ($N = 10$). Statistics for significant comparisons ($*P < 0.05$): (a) 12 h: $t_9 = 3.920$, $P = 0.004$; 24 h: $t_9 = 3.318$, $P = 0.009$; 48 h: $t_9 = 2.709$, $P = 0.024$; 72 h: $t_9 = 2.509$, $P = 0.033$; (c) 0 h: $t_9 = 2.840$, $P = 0.019$; 12 h: $t_9 = 2.731$, $P = 0.023$; 24 h: $t_9 = 3.361$, $P = 0.008$; 48 h: $t_9 = 3.128$, $P = 0.012$; 72 h: $t_9 = 2.627$, $P = 0.027$; (d) 0 h: $t_9 = 5.464$, $P < 0.001$.

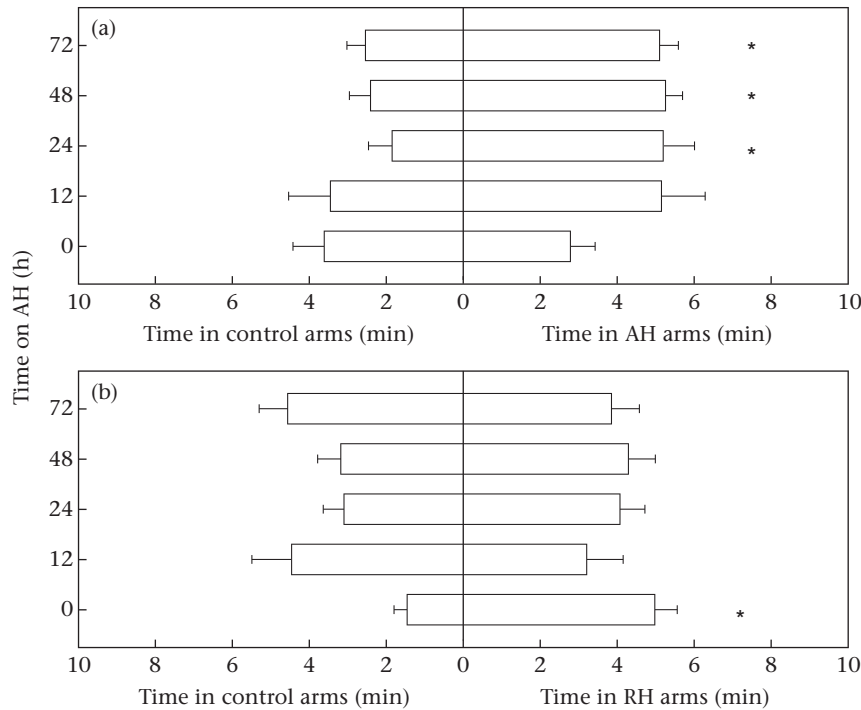


Figure 3. Test of the ability of *Myzus* spp. to learn new hosts and their propensity to forget old hosts (retroactive interference): olfactory responses (time in treatment arms; mean \pm SE) of *Myzus persicae nicotianae* reared on tobacco (rearing host, RH) after different durations of experience on the alternative host (AH) sweet pepper and tested with (a) AH volatiles and (b) RH volatiles. Plants were absent in the control arms of the olfactometer. Data were compared by a Student's *t* test ($N = 10$). Statistics for significant comparisons ($^*P < 0.05$): (a) 24 h: $t_9 = 2.525$, $P = 0.032$; 48 h: $t_9 = 3.145$, $P = 0.012$; 72 h: $t_9 = 2.708$, $P = 0.024$; (b) 0 h: $t_9 = 5.464$, $P < 0.001$.

previous experience on the alternative host, individuals of *M. persicae s. str.* showed no behavioural differences on the rearing host and the alternative host, whereas individuals of *M. p. nicotianae* initiated probing later, spent more time probing before a long-duration probe, reached a long-duration probe later, performed more probes before a long-duration probe and took longer to take-off from the plant when tested on the rearing host than when tested on the alternative host. It is worth noting that individuals of *M. persicae s. str.* (the generalist) never took-off from the plant, while in *M. p. nicotianae* (the specialist), a higher proportion of individuals took-off from the alternative host plants (Table 2), although the mean time to take-off did not exhibit a clear pattern.

