Selective attention by priming in host search behavior of two generalist butterflies.

Short title: Butterfly search behavior.

Abstract
In phytophagous insects such as butterflies, there is an evolutionary trend towards specialization in host plant use. One contributing mechanism for this pattern may be found in female host search behavior. Since search attention is limited, generalist females searching for hosts for oviposition may potentially increase their search efficacy by aiming their attention on a single host species at a time, a behavior consistent with search image formation. Using laboratory reared and mated females of two species of generalist butterflies, the comma, *Polygonia c-album*, and the painted lady, *Vanessa cardui* (Lepidoptera: Nymphalidae), we investigated the probability of finding a specific target host (among non-host distractors) immediately after being primed with an oviposition experience of the same host as compared to different host in indoor cages. We used species-specific host plants that varied with respect to growth form, historical age of the butterfly-host association, and relative preference ranking. We found improved search efficacy after previous encounters of the same host for some but not all host species. Positive priming effects were found only in hosts with which the butterfly has a historically old relationship and these hosts are sometimes also highly preferred. Our findings provide additional support for the importance of behavioral factors in shaping the host range of phytophagous
insects, and show that butterflies can attune their search behavior to compensate for negative effects of divided attention between multiple hosts.

**key-words:** search behavior; limited attention; priming; specialization; host-plant; diet breadth

**Ley summary:**
We show that females of two generalist butterflies improve their search efficacy after previous encounters of the same host in a way similar to search-image formation, especially if the butterfly-host relationship is historically old. Thus, by targeting a single host at a time, host search efficacy may be improved and constitute a selection pressure for specialization. This result can help explain the evolutionary trend towards host specialization in phytophagous insects that is not well understood.
INTRODUCTION

The relative costs and benefits of resource specialization versus generalization are of major importance for understanding the evolution of host range in herbivorous insects. The potential benefits to each strategy are many, yet there is a notable tendency towards specialization in plant-feeding insects (Futuyma and Moreno 1988; Jaenike 1990; Forister et al. 2015) even though a generalist strategy for instance leads to a higher frequency of potential host targets (Johansson et al. 2007), and is less sensitive to fluctuating environments by providing more opportunities for risk spreading (Hopper 1999; Wiklund and Friberg 2009). There are both physiological and behavioral reasons suggesting that insects benefit by restricting their diet. The physiological aspects mainly include that generalists, having the ability to digest many types of plants (implicitly with different digestive requirements), have a lower performance on each of the hosts, whereas specialists trade-off this ability with a higher performance on the one host (Dethier 1954; Mackenzie 1996; Via and Hawthorne 2002). However, experimental evidence of performance trade-offs between hosts is at the best inconclusive since numerous studies show no, or even positive correlations between hosts (e.g. Futuyma and Philippi 1987; Carriere and Roitberg 1994; Fox and Caldwell 1994; Janz and Nylin 1997; Friberg and Wiklund 2009; Agosta and Klemens 2009; Gompert et al. 2015). Also of relevance is the fact that larvae of many butterfly species can readily survive on plants that are not normally in the repertoire of ovipositing females (Wiklund, 1975; Janz et al. 2001; Lehnert and Sribner, 2012; Nylin et al. 2015). These findings suggest that, although physiological reasons may sometimes be primary, the behavioral aspects of female host search may be of greater importance in specialization.
Although a generalist butterfly female searching for host plants to oviposit on has a greater number of individual targets as compared to a female of her specialist sister species, she might yet be at a disadvantage because she is potentially less effective in her search and may make poorer choices. Several very similar hypotheses have been put forward explaining this relationship, implicit already in the model put forward by Levins and MacArthur (1969) to explain monophagy. For instance, the “information processing hypothesis” (Courtney 1983; Futuyma 1983) and the “neural limitations hypothesis” (Dall and Cuthill 1997; Bernays 2001; Tosh et al. 2009) both argue limitations to the information system that correctly separates a good host from an unsuitable host, namely decision accuracy. There are several experiments supporting the superiority of specialists in choosing the host of better quality (fitness wise) (e.g. Janz and Nylin 1997; Bernays and Funk 1999; Egan and Funk 2006; Schäpers et al. 2016), and search speed and decision time also seem to be positively affected by having a neural system that is focused on a smaller host repertoire (Bernays and Funk 1999; Bernays 2001; Janz 2003). An additional hypothesis, the “limited attention” hypothesis, focuses on the dynamics of search behavior rather than the specialization of the neural system. It states that generalist females, by aiming their limited attention on a single host species at a time, may increase their search rate. This behavioral benefit of selective attention may therefore select for a more restricted diet (Dukas 2002).

