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

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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...
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; Reáume, 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 (Reáume 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, 2005), 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, Olivas-Donoso, & Niemeyer, 2005) (but see Tosh, Powell, & Hardie, 2003; Troncoso, Vargas, Tapia, Olivas-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, Tjallinii, & 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 unresolved 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 nicotioidae (Blackman & Eastop) has been described as an ecological tobacco specialist (Blackman, 1987; Cabrera-Brandeit, Fuentes-Contreras, & Figueroa, 2010; Margaritopoulos, Malarkey, Tsiptis, & Blackman, 2007; Olivas-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 of 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-alignment 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 (Reáume 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, Acyrthosiphon 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. (Solanaeaceae), and M. p. nicotianae lineages were reared on tobacco plants, Nicotiana tabacum L. cv. BY 64 (Solanaeaceae). 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. (Solanaeaceae), 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
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 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 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

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**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.
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 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 (probing lasting more than 5 min), or until a preset 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 using the AfinityScript 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) or AmpliSeq (v.1.3.7) packages, and checked in NCBI/Primer-BLAST (forward: ACTGCGAGGATAACCACCCAGATA; reverse: AGTGCACAA-GACCTGTAGACTCCG). 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 μM of SYBR Green PCRMaster 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 55–95 °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 (Legea et al., 2010). Among glyceraldehyde-3-phosphate dehydrogenase GAPDH (DW011095), cyclophilin-10-like (EC388830), actin (EE262754), ribosomal protein LPO (DW011949) and ribosomal protein L7 (DW361765), NormFinder identified GAPDH (for primers details see Silva et al., 2012) as the most stable expressed housekeeping gene followed by cyclophilin-10-like. We used only the data obtained using GAPDH as the reference gene, since both reference genes showed similar results. The log2 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 log2-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 μg/μl and 2.0 μg/μl 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 M. p. nicotianae, using the ThermoScript™-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 μg/μl for M. persicae s. str. and M. p. nicotianae, respectively) as template for the amplification of mpfor. Specific primers for mpfor (forward: 5’ AGTACGGACTTCCGTTCAC 3’; reverse: 5’ GCAAAGTATGAGGATTGG 3’) were designed based on the predicted sequences from the aphid Acyrthosiphon pisum recovered from Aphidbase (Legeari et al., 2010) (Gbrowse accession numbers: for: orthodoxous 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 Mg2+, 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 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 Genetec (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).

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 ± 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: t0 = 3.920, P = 0.004; 24 h: t0 = 3.318, P = 0.009; 48 h: t0 = 2.709, P = 0.024; 72 h: t0 = 2.509, P = 0.033; (c) 0 h: t0 = 2.640, P = 0.019; 12 h: t0 = 2.731, P = 0.023; 24 h: t0 = 3.361, P = 0.008; 48 h: t0 = 3.128, P = 0.012; 72 h: t0 = 2.627, P = 0.027; (d) 0 h: t0 = 5.464, P < 0.001.

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
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.

**Mpf or Gene Expression**

The sequence obtained for the putative gene for confirmed that *mpf or* transcripts are orthologous with the *for* gene, 99% and 96% 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 *mpf or* and *mpn for*). The *mpf or* sequences for each taxa were deposited in GenBank under the following accession numbers: *mpf or*: JF776573, *mpn for*: JF776572. When aphids were reared on their optimal host plant (see Methods), the *mpf or* 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 *mpf or* 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...
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, & Glenwood, 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 aphids 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 & Niemeyer, 2000). However, this effect was reversed in the specialist Myzus 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, Skorupska, & 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
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; Geoghegan & 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 (Nery 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; Tobbback 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

**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 CND expression. Bars correspond to independent biological replicates (means ± 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 CND expression. Bars correspond to means ± SE. *P < 0.05 for comparisons using a nested ANOVA.
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 mtpor, 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.

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