Mppfor Gene Expression

The sequence obtained for the putative gene *for* confirmed that *mpfor* transcripts are orthologous with the *for* gene, 99% and 96%

Table 1

Three-way MANOVA on ranked data of behaviours during the post-alighting phase of host searching by the generalist *Myzus persicae s. str.* and the tobacco specialist *Myzus persicae nicotianae* ($N = 30$)

	Wilks' λ	<i>F</i>	Effect	Error	<i>P</i>
Specialization	0.777	15.11	4	211	<0.0001
Experience on AH	0.951	2.73	4	211	0.0302
Host	0.973	1.48	4	211	0.2081
Specialization * experience	0.969	1.65	4	211	0.1634
Specialization * host	0.958	2.33	4	211	0.0572
Experience * host	0.603	34.68	4	211	<0.0001
Specialization * experience * host	0.834	10.47	4	211	<0.0001

Factors were degree of specialization (specialist versus generalist), experience (with versus without experience on the alternative host) and test plant (rearing host, RH, versus alternative host, AH).

identical to *A. pisum* and *D. melanogaster for* genes, respectively. These transcripts, although incomplete, corresponded to the coding region of the gene and did not differ between aphid taxa (884 bp both in *mppfor* and *mpnfor*). The *mpfor* sequences for each taxa were deposited in GenBank under the following accession numbers: *mppfor*: JF776573, *mpnfor*: JF776572. When aphids were reared on their optimal host plant (see Methods), the *mpfor* showed a significantly higher transcriptional expression in *M. persicae s. str.* than in *M. p. nicotianae* in two of the three biological replicates ($t = 11.4$, $P = 0.001$ and $t = 9.4$, $P = 0.001$; see Fig. 4a).

In a second gene expression analysis, this time including *M. persicae s. str.* apterous individuals of G or N genotypes, the relative gene expression (estimated as the winged/apterous ratio of the expression) of *mpfor* appeared significantly upregulated in winged individuals of *M. persicae s. str.* compared to that of *M. p. nicotianae* (nested ANOVA with significant effect only for taxa: $F_{1,8} = 10.9$, $P = 0.011$; Fig. 4b).

DISCUSSION

Learning Capacity and Retroactive Interference during the Pre-alighting Behaviour

In this study, differences in learning ability and retroactive interference were found between a generalist and a specialist subspecies of a phloem-feeding insect at pre- and post-alighting phases of host searching. In the pre-alighting phase, the generalist aphid *M. persicae s. str.* was able to learn novel olfactory information after just 12 h of experience on an alternative host and to retrieve information of its rearing host even after 72 h of experience on an alternative host, thus suggesting no retroactive interference effect under the conditions of this set of experiments. The specialist *M. p. nicotianae* was also able to learn, but such capacity

Table 2Behavioural variables (means \pm SE, and proportions) during the post-alighting phase of host searching by the generalist *Myzus persicae* s. str. and the tobacco specialist *Myzus persicae nicotianae*

Variables	<i>M. persicae</i> s. str.				<i>M. p. nicotianae</i>			
	With experience on AH		Without experience on AH		With experience on AH		Without experience on AH	
	Test on RH	Test on AH	Test on RH	Test on AH	Test on RH	Test on AH	Test on RH	Test on AH
Time to first probe (min)	1.01 \pm 0.16 e	0.84 \pm 0.16 e	0.78 \pm 0.12 e	1.34 \pm 0.13 d	6.65 \pm 1.66 a	1.32 \pm 0.26 d	1.84 \pm 0.36 bc	2.69 \pm 0.53 ab
Time probing before a long-duration probe (min)	1.1 \pm 0.29 c	0.6 \pm 0.18 c	0.62 \pm 0.16 c	2.03 \pm 0.34 b	3.81 \pm 0.58 a	0.69 \pm 0.21 c	0.51 \pm 0.15 c	2.09 \pm 0.34 b
Time to long-duration probe (min)	2.81 \pm 0.44 cd	2.16 \pm 0.42 d	1.58 \pm 0.28 d	4.77 \pm 0.74 c	16.48 \pm 1.64 a	2.36 \pm 0.48 d	2.03 \pm 0.3 d	9.11 \pm 0.92 b
Number of probes before a long-duration probe	0.9 \pm 0.15 c	0.7 \pm 0.17 c	0.53 \pm 0.12 c	2.03 \pm 0.33 b	3.3 \pm 0.54 a	1.27 \pm 0.32 c	0.73 \pm 0.15 c	2.63 \pm 0.28 ab
Proportion of individuals performing a long-duration probe*	1 a	1 a	1 a	0.97 ab	0.77 b	0.73 c	1 a	0.70 c
Proportion of individuals that left the plant	0 b	0 b	0 b	0 b	0.03 b	0.27 a	0 b	0.27 a
Time to take-off (min)					27.11 a	4.32 \pm 1.26 c		10.15 \pm 2.18 b

Variables were compared across aphid/experience/test plant combinations. Within a row, different letters denote significant differences across aphid/experience/test plant combinations (see text for details of the statistical tests performed). Rearing hosts (RH) were sweet pepper for *Myzus persicae* s. str. and tobacco for *Myzus persicae nicotianae*. The alternative host (AH) was thorn apple for both taxa.