One effect of selective attention in search behavior may be **Sequential priming**, a phenomenon studied in visual search theory (e.g. Blough, 1989; 1991 Reid and
Shettleworth 1992; Dukas and Camil 2001), where by finding one target an individual’s attention becomes temporarily attuned to the features of that target. This selective attention by priming has been suggested to be the mechanism behind the formation of search images (Blough 1989, 1991; Langley 1996), a hypothesis originally explaining birds’ tendency to prefer abundant prey and select them at higher proportions than their actual frequencies (Tinbergen, 1960; Bond 1983). In generalist butterflies searching for hosts, sequential priming would entail that a female, after interacting with a specific host, would prime or attune her attention to that specific host and increase her search efficacy by concentrating search to that single (more abundant) host. The attentional priming would entail an increased ability to find a host species that have recently been encountered, as well as a decreased ability to find other hosts in their repertoire (Blough 1989). There is some circumstantial evidence suggesting that sequential priming may happen in ovipositing butterflies. For instance, females of the pipevine butterfly (*Battus philenor*) learn from chemical reinforcement to discriminate hosts by using leaf shape (Papaj 1986) and they more easily find the host with a leaf shape they have previously experienced (Rausher 1978). Also, a field study of *Colias* butterflies show a more effective search in females when they divide their time into longer foraging bouts and oviposition bouts, with as few switches as possible (Stanton 1984).

The aim of our study was to, in controlled experiments, investigate effects of prior host exposure on the search behavior of ovipositing females. More specifically, we aimed to investigate if a prior positive exposure to a specific host, a priming event, may affect the probability of finding that same host species again. Such effects would suggest that
generalist butterflies could temporarily focus their search attention towards specific host species, which would result in a more effective search behavior. We use two polyphagous species, the comma (*Polygonia c-album*) and the painted lady (*Vanessa cardui*, Lepidoptera: Nymphalidae) that both can be considered to be relative generalists when searching for hosts. Since the different host species used by polyphagous insects often has different ranking in a preference hierarchy, have a longer or shorter evolutionary history as hosts (with corresponding variation in time for adaptation), or require different search behaviors depending on growth form, we chose to include host species that would provide information about possible effects of these factors on search behavior. A variation in host value is present in most generalist insects, and can be manifested as a more or less strict preference hierarchy which may or may not reflect the fitness consequences of feeding on the hosts (Wiklund 1975; Thompson 1988; Courtney et al. 1989; Gripenberg et al. 2010). A variation among target hosts in preference may affect the attractiveness, or the willingness to pursue the host, to the searching female. Another level of complexity is the historical age of the butterfly-host association. It is possible that a longer association will have allowed for more specific host recognition systems to evolve than would be present in a younger association and this may affect search capacity. Additionally, since comma butterflies also include trees among their hosts, it is possible that they may adopt different search behaviors when searching for a large tree, as compared to a herb. Thus, these three factors may affect the individual female’s motivation to search for each specific host, as well as the conspicuousness of different host species in an experimental setting, so we aimed to control for these factors in the study. In short, we expected that a positive exposure to a plant
should increase the ability of butterfly females to find that same host again, especially if it
is a high ranked plant or a host with long evolutionary history.

METHODS

The study consisted of three separate experiments that took place during spring and early
summer of 2015 and 2016. Generally, to investigate effects of immediate prior host
experience on search behavior, the experiments were set up so that experienced egg-laying
females first were subjected to one host plant (the ‘priming host’), landed and were allowed
to oviposit. Immediately afterwards they were allowed to search for a second host plant (the
‘experimental host’) in the arena. The priming host and the experimental host were either
the same host species or a different host, giving each female the priming host-experimental
host combinations A-A, A-B, B-B, and B-A.

