



## Original Article

# Selective attention by priming in host search behavior of 2 generalist butterflies

Gabriella Gamberale-Stille<sup>✉</sup>, Alexander Schäpers, Niklas Janz, and Sören Nylin

Department of Zoology, Stockholm University, 10691 Stockholm, Sweden

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In phytophagous insects such as butterflies, there is an evolutionary trend toward 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 2 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 nonhost distractors) immediately after being primed with an oviposition experience of the same host as compared with 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 provides 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:** diet breadth, host plant, limited attention, priming, search behavior, specialization.

## 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 toward specialization in plant-feeding insects (Futuyma and Moreno 1988; Jaenike 1990; Forister et al. 2015). This is true 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 should 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; Agosta and Klemens 2009; Friberg and Wiklund 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 Scriber 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 a related specialist 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

Address correspondence to G. Gamberale-Stille. E-mail: [gabriella.gamberale@zoologi.su.se](mailto:gabriella.gamberale@zoologi.su.se)

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), whereby 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 (Rauscher 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 toward specific host species, which would result in a more effective search behavior. We use 2 polyphagous species, the comma (*Polygona 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 with a herb. Thus, these 3 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 highly ranked plant or a host with long evolutionary history.

## METHODS

The study consisted of 3 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 2 single-egg laying, relatively generalist species of Nymphalidae (Lepidoptera) butterflies. The comma butterfly (*P. c-album*) is polyphagous on a few families belonging to the orders Rosales (including urticalean rosids), Saxifragales, Fagales, and Malpighiales (Seppänen 1970) including trees, shrubs, and herbs, whereas the painted lady (*V. 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 3 experiments. They were chosen based on 3 criteria: the relative preference ranking, the relative age of the butterfly–host association (see separate section later) and the growth form. For *P. c-album*, we contrasted the highly ranked *Urtica dioica* with the lower ranked host *Salix caprea* in 2015 (Experiment 1), and in 2016 *U. dioica* was contrasted with the highly ranked *Ulmus glabra* and the lower ranked *Ribes alpinum* (Experiment 2, Table 1). The *V. cardui* females were presented with the highly ranked *Circium arvense* contrasted against the lower ranked *U. dioica* and *Plantago lanceolata* (Experiment 3, Table 1) in both years. Here, 2 years were 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 preference 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). *Vanessa 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

**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**

Host	Growth form	Relative ranking <sup>a</sup>	Approximate age of association <sup>b</sup>
<b><i>Polygonia c-album</i> (the comma) hosts</b>			
Urticalean rosid			
<i>Urtica dioica</i> (Ur)	Herb	High	>90 Ma
<i>Ulmus glabra</i> (Ul)	Tree	High	
Malpighiales			
<i>Salix caprea</i> (Sa)	Tree	Medium	<11 Ma
Saxifragales			
<i>Ribes alpinum</i> (Ri)	Shrub	Medium	<7 Ma
<b><i>Vanessa cardui</i> (the painted lady) hosts</b>			
Urticalean rosid			
<i>U. dioica</i> (Ur)	Herb	Low	>90 Ma
Asterales			
<i>Cirsium arvense</i> (Ci)	Herb	High	<20 Ma
Lamiales			
<i>Plantago lanceolata</i> (Pl)	Herb	Low	<10.5 Ma

<sup>a</sup>For example, see Nylin (1988) and Celorio-Manchera et al. (2016).

<sup>b</sup>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.

selection in *P. c-album* (Janz et al. 2009), and given the high mobility and migratory behavior 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 cm × 52 cm × 48 cm (width × length × 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 2–3 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 *U. dioica* (Urticaceae) and *U. 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 million years ago (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. 2014). 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 *S. 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 2 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).

*Cirsium arvense* (Asteraceae) used by *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 subsection of the tribe Melitaeni (Nylin and Wahlberg 2008) and by *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 *Vanessa* at about 20 Ma (Chazot et al. 2018).

The final host *P. 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 *Vanessa* is a separate colonization, and the order is probably used only by the

most polyphagous species in the genus: *V. cardui* and *V. virginensis*. This still puts the age of the association at  $<10.5$  Ma if these are not independent events (dating from Wahlberg and Rubinoff 2011). However, use of the genus *Plantago* seems to be unique to *V. cardui* in the genus and is thus a considerably younger association.