* ab vs c: $Z = 2.170$, $P = 0.03$; a vs c: $Z = 2.659$, $P = 0.008$.

depended on the alternative host (Figs 2b, 3a); moreover, learning novel olfactory information from the alternative host took longer in the specialist than in the generalist (Figs 3a, 2a). Whether or not the host-dependent ability of the specialist to learn is also exhibited by the generalist remains to be tested. Further experiments using a range of alternative hosts in the generalist would shed some light on this. Nevertheless, these experiments must consider that plant odours together with other plant features (e.g. visual stimuli) might be learned by aphids and used during host selection (Webster, Qvarfordt, Olsson, & Glinwood, 2013).

Interestingly, in contrast to the generalist (Fig. 2c), retroactive interference was apparent in the specialist, as the specialist's preference for the rearing host was lost after 12 h of experience on both alternative hosts tested (Figs 2d, 3b). However, this aphid was not able to learn one of the alternative hosts (thorn apple) after spending a long time on its rearing host (tobacco) (Fig. 2b), but it was indeed able to 'forget' its rearing host when exposed to the alternative host (Fig. 2d). Although that new host was not learned, some of its features could have interfered with the expected preference for the rearing host. Other studies have shown that this same aphid taxon prefers the odours of the rearing host (Vargas et al., 2005). Because retroactive interference refers to defects when retrieving previously learned tasks, this experiment provides weak evidence of retroactive interference in the specialist. Unambiguous evidence of retroactive interference in this specialist aphid was exhibited when another alternative host (sweet pepper), which was previously learned (Fig. 3a), interfered with preference for the rearing host (Fig. 3b).

Learning Capacity and Retroactive Interference during the Post-alighting Behaviour

The experiments of post-alighting behaviour also provide evidence that generalist and specialist aphids differ in their learning capacity and the occurrence of retroactive interference. As expected (Fig. 1), when aphids had no experience on an alternative host, both taxa performed more 'efficiently' (accessed feeding sites more quickly) on their respective rearing hosts than on alternative hosts. Thus, both taxa were able to use previous experience to guide current behaviour. Because the total time spent on the rearing host

involved more than three generations, maternal and pre-adult experience on the rearing host may explain this result, as described for these taxa in previous studies (Olivares-Donoso, et al., 2007) and also in other aphid species (Liu, Zhai, & Zhang, 2008; Ortiz-Martínez, Ramírez, & Lavandero, 2013; Ramírez & Niemeier, 2000). However, this effect was reversed in the specialist *M. p. nicotianae* after only 24 h of experience on the alternative host, leading to a more efficient probing on the alternative host than on the rearing host.

Since there is a relationship between the difficulty of the task and the speed of the final decision (Chittka, Skorupski, & Raine, 2009), less efficient probing by the specialist aphid on their rearing host after 24 h of experience on the alternative host may reflect difficulties in the process of host recognition as a result of failures in the retrieval of previous information. Taking into account that these failures are concomitant with learning of new information from a novel host, this response may reflect the occurrence of retroactive interference in the specialist aphid. In contrast, when the generalist aphids were given experience or no experience on an alternative host and tested on the rearing host, they showed no significant differences in behaviour; thus, retrieving failures of previous information were not the general tendency in the generalist. These results from pre- and post-alighting behaviours are consistent and suggest a greater relative impact of retroactive interference on the specialist aphid. Nevertheless, these results should be interpreted with caution because retroactive interference, as found in the pre-alighting bioassays, might vary depending on the alternative host used. On the other hand, a plant used to test retroactive interference in the pre-alighting phase will not necessarily produce comparable retroactive interference in the post-alighting phase. For instance, sweet pepper was used as the alternative host for the specialist in the pre-alighting study since it did not show learning with thorn apple as the alternative host; regrettably, this host was not used for another set of post-alighting experiments. Note, however, that the cues involved in each process differ (e.g. volatile compounds in the pre-alighting phase versus internal metabolites in the post-alighting phase), and thus possibly induce different learning processes; therefore, effects of retroactive interference for a given host plant may differ during pre- and post-alighting phases. Further research is needed to decipher the specific stimuli