Butterfly subjects and hosts

We used two single-egg laying, relatively generalist species of Nymphalidae (Lepidoptera)
butterflies. The comma butterfly (*Polygonia c-album*) is polyphagous on a few families
belonging to the orders Rosales (including urticalean rosids), Saxifragales, Fagales and
Malphigiales (Seppänen 1970) including trees, shrubs and herbs, whereas the painted lady
(*Vanessa cardui*) is one of the most polyphagous butterflies and can use over 100 host-
plant species, mainly herbs, from about 25 families (Scott 1986). Table 1 summarizes the
experimental host plants we used in the three experiments. They were chosen based on
three criteria: the relative preference ranking, the relative age of the butterfly-host
association (see separate section below) and the growth form. For *P. c-album*, we
contrasted the highly ranked *U. dioica* with the lower ranked host *S. caprea* in 2015 (Experiment 1), and in 2016 *U. dioica* was contrasted with the highly ranked *U. glabra* and the lower ranked *R. alpinum* (Experiment 2, table 1). The *V. cardui* females were presented with the highly ranked *C. arvense* contrasted against the lower ranked *U. dioica* and *P. lanceolata* (Experiment 3, table 1) in both years. Here, two years was needed because we had trouble reaching a good sample size the first year with this species. The ranking scores in table 1 represent the female preferences, but in these cases also larval performance on the specific hosts corresponds rather well with the scores (Nylin 1988; Celorio-Manchera et al. 2016).

The *P. c-album* females were laboratory-reared offspring of wild-caught gravid females. When hatched, the larvae were reared in small groups on *U. dioica* in plastic jars that provided a water-culture for the host plants. Plants were replaced with fresh ones when needed. Light and temperature conditions were set to induce the directly developing morph (Nylin, 1989). The *V. cardui* females used were the offspring of individuals we obtained as pupae from a commercial breeder (World Wide Butterflies). *V. cardui* larvae were reared in the same fashion as *P. c-album*, but we used *C. arvense* (2015) and *Arctium minus* (2016) as food. Larval experience of rearing plant has been shown to not affect subsequent oviposition selection in *P. c-album* (Janz et al. 2009), and given the high mobility and migratory behaviour of *V. cardui*, meaning that subsequent larval generations will seldom experience the same environment, there is no reason to expect such an effect in that species either. We reared larvae in batches over a longer time interval to continuously have fresh emerging experimental animals available.
After eclosion, adult individuals of each species were sexed, marked, and released into mating cages for mating. Mating pairs were extracted from the cages and when separating they were marked individually and the females were collected for the experiment whereas the males were returned to the mating cages. Mated females were placed individually into cages measuring approximately 36 x 52 x 48 cm (width x length x height) with moist paper towels on the floor to ensure high humidity in the cages. The cages had transparent plastic roofs, green cloth sides and back, and a transparent net in the front. Each cage had a heat and light source above and was equipped with a food source (a sponge submerged into sugar solution placed into a highly positioned small jar), as well as a number of bottles containing one of each of the experimental host plants that the butterflies would encounter later in the experiment (table 1). After approximately two-three days, the females started ovipositing regularly and were then moved together into the “priming cage” and used in the experiment.

Age of plant associations

In the study we use *Urtica dioica* (Urticaceae) and *Ulmus glabra* (Ulmaceae), both from the Urticalean rosids (part of Rosales). Phylogenetic reconstructions suggest that the “urticalean rosids” (formerly Urticales: families Urticaceae, Ulmaceae, Cannabaceae and Moraceae) were the ancestral larval hosts for the entire butterfly family Nymphalidae (Nylin et al. 2014), putting the age of the association at > 90 Ma (Wahlberg et al. 2009; Chazot et al. 2018). They are used by the subfamily Libytheinae, sister to the remaining nymphalids, as well as by basal branches in several major clades in the family (Nylin et al.
Closer to the study species, specialization on urticalean rosids remained the ancestral state for the tribe Nymphalini, containing both of the butterfly species used in the present study (Janz et al. 2001; Nylin and Wahlberg 2008).

We also use *Salix caprea* (Salicaceae), as a host for *P. c-album*. It belongs to the order Malpighiales. The history of association with this order among nymphalid butterflies is more complex. It is widely used in the family and the age of the association is difficult to assess. It could be as old as 90 Ma (Wahlberg et al. 2009; Nylin et al. 2014; Chazot et al. 2018), but given the very long period of specialization on urticalean rosids in the ancestors of the study species, we suggest that a more relevant age is < 11 Ma. This is when the *Nymphalis* + *Polygonia* clade diverged from the lineages specialized on urticalean rosids (Chazot et al. 2018). Genera in this clade share a range of host families other than urticalean rosids, including the tested host family Salicaceae in the Malpighiales, indicating an evolutionary event when the host range was broadened to include these families (Nylin 1988; Janz et al. 2001).