## Arena

The experiments took place in 2 larger cages that measured 80 cm × 80 cm × 50 cm (width × length × 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 cutoff plants placed in bottles. There were 12 nonhosts, 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 min. 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 min, the trial was repeated after a while with the same individual female. If a female showed search behavior at some point during the 10 min, that is, 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, Ur-Ur, Ul-Ur, Ri-Ur, Ri-Ri, and Ur-Ri. In Experiment 3 (2015 and 2016), *V. 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 to 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,  $N = 45$ ; and 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,  $N = 41$ ; and 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,  $N = 21$  and Ci-Pl,  $N = 25$ .

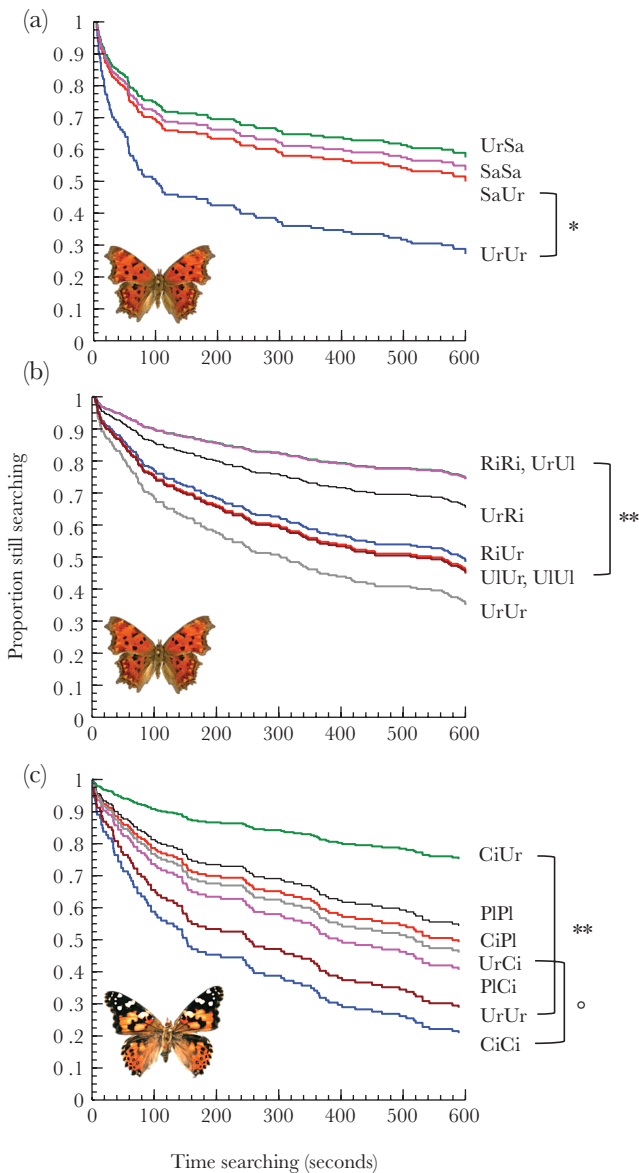
We noted whether a host was found or not during the whole trial and the time to finding the host. We first compared the tendency to find a certain host between host species by comparing found or not found frequencies using contingency tables (2-tailed Pearson's goodness of fit chi-square or Fisher's exact tests when necessary).

The detection time data included right-censored data: a butterfly that found the host during the 600 s 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. Presentation order (Experiments 1–3) and year (Experiment 3) were included as covariates in the models for each experiment. We also conducted a priori decided pairwise contrasts within the limits of degrees of freedom, to compare the specific treatments relevant when testing for priming effects. Presentation order and year were excluded from these tests, being nonsignificant in the complete models.

## 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, that is, 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 with the lower ranked and relatively younger hosts *S. caprea* (Ur-Ur: 34/48 vs. Sa-Sa: 21/45)  $\chi^2 = 5.613$ ,  $df = 1$ ,  $P = 0.018$ ). Similarly in Experiment 2, *U. dioica* was more easily found than *R. alpinum* (Ur-Ur: 27/42 vs. Ri-Ri: (11/41),  $\chi^2 = 11.726$ ,  $df = 1$ ,  $P = 0.00062$ ). Also the highly ranked and old *U. glabra* was found significantly more frequently than *R. alpinum* (Ul-Ul: 22/41 vs. Ri-Ri,  $\chi^2 = 6.136$ ,  $df = 1$ ,  $P = 0.013$ ) whereas there was no significant difference between *U. dioica* and *U. glabra* ( $\chi^2 = 0.969$ ,  $df = 1$ ,  $P = 0.324958$ ). In Experiment 3, fewer *V. cardui* females found the lower ranked *P. lanceolata* as compared with the higher ranked *C. arvensis* (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 with either of the other hosts (Ur-Ur: 16/23 vs. Pl-Pl  $\chi^2 = 2.187$ ,  $df = 1$ ,  $P = 0.139$  and Ur-Ur vs. Ci-Ci, Fisher exact  $P = 0.517$ ).