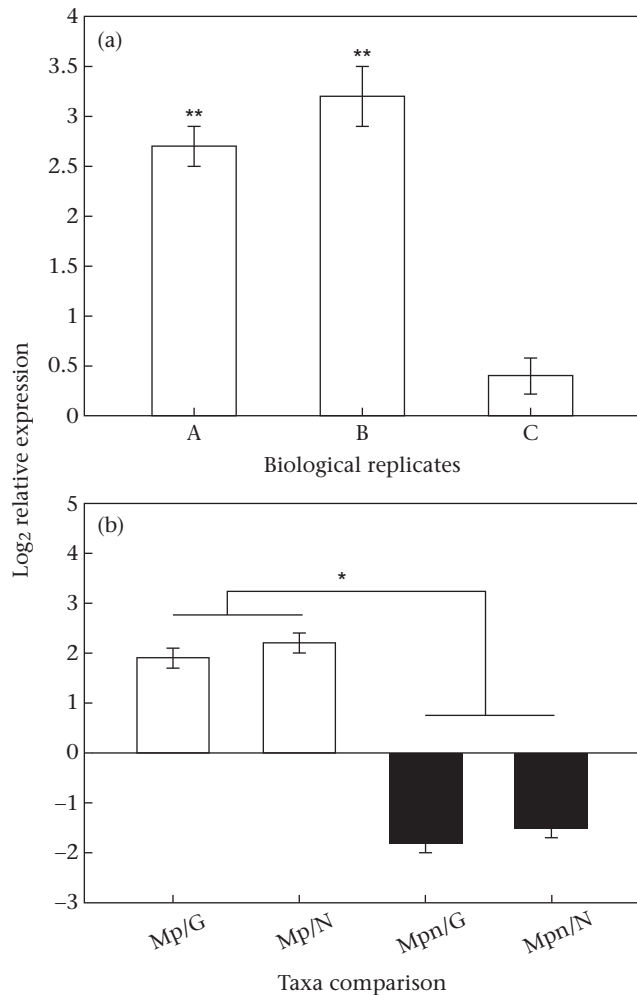


Figure 4. Expression levels in the generalist (*Myzus persicae* s. str.) and the tobacco specialist (*Myzus persicae nicotianae*) in the *for* gene (a) and quantification of *for* gene expression levels (b). In (a), the results represent the relative mRNA expression for three biological replicates (*M. persicae* s. str. relative to *M. p. nicotianae*; both winged morphs), with transcripts expressed by *M. p. nicotianae* as calibrator. Data were normalized for variation using GAPDH expression. Bars correspond to independent biological replicates (means \pm SE). ** $P < 0.01$ indicates significance level relative to 1, used as a reference value for no change in expression in a Student's *t* test. In (b), expression was estimated in winged morphs of *Myzus persicae* s. str. (Mp) and *Myzus p. nicotianae* (Mpn) relative to apterous individuals of *Myzus persicae* s. str. (genotype G or N). The results represent the relative mRNA expression in the pooled biological replicates (A, B and C in panel (a)), with transcripts expressed by apterous individuals of *Myzus persicae* s. str. as calibrator. Data were normalized for variation using GAPDH expression. Bars correspond to means \pm SE. * $P < 0.05$ for comparisons using a nested ANOVA.

generating learning and retroactive interference on these host selection phases.

Our findings are in agreement with the prediction from the neural limitation hypothesis of greater learning capacities in a generalist insect (Bernays, 2001; Bernays & Wcislo, 1994). Although retroactive interference is not explicitly included in the neural limitation hypothesis, the present empirical evidence suggests that other cognitive processes could be included in this framework. Retroactive interference may play a crucial role in decision accuracy when, for instance, some information can be ignored as a result of retrieving failures, thus avoiding disruption in focus and accuracy of host selection decisions. This proposal may represent a mechanism to reduce the sensory input and the potential occurrence of an overloading of the neural capabilities of the individual (sensu

Bernays & Wcislo, 1994), whose effects may prevent efficient searching behaviours. This mechanism could be particularly relevant when an insect has been exposed to more than one plant species during its ontogeny. When previous information can be disadvantageous, retroactive interference could be beneficial to an insect as a form of adaptive forgetting (Kraemer & Golding, 1997); these particular conditions could extend the framework of the neural limitation hypothesis to situations where specialization may evolve under environmental heterogeneity in terms of the potential hosts available. Note that failures in retrieving learnt information and memory limitations have been proposed to promote constancy in resource exploitation by pollinators (Chittka & Thomson, 1997; Geegar & Laverty, 1998; Ishii, 2005).