*Ribes alpinum* (Grossulariaceae), also used by *P. c-album* belongs to the order Saxifragales. This plant order is very rarely used as host by nymphalid butterflies (Nylin et al. 2014). The genus *Ribes* in the order is used by several species of *Polygonia* in two separate sections of the clade, but not by any other nymphalids, and it is thus not likely that it was colonized independently twice (Weingartner et al. 2006; Nylin et al. 2015). Rather, it was probably colonized near the base of *Polygonia* at < 7 Ma (dating from Chazot et al. 2018).
\textit{Circium arvense} (Asteraceae) used by \textit{V. cardui} is of the order Asterales that originated relatively recently at geological time scales, and is consequently used apically among nymphalid butterflies in a scattered manner. Asterales seems to have been colonized twice in the subfamily: in a sub-section of the tribe Melitaeni (Nylin and Wahlberg 2008) and by \textit{Vanessa} butterflies in the Nymphalini (Nylin et al. 2014). In the latter genus we see this as a single colonization, putting the age of the association near the base of \textit{Vanessa} at about 20 Ma (Chazot et al. 2018).

The final host \textit{Plantago lanceolata} (Plantaginaceae) is of the order Lamiales. Although there are scattered uses of this host order in several parts of the nymphalids, the use of Lamiales by \textit{Vanessa} is a separate colonization, and the order is probably used only by the most polyphagous species in the genus: \textit{V. cardui} and \textit{V. virginiensis}. This still puts the age of the association at < \textit{10.5 Ma} if these are not independent events (dating from Wahlberg and Rubinoff 2011). However, use of the genus \textit{Plantago} seems to be unique to \textit{V. cardui} in the genus and is thus a considerably younger association.

\textbf{Arena}

The experiments took place in two larger cages that measured 80 x 80 x 50 cm (width x length x height), with green cloth sides, transparent plastic roof and back, a net front and a floor covered with moist paper towels. In the first of the cages, the “priming cage”, we supplied several feeding sources, but no plants were present. In the other, the “experimental cage”, we created a search environment from cut-off plants placed in bottles. There were 12
non-hosts, used as distractors, spread out in the cage (10-15 cm in between plants) and surrounding one centrally placed bottle with the experimental host plant. The bottles and leaved plant stalks reached approximately two thirds of the height of the cages, leaving the top third free for flying. There was also some flying space between the plants. We chose to use cuts of garlic mustard (*Alliaria petiolata*, Brassicaceae) as distractors since they are abundant in localities where many of the host-plants grow and are quite aromatic. It also has a dented leaf shape similar to several of the *P. c-album* host-plants, including *U. dioica*, an old host of both butterfly species with respectively high or low ranking.

**Procedure and data collection**

Experiments were conducted continuously, and as soon as a female was starting to oviposit readily she took part in the experiment. We presented one host plant at a time in the priming cage, a cutting placed in a bottle with water. One experimental trial started when a butterfly landed and started to lay an egg on the priming host. The host together with the ovipositing female was then carefully transferred into the experimental cage, and when the butterfly flew up after laying an egg, the priming host was quickly removed. The butterfly was then allowed to search for the experimental host for a maximum of 10 minutes. A search was considered successful if the butterfly landed and oviposited on the host. If the female did not show search behavior during the whole 10 minutes, the trial was repeated after a while with the same individual female. If a female showed search behavior at some point during the 10 minutes, i.e. flying close to the plants, circling over them and drumming with the forelegs when landing (tasting the substrate), but did not find the host, the search was considered unsuccessful. In Experiment 1 (2015), each female of *P. c-album*
encountered the priming host-experimental host combinations Ur-Ur, Sa-Ur, Sa-Sa, and Ur-Sa (see Table 1 for host codes), and in Experiment 2 (2016), each female encountered the combinations Ul-Ul, Ur-Ul, Ul-Ur, Ri-Ur, Ri-Ri and Ur-Ri. In Experiment 3 (2015 & 2016), *Vanessa cardui* females each encountered the combinations Ur-Ur, Ci-Ur, Ci-Ci, Ur-Ci, Pl-Ci, Pl-Pl and Ci-Pl. The order of host pair presentations varied between females and most females searched in all treatments they were subjected to, but a few did not survive throughout, did not accept some hosts or did not search in one or a few treatments. Females were only included in the data analysis if they had successfully searched in more than half of the treatments (3/4 and 4/7 treatments respectively), and thus 6/54, 13/58 and 5/30 females were excluded from Experiments 1-3 respectively. This left the sample sizes of searching females of each treatment group as follows, Experiment 1: Ur-Ur, N=48; Sa-Ur, N=47; Sa-Sa, N=45 & Ur-Sa, N=46. Experiment 2: Ul-Ul, N=41; Ur-Ul, N=43; Ur-Ur, N=42; Ul-Ur, N=41; Ri-Ur, N=41; Ri-Ri, N=41 & Ur-Ri, N=41. Experiment 3: Ur-Ur, N=23; Ci-Ur, N=20; Ci-Ci, N=24; Ur-Ci, N=21; Pl-Ci, N=23; Pl-Pl, N=21 & Ci-Pl, N=25.