More importantly, if previous host experience positively affects the attention of female butterflies through priming 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 in the complete model (Wald  $\chi^2 = 12.70$ ,  $df = 3$ ,  $P = 0.005$ ) but not of the order of host presentation (Wald  $\chi^2 = 1.40$ ,  $df = 1$ ,  $P = 0.236$ ). When primed with *U. dioica*, *P. c-album* females found *U. dioica* faster than when primed with *S. caprea* (Ur-Ur vs. Sa-Ur,  $\beta = 0.310$ ,  $\chi^2 = 5.24$ ,  $df = 1$ ,  $e^\beta = 1.85$ ,  $P = 0.02$ ), but no priming effect could be found



**Figure 1**

Survival plot showing the detection rate of experimental hosts as the proportion butterflies still searching as a function of time (seconds). The graphs represent the search behavior of *Polygonia c-album* when (a) in Experiment 1, *Urtica dioica* (Ur) is contrasted with *Salix caprea* (Sa), and (b) in Experiment 2, *U. dioica* (Ur) is contrasted with *Ulmus glabra* (Ul) and *Ribes alpinum* (Ri), and the search behavior of *Vanessa cardui* when (c) in Experiment 3, *U. dioica* (Ur) and *Plantago lanceolata* (Pl) were contrasted with *Cirsium arvense* (Ci). The labels on the curves represent the treatments, showing priming host-experimental host pairs. Brackets highlight the planned pairwise 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  $*0.01 < P \leq 0.05$ ,  $**0.001 < P \leq 0.01$  and  $^{\circ}0.05 < P > 0.10$  (NS).

in females searching for *S. caprea* (Sa-Sa vs. Ur-Sa,  $\beta = 0.066$ ,  $\chi^2 = 0.18$ ,  $df = 1$ ,  $e^{\beta} = 1.14$ ,  $P = 0.7$ ).

Experiment 2 (Figure 1b) shows a similar pattern. Again, there was a significant effect of treatment (Wald  $\chi^2 = 24.23$ ,  $df = 6$ ,  $P = 0.0005$ ) but not the order of presentation (Wald  $\chi^2 = 0.71$ ,  $df = 1$ ,  $P = 0.398$ ). *Ulmus glabra* was found faster when primed

with the same host than when primed with *U. dioica* (Ul-Ul vs. Ur-Ul,  $\beta = 0.482$ ,  $\chi^2 = 6.79$ ,  $df = 1$ ,  $e^{\beta} = 2.62$ ,  $P = 0.009$ ). No other planned comparisons investigating priming in Experiment 2 were significant (Ur-Ur vs. Ul-Ur,  $\beta = 0.140$ ,  $\chi^2 = 0.97$ ,  $df = 1$ ,  $e^{\beta} = 1.32$ ,  $P = 0.3$ ; Ur-Ur vs. Ri-Ur,  $\beta = -0.172$ ,  $\chi^2 = 1.41$ ,  $df = 1$ ,  $e^{\beta} = 0.71$ ,  $P = 0.2$ ; Ri-Ri vs. Ur-Ri,  $\beta = -0.180$ ,  $\chi^2 = 0.80$ ,  $df = 1$ ,  $e^{\beta} = 0.70$ ,  $P = 0.4$ ).

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  $\chi^2 = 15.63$ ,  $df = 6$ ,  $P = 0.016$ ) but not the order of host presentation (Wald  $\chi^2 = 1.20$ ,  $df = 1$ ,  $P = 0.273$ ) or the experimental year (Wald  $\chi^2 = 0.78$ ,  $df = 1$ ,  $P = 0.376$ ). A priming effect on *U. dioica* could be seen as a previous encounter with *U. dioica* significantly increased detection compared with a previous encounter with *C. arvense* (Ur-Ur vs. Ci-Ur,  $\beta = -0.697$ ,  $\chi^2 = 7.36$ ,  $df = 1$ ,  $e^{\beta} = 0.25$ ,  $P = 0.007$ ). Although there was a tendency toward significant priming on *C. arvense* (Ci-Ci vs. Ur-Ci,  $\beta = 0.334$ ,  $\chi^2 = 3.23$ ,  $df = 1$ ,  $e^{\beta} = 1.95$ ,  $P = 0.07$ ), no other planned comparisons of priming in *V. cardui* was significant (Ci-Ci vs. Pl-Ci,  $\beta = 0.251$ ,  $\chi^2 = 2$ ,  $df = 1$ ,  $e^{\beta} = 1.65$ ,  $P = 0.2$ ; Pl-Pl vs. Ci-Pl,  $\beta = 0.013$ ,  $\chi^2 = 0$ ,  $df = 1$ ,  $e^{\beta} = 1.03$ ,  $P = 1$ ).