Differences between Specialist and Generalist in Gene for Expression

The *for* gene encodes a cGMP-dependent protein kinase (PKG); although the mode of action of this enzyme is not fully understood, it seems to be associated with memory and learning performance (Mery et al., 2007). PKG is expressed in the mushroom bodies in insects; it modulates the concentration of cAMP (essential for synapsis formation and efficacy) and is important for olfactory and visual signal transduction by activating cyclic nucleotide-gated (CNG) cation channels, and also for the neuronal wiring by guiding arborization through signalling elements, among other functions (Hofmann, Feil, Kleppisch, & Schlossmann, 2006). Also, individuals with higher levels of PKG show an improved short-term memory and faster olfactory learning (Kaun & Sokolowski, 2009). Structural and functional differences of the *for* gene were explored in the *M. persicae* complex; while no sequence differences were found between taxa, the levels of gene expression under optimal rearing conditions differed between taxa. Indeed, the *mpfor* gene was found to be transcribed in comparatively higher levels in the generalist *M. persicae* s. str., an aphid that in our bioassays did not show retrieval failures, thus providing evidence for associative learning, retroactive interference and *for* gene expression. In fact, in our case the aphid taxon with higher *for* gene expression and learning performance showed a lower occurrence of retroactive interference. Likewise, differences in *for* gene expression also agree with predictions of the neural limitation hypothesis, which states that generalists should show greater learning performance than specialists. Furthermore, the *for* gene is also a highly pleiotropic gene with effects not only on neuronal functions, but also on foraging activity, animal dispersal and locomotion, feeding, nutrient absorption and stress resistance (Ben-Shahar, Robichon, Sokolowski, & Robinson, 2002; Ingram, Oefner, & Gordon, 2005; Kaun & Sokolowski, 2009; Lucas & Sokolowski, 2009; Reaume & Sokolowski, 2009; Tobback et al., 2008). Hence, low levels of *for* gene could imply a reduced PKG expression (although protein levels or activity of PKG were not studied in this work), resulting in reduced mobility, foraging activity and dispersal. These behavioural differences could in turn reinforce differences in diet breadth and ecological specialization by, for instance, reducing resource searching behaviours and mobility in PKG-downregulated individuals (in our case *M. p. nicotianae*), thereby increasing host fidelity in phytophagous insects. All these factors can explain the differences in expression levels of *mpfor* gene between alate and apterous morphs (for both taxa), because only the former are responsible for host searching and dispersal; thus, they should require comparatively higher neural and dispersal (i.e. metabolic and locomotory) capacities than apterous morphs.

In summary, we have provided evidence for differences in learning performance between a specialist and a generalist phytophagous insect (of the same species but differing at the subspecies level), which in part may be underlying host

specialization. Other mechanisms such as upregulation of detoxification-related genes may be also participating in this specialization (Bass et al., 2013). Here, we have identified differences in gene expression of *mpfor*, a gene related to learning and putatively promoting ecological specialization. Our findings are in line with ideas proposing that specialist insects are neurally constrained and display lower learning capacity and higher retroactive interference. We have also provided a potential molecular genetic basis that may introduce the necessary variation to explain the evolution of ecological specialization in *M. p. nicotianae*, and this could be acting in other systems with contrasting degrees of ecological specialization. It seems crucial to expand this comparison to other taxa in order to assess how widespread within the insect world our findings are.

Acknowledgments

We thank Daniel Benítez, Marcela Cordero and Carolina Mendoza for their logistical support during this research and Víctor Cifuentes for his valuable comments. This work was supported by an International Foundation for Science grant C/4712-1 to D.H.T., Fondecyt 1100746 to C.C.R and Fondecyt 1090378 to C.C.F., and partially funded by Iniciativa Científica Milenio grant NC120027.