We noted whether a host was found or not during the whole trial and the time to finding the host. We first compared the tendency to find a certain host between host species by comparing found or not found frequencies using contingency tables (two-tailed Pearson's Goodness of Fit Chi-square, or Fisher’s exact tests when necessary). The detection time data included right-censored data: a butterfly that found the host during the 600 seconds of treatment time represented a complete observation, whereas a butterfly searching but not finding the host during the allotted time represented an observation that was right-censored. Therefore we used survival analysis for the detection times, performed
with Cox proportional hazards regression (Cox 1972), using Dell Statistica, version 13 (2015) software with default settings and presentation order (Experiments 1-3) and year (Experiment 3) were included as factors in the models. We also conducted a priori decided pairwise contrasts within the limits of degrees of freedom, to compare the specific treatments relevant for priming.

RESULTS

The probability of finding a certain host species in our experiment reflects the preference hierarchy and/or age of the butterfly-host association. When comparing between host species the treatments where females were primed on the same host as the experimental host, in Experiment 1, *P. c-album* females more easily found the highly ranked, old host *U. dioica* as compared to the lower ranked and relatively younger hosts *S. caprea* (Ur-Ur: 34/48, vs. Sa-Sa: 21/45) $X^2 = 5.613$, d.f. = 1, $p = 0.018$). Similarly in Experiment 2, *U. dioica* was easier found than *R. alpinum* (Ur-Ur: 27/42, vs. Ri-Ri: (11/41), $X^2 = 11.726$, d.f. = 1, $p = 0.00062$). Also the highly ranked and old *U. glabra* was found significantly more frequently than *R. alpinum* (Ul-Ul: 22/41 vs. Ri-Ri, $X^2 = 6.136$, d.f. = 1, $p = 0.013$) whereas there was no significant difference between *U. dioica* and *U. glabra* ($X^2 = 0.969$, d.f. = 1, $p = 0.324958$). In Experiment 3, fewer, *V. cardui* females found the lower ranked *P. lanceolata* as compared to the higher ranked *C. arvense* (Pl-Pl: 10/21, vs. Ci-Ci: 19/24, Fisher exact $p = 0.0345$). However, there was no significant difference in the probability of finding *U. dioica* as compared to either of the other hosts (Ur-Ur: 16/23, vs Pl-Pl $X^2 = 2.187$, d.f. = 1, $p = 0.139$ and Ur-Ur vs Ci-Ci, Fisher exact $p = 0.517$).
More importantly, if previous host experience positively affects the attention of female butterflies we would expect that the search for a specific host would be more effective in the treatments where they had just encountered the same host species as opposed to a different host. In Experiment 1 (figure 1a), there was a significant effect of treatment (Wald $X^2 = 12.70$, d.f. =3, $p = 0.005$) but not the order of host presentation (Wald $X^2 = 1.40$, d.f. =1, $p = 0.236$). When primed with *U. dioica*, *P. c-album* females found *U. dioica* faster than when primed with *S. caprea* (Ur-Ur vs. Sa-Ur, $\beta = 0.310$, $X^2 = 5.24$ d.f. =1, $e^\beta = 1.85$, $p = 0.02$), but no priming effect could be found in females searching for *S. caprea* (Sa-Sa vs. Ur-Sa, $\beta = 0.066$, $X^2 = 0.18$, d.f. = 1, $e^\beta = 1.14$, $p = 0.7$).

