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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 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.

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

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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, Tjallinjii, & 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-alightening 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-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 (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, *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. (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