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
- Bass, C., Zimmer, C. T., Riveron, J. M., Wilding, C. S., Wondji, C. S., Kaussmann, et al. (2013). Gene amplification and microsatellite polymorphism underlie a recent insect host shift. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 19460–19465.
- Ben-Shahar, Y., Robichon, A., Sokolowski, M. B., & Robinson, G. E. (2002). Influence of gene action across different time scales on behavior. *Science*, 296, 741–744.
- Bernays, E. A. (1998). The value of being a resource specialist: behavioral support for a neural hypothesis. *American Naturalist*, 151, 451–464.
- Bernays, E. A. (1999). When host choice is a problem for a generalist herbivore: experiments with the whitefly, *Bemisia tabaci*. *Ecological Entomology*, 24, 260–267.
- Bernays, E. A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology*, 46, 703–727.
- Bernays, E. A., & Bright, K. L. (2001). Food choice causes interrupted feeding in the generalist grasshopper *Schistocerca americana*: further evidence for inefficient decision-making. *Journal of Insect Physiology*, 47, 63–71.
- Bernays, E. A., & Bright, K. L. (2005). Distinctive flavours improve foraging efficiency in the polyphagous grasshopper, *Taeniopoda eques*. *Animal Behaviour*, 69, 463–469.
- Bernays, E. A., Singer, M. S., & Rodrigues, D. (2004). Foraging in nature: foraging efficiency and attentiveness in caterpillars with different diet breadths. *Ecological Entomology*, 29, 389–397.
- Bernays, E. A., & Wcislo, W. T. (1994). Sensory capabilities, information-processing, and resource specialization. *Quarterly Review of Biology*, 69, 187–204.
- Blackman, R. L. (1987). Morphological discrimination of a tobacco-feeding form from *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (Nectarosiphon) species. *Bulletin of Entomological Research*, 77, 713–730.
- Blackman, R. L., & Eastop, V. F. (2000). *Aphids on the world's crops. An identification guide*. Chichester, U.K.: J. Wiley.
- Cabrera-Brandt, M. A., Fuentes-Contreras, E., & Figueroa, C. C. (2010). Differences in the detoxification metabolism between two clonal lineages of the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) reared on tobacco (*Nicotiana tabacum* L.). *Chilean Journal of Agricultural Research*, 70, 567–575.
- Cheng, K. (2005). Context cues eliminate retroactive interference effects in honeybees *Apis mellifera*. *Journal of Experimental Biology*, 208, 1019–1024.
- Cheng, K., & Wignall, A. E. (2006). Honeybees (*Apis mellifera*) holding on to memories: response competition causes retroactive interference effects. *Animal Cognition*, 9, 141–150.
- Chittka, L., Skorupski, P., & Raine, N. E. (2009). Speed–accuracy tradeoffs in animal decision making. *Trends in Ecology & Evolution*, 24, 400–407.
- Chittka, L., & Thomson, J. D. (1997). Sensory-motor learning and its relevance for task specialization in bumble bees. *Behavioral Ecology and Sociobiology*, 41, 385–398.
- Conover, W. J., & Iman, R. L. (1985). Rank transformations as a bridge between parametric and nonparametric statistics. *American Statistician*, 35, 124–129.
- Daly, K. C., & Smith, B. H. (2000). Associative olfactory learning in the moth *Manduca sexta*. *Journal of Experimental Biology*, 203, 2025–2038.
- Dixon, A. F. G. (1998). *Aphid ecology* (2nd ed.). London, U.K.: Chapman & Hall.
- Dukas, R. (2004). Evolutionary biology of animal cognition. *Annual Review of Ecology and Systematics*, 35, 347–374.
- Dukas, R. (2008). Evolutionary biology of insect learning. *Annual Review of Entomology*, 53, 145–160.
- Dukas, R., & Bernays, E. A. (2000). Learning improves growth rate in grasshoppers. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 2637–2640.
- Egan, S. P., & Funk, D. J. (2006). Individual advantages to ecological specialization: insights on cognitive constraints from three conspecific taxa. *Proceedings of the Royal Society B: Biological Sciences*, 273, 843–848.
- Egas, M., & Sabelis, M. W. (2001). Adaptive learning of host preference in a herbivorous arthropod. *Ecology Letters*, 4, 190–195.
- Ewing, B., Hillier, L., Wendl, M. C., & Green, P. (1998). Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Research*, 8, 175–185.
- Faber, T., Joerges, J., & Menzel, R. (1999). Associative learning modifies neural representations of odors in the insect brain. *Nature Neuroscience*, 2, 74–78.