Experiment 2 (figure 1b) shows a similar pattern. Again, there was a significant effect of treatment (Wald $X^2 = 24.23$, d.f. = 6, $p = 0.0005$) but not the order of presentation (Wald $X^2 = 0.71$ d.f. = 1, $p = 0.398$). *U. glabra* was found faster when primed with the same host than when primed with *U. dioica* (Ul-Ul vs. Ur-Ul, $\beta = 0.482$, $X^2 = 6.79$, d.f. = 1, $e^\beta = 2.62$, $p = 0.009$). No other planned comparisons investigating priming in Experiment 2 were significant (Ur-Ur vs. Ul-Ur, $\beta = 0.140$, $X^2 = 0.97$, d.f. = 1, $e^\beta = 1.32$, $p = 0.3$; Ur-Ur vs. Ri-Ur, $\beta = -0.172$, $X^2 = 1.41$, d.f. = 1, $e^\beta = 0.71$, $p = 0.2$; Ri-Ri vs. Ur-Ri, $\beta = -0.180$, $X^2 = 0.80$, d.f. = 1, $e^\beta = 0.70$, $p = 0.4$).

In Experiment 3 (figure 1c) investigating the painted lady, *V. cardui*, while the sample sizes were quite low there was a significant effect of treatment (Wald $X^2 = 15.63$, d.f. =6, $p = 0.016$) but not the order of host presentation (Wald $X^2 = 1.20$, d.f. = 1, $p = 0.273$) or the experimental year (Wald $X^2 = 0.78$ d.f. =1, $p = 0.376$). A priming effect on *U. dioica* could
be seen as a previous encounter with *U. dioica* significantly increased detection compared
to a previous encounter with *C. arvense* (Ur-Ur vs. Ci-Ur, $\beta = -0.697$, $X^2 = 7.36$, d.f. = 1, $e^\beta = 0.25$, $p = 0.007$). Although there was a tendency towards significant priming on *C.

arvense* (Ci-Ci vs Ur-Ci, $\beta = 0.334$, $X^2 = 3.23$, d.f. = 1, $e^\beta = 1.95$, $p = 0.07$), no other planned comparisons of priming in *V. cardui* was significant (Ci-Ci vs. Pl-Ci, $\beta = 0.251$, $X^2 = 2$, d.f. = 1, $e^\beta = 1.65$, $p = 0.2$; Pl-Pl vs. Ci-Pl, $\beta = 0.013$, $X^2 = 0$, d.f. =1, $e^\beta = 1.03$, $p = 1$).

**DISCUSSION**

The main finding of this study is that butterflies can decrease host search times by priming their attention to a target host, shortly following a prior positive encounter. These findings provide additional support for the importance of behavioral factors in shaping the host range of phytophagous insects, and show that generalist butterflies can adjust their search behavior to compensate for the possible disadvantage of divided attention between multiple target hosts. However, the results also have some additional interesting implications. The data suggest that attentional priming does not happen to all hosts in the repertoire. In the comma (*P. c-album*), the lesser generalist of the pair, priming was found only in hosts that are highly preferred and/or with which they have a historically old relationship. The family Nymphalidae has a very long history of association with the “urticalean rosids” section of Rosales (Nylin et al. 2014), and this plant group is with high probability the ancestral host for the tribe Nymphalini, to which both study species belong (Janz et al. 2001; Nylin and Wahlberg 2008). Both urticalean rosids tested here with *P. c-album* (*U. dioica* and *U. glabra*) induced increased search efficacy for these hosts, whereas *S. caprea* and *R.
alpinum, did not (Figure 1ab). The probability to find the most recently colonized host R. alpinum was low, in fact especially when primed for it.