## 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 (Nylín 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; Nylín 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 1a,b). 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* (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, that is, 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 toward 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 1 egg at a time on the same host. There is no evidence of performance on *R. alpinum* being particularly variable in the laboratory (e.g., Nylín 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 Nylín 1998). Of course, specialization toward relatively newer hosts also does occur, but is not as common (Janz et al. 2001; Nylín 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 with 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 previously, 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 toward 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.

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## REFERENCES

- Agosta SJ, Klemens JA. 2009. Resource specialization in a phytophagous insect: no evidence for genetically based performance trade-offs across hosts in the field or laboratory. *J Evol Biol.* 22:907–912.
- Atema J, Holland K, Ikehara W. 1980. Olfactory responses of yellowfin tuna (*Thunnus albacares*) to prey odors: chemical search image. *J Chem Ecol.* 6:457–465.
- Bernays EA. 2001. Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annu Rev Entomol.* 46:703–727.
- Bernays EA, Funk DJ. 1999. Specialists make faster decisions than generalists: experiments with aphids. *Proc Biol Sci.* 266:151–156.
- Blough DS. 1991. Selective attention and search images in pigeons. *J Exp Psych Anim Behav Proc.* 1:3–21.
- Blough PM. 1989. Attentional priming and visual search in pigeons. *J Exp Psychol Anim Behav Process.* 15:358–365.
- Bond AB. 1983. Visual search and selection of natural stimuli in the pigeon: the attention threshold hypothesis. *J Exp Psychol Anim Behav Process.* 9:292–306.