- Farris, S. M., & Roberts, N. S. (2005). Coevolution of generalist feeding ecologies and gynecephalic mushroom bodies in insects. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 17394–17399.
- Frasnelli, E., Vallortigara, G., & Rogers, L. J. (2010). Response competition associated with right–left antennal asymmetries of new and old olfactory memory traces in honeybees. *Behavioural Brain Research*, 209, 36–41.
- Gegeer, R. J., & Lavery, T. M. (1998). How many flower types can bumble bees work at the same time? *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 76, 1358–1365.
- Giurfa, M. (2013). Cognition with few neurons: higher-order learning in insects. *Trends in Neurosciences*, 36, 285–294.
- Hofmann, F., Feil, R., Kleppisch, T., & Schlossmann, J. (2006). Function of cGMP-dependent protein kinases as revealed by gene deletion. *Physiological Reviews*, 86, 1–23.
- Huang, X., & Madan, A. (1999). CAP3: a DNA sequence assembly program. *Genome Research*, 9, 868–877.
- Ingram, K. K., Oefner, P., & Gordon, D. M. (2005). Task-specific expression of the foraging gene in harvester ants. *Molecular Ecology*, 14, 813–818.
- Ishii, H. S. (2005). Analysis of bumblebee visitation sequences within single bouts: implication of the overstrike effect on short-term memory. *Behavioral Ecology and Sociobiology*, 57, 599–610.
- Janz, N., & Nylin, S. (1997). The role of female search behaviour in determining host plant range in plant feeding insects: a test of the information processing hypothesis. *Proceedings of the Royal Society B: Biological Sciences*, 264, 701–707.
- Kaun, K. R., & Sokolowski, M. B. (2009). cGMP-dependent protein kinase: linking foraging to energy homeostasis. *Genome*, 52, 1–7.
- Kraemer, P. J., & Golding, J. M. (1997). Adaptive forgetting in animals. *Psychonomic Bulletin & Review*, 4, 480–491.
- Legeai, F., Shigenobu, S., Gauthier, J. P., Colbourne, J., Rispe, C., Collin, O., et al. (2010). AphidBase: a centralized bioinformatic resource for annotation of the pea aphid genome. *Insect Molecular Biology*, 19, 5–12.
- Levins, R., & MacArthur, R. H. (1969). An hypothesis to explain the incidence of monophagy. *Ecology*, 50, 910–911.
- Liu, X. D., Zhai, B. P., & Zhang, X. X. (2008). Specialized host-plant performance of the cotton aphid is altered by experience. *Ecological Research*, 23, 919–925.
- Lucas, C., & Sokolowski, M. B. (2009). Molecular basis for changes in behavioral state in ant social behaviors. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 6351–6356.
- Margaritopoulos, J. T., Malarky, G., Tsitsipis, J. A., & Blackman, R. L. (2007). Microsatellite DNA and behavioural studies provide evidence of host-mediated speciation in *Myzus persicae* (Hemiptera: Aphididae). *Biological Journal of the Linnean Society*, 91, 687–702.
- Mery, F., Belay, A. T., So, A. K. C., Sokolowski, M. B., & Kawecki, T. J. (2007). Natural polymorphism affecting learning and memory in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 13051–13055.
- Moreau, J., Rahme, J., Benrey, B., & Thiery, D. (2008). Larval host plant origin modifies the adult oviposition preference of the female European grapevine moth *Lobesia botrana*. *Naturwissenschaften*, 95, 317–324.
- Niemeyer, H. M. (1990). Secondary plant chemicals in aphid–host interactions. In D. C. Peters, J. A. Webster, & C. S. Chlouber (Eds.), *Aphid–plant interactions: Populations to molecules* (pp. 101–111). Stillwater, OK: USDA/Agricultural Research Service, Oklahoma State University.
- Olivares-Donoso, R., Troncoso, A. J., Tapia, D. H., Aguilera-Olivares, D., & Niemeyer, H. M. (2007). Contrasting performances of generalist and specialist *Myzus persicae* (Hemiptera: Aphididae) reveal differential prevalence of maternal effects after host transfer. *Bulletin of Entomological Research*, 97, 61–67.
- Oppenheim, S. J., & Gould, F. (2002). Behavioral adaptations increase the value of enemy-free space for *Heliothis subflexa*, a specialist herbivore. *Evolution*, 56, 679–689.
- Ortiz-Martínez, S. A., Ramírez, C. C., & Lavandero, B. (2013). Host acceptance behavior of the parasitoid *Aphelinus mali* and its aphid-host *Eriosoma lanigerum* on two Rosaceae plant species. *Journal of Pest Science*, 86, 659–667.
- Osborne, K. A., Robichon, A., Burgess, E., Butland, S., Shaw, R. A., Coulthard, A., et al. (1997). Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science*, 277, 834–836.
- Papaj, D. R., & Prokopy, R. J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology*, 34, 315–350.