The data from the ‘broad-generalist’, the painted lady (V. cardui), suggest a similar pattern. Attentional priming was shown in search for the old and low ranked U. dioica, but not for the newly incorporated and low ranked P. lanceolata (see Celorio-Mancera et al. 2016). The search for C. arvense, the much-preferred host, was generally quite effective and the effect of priming was in the expected direction (Figure 1c). A possible priming on C. arvense cannot be ruled out as it could at least partly explain the very low probability of finding U. dioica after encountering C. arvense as priming host (Figure 1b). However, the age of the butterfly-host association seems to have the most explanatory power. Taken together, these data suggest that butterflies have more developed search mechanisms for older and sometimes more preferred hosts and suggest that butterflies may have evolved to perceive these hosts’ characteristics as more salient, more conspicuous, than traits of other hosts in their repertoire. This would also mean that the more salient hosts receive more attention both during priming and during host search, which could easily overshadow any potential attention towards less salient hosts.

It is possible that such overshadowing effects can explain the lack of evidence for attentional priming in the more recently colonized, less preferred hosts, and we might have gotten a different result if these hosts were contrasted with less salient hosts in the experiment. Such a possibility is interesting for the general understanding of host search mechanisms, but nevertheless the potential imbalances in host conspicuousness in our
experiment would also be present in nature and would most probably have similar consequences on the natural host search behavior. It can be noted from figure 1b that *P. c-album* females did not find *R. alpinum* as often as the other hosts, especially not after being primed with *R. alpinum*. This finding could reflect its only intermediate preferability as well as the relatively short time of association with this host. An additional reason for a low detection rate of a host after priming would be if females were risk spreading, and actively avoiding laying more than one egg at a time. There is no evidence of performance on *R. alpinum* being particularly variable in the laboratory (e.g. Nylin et al. 2015), yet, temporal and spatial fitness variation in the field due to climatic or other factors, such as risk of predation and parasitoid exposure, may also affect risk spreading in oviposition behavior (Thompson 1988).

The limited attention hypothesis suggests that benefits to attentional priming select for specialization (Dukas 2002). If our findings reflect a general pattern in butterflies and perhaps other phytophagous insects with similar search strategies, it would infer that specialization could relatively quickly and more easily occur on host species that the insect has a long prior historical relationship with. Thus, the priming effects shown here could be a mechanism that would ultimately benefit conservatism in insect-host associations, a pattern that has been shown to be true in butterflies at large (Ehrlich and Raven 1964; Janz and Nylin 1998). Of course, specialization towards relatively newer hosts also does occur, but is not as common (Janz et al. 2001, Nylin et al. 2015). In these cases, we would expect attentional priming to only be important later in the specialization process, after the
butterflies have already evolved specific search mechanisms and strong preference for these younger hosts.

As the comma, *P. c-album*, has a host repertoire that includes both herbs and trees, we were able to include a highly ranked tree (*U. glabra* in 2016, to complement the data from 2015 that showed that the medium ranked tree *S. caprea*, did not induce attentional priming when compared to the herb *U. dioica*. As the results show that the butterflies primed their attention to *U. glabra*, we could rule out the possibility that it was differences in search strategy based on the host growth-form that affected the behavior in the experimental setting. Thus, at least when presented in a similar way to herbs, an admittedly rather unnatural situation, the butterflies treated the trees in a similar way to herbs in our search experiment.

It was interesting to see that also the painted lady (*V. cardui*), a very opportunistic, migrating species with a very large host repertoire, showed the same patterns of attentional priming as the comma (*P. c-album*). As mentioned above, significant search effects of priming could be seen only for the historically old but not highly preferred *Urtica* host, but sample sizes were quite low. It would be interesting to see how general the priming effects are with respect to other hosts in their large host repertoire. However, this study and others (e.g. Stefanescu 1997; Janz 2005; Celorio-Mancera et al. 2016), clearly show that although the painted lady is an extreme generalist whose ability to use such a large host range allow it to migrate to novel areas with a completely different set of host species, it still has a
rather strong host preference hierarchy together with both physiological and behavioral search mechanisms that allow it to fine-tune its search towards some hosts at the expense of others.

Alongside the use of visual cues (Raucher 1978; Kelber 1999), recent evidence shows that butterflies may also use olfactory cues when locating host plants (Schäpers et al. 2015; Mozuraitis et al. 2016). We do not know to what extent the different modalities played a role in the present experiment, but both probably had some influence on the search of the butterflies. Although most studies on animal search and attention have been made in well-controlled visual settings, some evidence exists for similar attentional trade-offs also in olfactory search (Atema et al. 1980; Nams 1997; Cross and Jackson 2010), suggesting attentional priming also in this modality.