- Carriere Y, Roitberg BD. 1994. Trade-offs in responses to host plants within a population of a generalist herbivore, *Choristoneura rosaeana*. *Entomol Exp Appl*. 72:173–180.
- Celorio-Manchera MD, Weet CW, Huss M, Vessi E, Neethiraj R, Reimegård J, Nylin S, Janz N. 2016. Evolutionary history of host use, rather than plant phylogeny, determines gene expression in a generalist butterfly. *BMC Evol Biol*. 16:59.
- Chazot N, Wahlberg N, Freitas AV, Mitter C, Labandeira C, Sohn J-C, Sahoo RK, Seraphim N, de Jong R, Heikkilä M. 2018. The trials and tribulations of priors and posteriors in Bayesian timing of divergence analyses: the age of butterflies revisited. *bioRxiv*. doi:10.1101/259184.
- Courtney SP. 1983. Models of hostplant location by butterflies: the effect of search images and search efficiency. *Oecologia*. 59:317–321.
- Courtney SP, Chen GK, Gardner A. 1989. A general model for individual host selection. *Oikos*. 55:55–65.
- Cox DR. 1972. Regression models and life-tables. *J R Stat Soc*. 34:187–220.
- Cross FR, Jackson RR. 2010. Olfactory search-image use by a mosquito-eating predator. *Proc Biol Sci*. 277:3173–3178.
- Dall SRX, Cuthill IC. 1997. The information costs of generalism. *Oikos*. 80:197–202.
- Dethier VG. 1954. Evolution of feeding preferences in phytophagous insects. *Evolution*. 8:33–54.
- Dukas R. 2002. Behavioural and ecological consequences of limited attention. *Philos Trans R Soc Lond B Biol Sci*. 357:1539–1547.
- Dukas R, Kamil AC. 2001. Limited attention: the constraint underlying search image. *Behav Ecol*. 12:192–199.
- Egan SP, Funk DJ. 2006. Individual advantages to ecological specialization: insights on cognitive constraints from three conspecific taxa. *Proc Biol Sci*. 273:843–848.
- Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution*. 18:586–608.
- Forister ML, Novotny V, Panorska AK, Baje L, Basset Y, Butterill PT, Cizek L, Coley PD, Dem F, Diniz IR, et al. 2015. The global distribution of diet breadth in insect herbivores. *Proc Natl Acad Sci U S A*. 112:442–447.
- Fox CW, Caldwell RL. 1994. Host-associated fitness trade-offs do not limit the evolution of diet breadth in the small milkweed bug *Lygaeus kalmii* (Hemiptera: Lygaeidae). *Oecologia*. 97:382–389.
- Friberg M, Wiklund C. 2009. Host plant preference and performance of the sibling species of butterflies *Leptidea sinapis* and *Leptidea reali*: a test of the trade-off hypothesis for food specialisation. *Oecologia*. 159:127–137.
- Futuyma DJ. 1983. Selective factors in the evolution of host choice by phytophagous insects. In: Ahmad S, editor. *Herbivorous insects: hosts seeking behavior and mechanisms*. New York: Academic Press. p. 227–244. doi:10.5061/dryad.9t4471k
- Futuyma DJ, Moreno G. 1988. The evolution of ecological specialization. *Ann Rev Ecol Syst*. 19:207–233.
- Futuyma DJ, Philippi TE. 1987. Genetic variation and covariation in responses to host plants by *Alsophila pometaria* (Lepidoptera: geometridae). *Evolution*. 41:269–279.
- Gamberale-Stille G, Schäpers A, Janz N, Nylin S. 2018. Data from: selective attention by priming in host search behavior of two generalist butterflies. Dryad Digital Repository. <http://doi.org/10.5061/dryad.9t4471k>.
- Gompert Z, Jahner JP, Scholl CF, Wilson JS, Lucas LK, Soria-Carrasco V, Fordyce JA, Nice CC, Buerkle CA, Forister ML. 2015. The evolution of novel host use is unlikely to be constrained by trade-offs or a lack of genetic variation. *Mol Ecol*. 24:2777–2793.
- Gripenberg S, Mayhew PJ, Parnell M, Roslin T. 2010. A meta-analysis of preference–performance relationships in phytophagous insects. *Ecol Lett*. 13:383–393.
- Hopper KR. 1999. Risk-spreading and bet-hedging in insect population biology. *Annu Rev Entomol*. 44:535–560.
- Jaenike J. 1990. Host specialization in phytophagous insects. *Ann Rev Ecol Syst*. 21:243–273.
- Janz N. 2003. The cost of polyphagy: oviposition decision time vs. error rate in a butterfly. *Oikos*. 100:493–496.
- 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.
- 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. *Proc R Soc Lond B*. 264:701–707.
- Janz N, Nylin S. 1998. Butterflies and plants: a phylogenetic study. *Evolution*. 52:486–502.
- Janz N, Nyblom K, Nylin S. 2001. Evolutionary dynamics of host–plant specialization: a case study of the tribe Nymphalini. *Evolution*. 55:783–796.
- Janz N, Söderlind L, Nylin S. 2009. No effect of larval experience on adult host preferences in *Polygonia c-album* (Lepidoptera: Nymphalidae): on the persistence of Hopkins' host selection principle. *Ecol Entomol*. 34:50–57.
- Johansson J, Bergström A, Janz N. 2007. The benefit of additional oviposition targets for a polyphagous butterfly. *J Insect Sci*. 7:3.
- Kelber A. 1999. Ovipositing butterflies use a red receptor to see green. *J Exp Biol*. 202(Pt 19):2619–2630.