- Pettersson, J. (1970). An aphid sex attractant. *Biological studies. Entomologica Scandinavica*, *1*, 63–73.
- Pettersson, J., Tjallingii, W., & Hardie, J. (2007). Host plant selection and feeding. In H. F. van Emden, & R. Harrington (Eds.), *Aphids as crop pests* (pp. 87–107). London, U.K.: CAB International.
- Pickett, J. A., Wadhams, L. J., Woodcock, C. M., & Hardie, J. (1992). The chemical ecology of aphids. *Annual Review of Entomology*, *37*, 67–90.
- Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, *51*, 309–330.
- Ramírez, C. C., Fuentes-Contreras, E., Rodríguez, L. C., & Niemeyer, H. M. (2000). Pseudoreplication and its frequency in olfactometric laboratory studies. *Journal of Chemical Ecology*, *26*, 1423–1431.
- Ramírez, C. C., & Niemeyer, H. M. (2000). The influence of previous experience and starvation on aphid feeding behavior. *Journal of Insect Behavior*, *13*, 699–709.
- Reaume, C. J., & Sokolowski, M. (2009). c-GMP-dependent protein kinase as a 700 modifier of behaviour. In H. H. W. Schmidt, F. Hofmann, & J. P. Stasch (Eds.), *cGMP: Generators, effects and therapeutic implications* (pp. 423–443). Berlin, Germany: Springer.
- Reaume, C. J., Sokolowski, M. B., & Mery, F. (2011). A natural genetic polymorphism affects retroactive interference in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 91–98.
- Schoonhoven, L. M., Jermy, T., & van Loon, J. J. A. (2006). *Insect–plant biology: From physiology to evolution* (2nd ed.). London: Chapman & Hall.
- Silva, A. X., Jander, G., Samaniego, H., Ramsey, J. S., & Figueroa, C. C. (2012). Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (Hemiptera: Aphididae): a transcriptomic survey. *PLoS One*, *7*, e36366.
- Steidle, J. L. M., & Van Loon, J. J. A. (2003). Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata*, *108*, 133–148.
- Thamm, M., & Scheiner, R. (2014). PKG in honey bees: spatial expression, *Amfor* gene expression, sucrose responsiveness, and division of labor. *Journal of Comparative Neurology*, *522*, 1786–1799.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673–4680.
- Tobback, J., Heylen, K., Gobin, B., Wenseleers, T., Billen, J., Arckens, L., et al. (2008). Cloning and expression of PKG, a candidate foraging regulating gene in *Vespula vulgaris*. *Animal Biology*, *58*, 341–351.
- Tosh, C. R., Krause, J., & Ruxton, G. D. (2009). Theoretical predictions strongly support decision accuracy as a major driver of ecological specialization. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 5698–5702.
- Tosh, C. R., Powell, G., & Hardie, J. (2003). Decision making by generalist and specialist aphids with the same genotype. *Journal of Insect Physiology*, *49*, 659–669.
- Troncoso, A. J., Vargas, R. R., Tapia, D. H., Olivares-Donoso, R., & Niemeyer, H. M. (2005). Host selection by the generalist aphid *Myzus persicae* (Hemiptera: Aphididae) and its subspecies specialized on tobacco, after being reared on the same host. *Bulletin of Entomological Research*, *95*, 23–28.
- Vargas, R. R., Troncoso, A. J., Tapia, D. H., Olivares-Donoso, R., & Niemeyer, H. M. (2005). Behavioural differences during host selection between alate virginoparae of generalist and tobacco-specialist *Myzus persicae*. *Entomologia Experimentalis et Applicata*, *116*, 43–53.
- Webster, B., Qvarfordt, E., Olsson, U., & Glinwood, R. (2013). Different roles for innate and learnt behavioral responses to odors in insect host location. *Behavioral Ecology*, *24*, 366–372.
- Wee, B., & Singer, M. C. (2007). Variation among individual butterflies along a generalist–specialist axis: no support for the ‘neural constraint’ hypothesis. *Ecological Entomology*, *32*, 257–261.
- Weiss, M. R., & Papaj, D. R. (2003). Colour learning in two behavioural contexts: how much can a butterfly keep in mind? *Animal Behaviour*, *65*, 425–434.
- West, S. A., & Cunningham, J. P. (2002). A general model for host plant selection in phytophagous insects. *Journal of Theoretical Biology*, *214*, 499–513.
- Wixted, J. T. (2004). The psychology and neuroscience of forgetting. *Annual Review of Psychology*, *55*, 235–269.
- Worden, B. D., Skemp, A. K., & Papaj, D. R. (2005). Learning in two contexts: the effects of interference and body size in bumblebees. *Journal of Experimental Biology*, *208*, 2045–2053.
- Wu, B. F., Shen, K., An, K. P., Huang, J., & Zhang, R. J. (2011). Effect of larval density and host species on preimaginal development of *Bactrocera tau* (Diptera: Tephritidae). *Journal of Economic Entomology*, *104*, 1840–1850.
- Zar, J. H. (1996). *Biostatistical analysis* (3rd ed.). Englewood Cliffs, NJ: Prentice Hall.