In conclusion, this study shows that the host search behavior of polyphagous butterflies may be affected by their previous exposure to a specific host, a priming event, in a way that enhances the search rate of that given host. This behavioral effect resembles the results of sequential priming and the formation of search images that have been studied in vertebrates (Bond 1983; Blough, 1989; 1991). Our data also suggests that a long evolutionary history of the butterfly–host association is of great importance for the priming to occur, possibly because of evolved attention to specific host cues. These results also suggest a behavioral mechanism that potentially can help explain the pattern of conservatism in insect-host associations.
REFERENCES


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Janz N. 2005. The relationship between habitat selection and preference for adult and larval food resources in the polyphagous butterfly Vanessa cardui (Lepidoptera: Nymphalidae) J Ins Behav, 18:767-780.


FIGURE LEGENDS

Figure 1. Survival plot showing the detection rate of experimental hosts as the proportion of butterflies still searching as a function of time (seconds). The graphs represent the search behavior of *P. c-album* when a) in Experiment 1, *U. dioica* (Ur) is contrasted with *S. caprea* (Sa), and b) in Experiment 2 *U. dioica* (Ur) is contrasted with *U. glabra* (Ul) and *R. alpinum* (Ri), and the search behavior of *V. cardui* when c) in Experiment 3, *U. dioica* (Ur) and *P. lanceolata* (Pl) were contrasted with *C. arvense* (Ci). The labels on the curves represent the treatments, showing priming host-experimental host pairs. Brackets highlight the planned pair-wise comparisons that differ significantly in the rate of host finding and asterisks represent the level of statistical significance of respective comparison (see text for details) where * = 0.01 < p ≤ 0.05, ** = 0.001 < p ≤ 0.01 and ° = 0.05 < p > 0.10 (NS).
Table 1. The growth form, relative ranking and approximate age of association with the orders of host plants used in the experiment for each species of butterfly

<table>
<thead>
<tr>
<th>Growth form</th>
<th>Rel. ranking*</th>
<th>Approx. age of association**</th>
</tr>
</thead>
</table>

**Polygonia c-album (the comma) hosts**

<table>
<thead>
<tr>
<th>Growth form</th>
<th>Rel. ranking*</th>
<th>Approx. age of association**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urticalean rosid</td>
<td>&gt;90 Ma</td>
<td></td>
</tr>
<tr>
<td><em>Urtica dioica (Ur)</em></td>
<td>herb</td>
<td>high</td>
</tr>
<tr>
<td><em>Ulmus glabra (Ul)</em></td>
<td>tree</td>
<td>high</td>
</tr>
<tr>
<td>Malphigales</td>
<td>&lt;11 Ma</td>
<td></td>
</tr>
<tr>
<td><em>Salix caprea (Sa)</em></td>
<td>tree</td>
<td>medium</td>
</tr>
<tr>
<td>Saxifragales</td>
<td>&lt;7 Ma</td>
<td></td>
</tr>
<tr>
<td><em>Ribes alpinum (Ri)</em></td>
<td>shrub</td>
<td>medium</td>
</tr>
</tbody>
</table>

**Vanessa cardui (the painted lady) hosts**

<table>
<thead>
<tr>
<th>Growth form</th>
<th>Rel. ranking*</th>
<th>Approx. age of association**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urticalean rosid</td>
<td>&gt;90 Ma</td>
<td></td>
</tr>
<tr>
<td><em>Urtica dioica (Ur)</em></td>
<td>herb</td>
<td>low</td>
</tr>
<tr>
<td>Asterales</td>
<td>&lt;20 Ma</td>
<td></td>
</tr>
<tr>
<td><em>Circium arvense (Ci)</em></td>
<td>herb</td>
<td>high</td>
</tr>
<tr>
<td>Lamiales</td>
<td>&lt;10.5 Ma</td>
<td></td>
</tr>
<tr>
<td><em>Plantago lanceolata (Pl)</em></td>
<td>herb</td>
<td>low</td>
</tr>
</tbody>
</table>

* e.g. see Nylin 1988 and Celorio-Manchera et al. 2016

** See text for references and a description of how the estimations of the approximate ages of association between the butterflies and the respective host plant orders was derived.
FIGURE 1

(a) Proportion still searching

(b) Proportion still searching

(c) Proportion still searching

Time searching (seconds)