- Langley CM. 1996. Search images: selective attention to specific visual features of prey. *J Exp Psychol Anim Behav Process*. 22:152–163.
- Lehnert MS, Scriber JM. 2012. Salicaceae detoxification abilities in Florida tiger swallowtail butterflies (*Papilio glaucus maynardi* Gauthier): novel ability or Pleistocene holdover? *Ins Sci*. 19:337–345.
- Levins R, MacArthur RH. 1969. An hypothesis to explain the incidence of monophagy. *Ecology*. 50:910–911.
- Mackenzie A. 1996. A trade-off for host plant utilization in the black bean aphid, *Aphis fabae*. *Evolution*. 50:155–162.
- Mozuraitis R, Radziute S, Apegaite V, Cravencenko A, Buda V, Nylin S. 2016. Volatiles released from foliar extract of host plant enhance landing rates of gravid *Polygonia c-album* females, but do not stimulate oviposition. *Entomol Exp Appl*. 158:275–283.
- Nams VO. 1997. Density-dependent predation by skunks using olfactory search images. *Oecologia*. 110:440–448.
- Nylin S. 1988. Host plant specialization and seasonality in a polyphagous butterfly, *Polygonia c-album* (Nymphalidae). *Oikos*. 53:381–386.
- Nylin S. 1989. Effects of changing photoperiods in the life cycle regulation of the comma butterfly, *Polygonia c-album* (Nymphalidae). *Ecol Entomol*. 14:209–218.
- Nylin S, Slove J, Janz N. 2014. Host plant utilization, host range oscillations and diversification in nymphalid butterflies: a phylogenetic investigation. *Evolution*. 68:105–124.
- Nylin S, Söderlind L, Gamberale-Stille G, Audesseau H, Celorio-Manchera MD, Janz N, Sperling FAH. 2015. Vestiges of an ancestral host plant: preference and performance in the butterfly *Polygonia faunus* and its sister species *P. c-album*. *Ecol Entomol*. 40:307–315.
- Nylin S, Wahlberg N. 2008. Does plasticity drive speciation? Host-plant shifts and diversification in nymphaline butterflies (Lepidoptera: Nymphalidae) during the Tertiary. *Biol J Linn Soc*. 94:115–130.
- Papaj DR. 1986. Conditioning of leaf-shape discrimination by chemical cues in the butterfly, *Battus philenor*. *Anim Behav*. 34:1281–1288.
- Rauscher MD. 1978. Search image for leaf shape in a butterfly. *Science*. 200:1071–1073.
- Reid PJ, Shettleworth SJ. 1992. Detection of cryptic prey: search image or search rate? *J Exp Psychol Anim Behav Process*. 18:273–286.
- Schäpers A, Carlsson MA, Gamberale-Stille G, Janz N. 2015. The role of olfactory cues for the search behaviour of a specialist and generalist butterfly. *J Ins Behav*. 28:77–87.
- Schäpers A, Nylin S, Carlsson MA, Janz N. 2016. Specialist and generalist oviposition strategies in butterflies: maternal care or precocious young? *Oecologia*. 180:335–343.
- Scott JA. 1986. *The butterflies of North America*. Stanford, CA: Stanford University Press.
- Seppänen EJ. 1970. Suurperhostoukkien ravintokasvit. (The food-plants of the larvae of the Macrolepidoptera of Finland). *Animalia fennica* 14. Porvoo-Helsinki: Werner Söderström.
- Stanton ML. 1984. Short-term learning and the searching accuracy of egg-laying butterflies. *Anim Behav*. 32:33–40.
- Stefanescu C. 1997. Migration patterns and feeding resources of the painted lady butterfly, *Cynthia cardui* (L.) (Lepidoptera, Nymphalidae) in the northeast of the Iberian peninsula. *Misc Zool*. 20:31–48.
- Thompson JN. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol Exp Appl*. 47:3–14.
- Timbergen N. 1960. The natural control of insects in pine woods: vol. I. Factors influencing the intensity of predation by songbirds. *Arch Neerl Zool*. 13:265–343.
- Tosh CR, Krause J, Ruxton GD. 2009. Theoretical predictions strongly support decision accuracy as a major driver of ecological specialization. *Proc Natl Acad Sci U S A*. 106:5698–5702.
- Via S, Hawthorne DJ. 2002. The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. *Am Nat*. 159(Suppl 3):S76–S88.

- Wahlberg N, Leneveu J, Kodandaramaiah U, Peña C, Nylin S, Freitas AV, Brower AV. 2009. Nymphalid butterflies diversify following near demise at the Cretaceous/Tertiary boundary. *Proc Biol Sci.* 276:4295–4302.
- Wahlberg N, Rubinoff D. 2011. Vagility across *Vanessa* (Lepidoptera: Nymphalidae): mobility in butterfly species does not inhibit the formation and persistence of isolated sister taxa. *Syst Entomol.* 36: 362–370.
- Weingartner E, Wahlberg N, Nylin S. 2006. Dynamics of host plant use and species diversity in *Polygonia* butterflies (Nymphalidae). *J Evol Biol.* 19:483–491.
- Wiklund C. 1975. The evolutionary relationship between adult oviposition preferences and larval host plant range in *Papilio machaon* L. *Oecologia.* 18:185–197.
- Wiklund C, Friberg M. 2009. The evolutionary ecology of generalization: among-year variation in host plant use and offspring survival in a butterfly. *Ecology.* 90:3406–3417.