Search Behavior and Selection of Innate Chemosensory Cues by Cabbage White (Pieris rapae) Larvae

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Abstract
Chemoreception of the cues that allow a caterpillar to locate and select a specific host plant reflects evolutionary constraints. In a stable environment, the evolution of innate preferences for specific host plants can increase feeding efficiency through stimulus filtering in a noisy environment. However, food choice plasticity, including the learning of new food cues, can allow survival when a population is faced with a changing environment. We used the caterpillars of cabbage whites, Pieris rapae, to test the hypothesis that preference for innate food cues would be stronger than for learned food cues. P. rapae caterpillars have sensitivity to a sugar compound, gluconasturtiin, found in their host plants, family Brassicaceae, which allows for search specificity to members of this family. We offered caterpillars, both in pairs and individually, choices between artificial food on which they had previously been reared (a learned cue) and kale, Brassica oleraceae, (an innate cue). Caterpillars grouped in pairs did not demonstrate a significant preference for either choice, although the first caterpillar in each pair to select a food item chose the innate cue of kale. Caterpillars tested individually showed a significant preference for the innate cue of kale, which persisted with experience. These individual results support our hypothesis that cabbage whites primarily use innate cues when choosing a food source. The presence of other caterpillars, however, may affect that choice, as observed in the initial group trials.

Keywords
prey choice, Lepidoptera, host specificity

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Chemoreception of the cues that allow a caterpillar to locate and select a specific host plant reflects evolutionary constraints. In a stable environment, the evolution of innate preferences for specific host plants can increase feeding efficiency through stimulus filtering in a noisy environment. However, food choice plasticity, including the learning of new food cues, can allow survival when a population is faced with a changing environment. We used the caterpillars of cabbage whites, Pieris rapae, to test the hypothesis that preference for innate food cues would be stronger than for learned food cues. P. rapae caterpillars have sensitivity to a sugar compound, gluconasturtiin, found in their host plants, family Brassicaceae, which allows for search specificity to members of this family. We offered caterpillars, both in pairs and individually, choices between artificial food on which they had previously been reared (a learned cue) and kale, Brassica oleracea, (an innate cue). Caterpillars grouped in pairs did not demonstrate a significant preference for either choice, although the first caterpillar in each pair to select a food item chose the innate cue of kale. Caterpillars tested individually showed a significant preference for the innate cue of kale, which persisted with experience. These individual results support our hypothesis that cabbage whites primarily use innate cues when choosing a food source. The presence of other caterpillars, however, may affect that choice, as observed in the initial group trials.

Keywords: prey choice, Lepidoptera, host specificity

Introduction

An insect must be able to filter complex stimuli to identify and locate food sources, mates, and ovipository sites, via visual, chemical, or mechanosensory cues in a large and noisy environment (Schäpers et al., 2015). Chemoreception is an important factor in an animal's umwelt, or sensory world, and is hypothesized to be one of the earliest types of animal perception to have evolved (Wicher, 2012). Chemoreception involves a recognized chemical signal in either an airborne (olfaction) or aquatic (gustation) medium that reacts with a receptor on a particular sensory structure; this reaction then allows for a behavioral response to be initiated (Schäpers et al., 2015). Odor-mediated responses – behavioral reactions that are evoked by chemical cues – occur in about 10-100 milliseconds in response to changes in concentration of odor plumes (Chapman, 2003).

Lepidopterans (butterflies) are insects that undergo the complex transformation known as metamorphosis, during which the organism goes through extensive developmental and physiological changes via cell differentiation and growth. In order for caterpillars, the larval stage of a butterfly’s life cycle, to become ready to metamorphose, they need to gain the appropriate nutrition to undergo an energetically expensive process. This requires them to have the ability to quickly recognize and act on the appropriate cues at the appropriate times.

Most ovipositing adult female butterflies are known to use olfaction in detecting a host-specific source to oviposit eggs (Schäpers et al., 2015). Ovipositing adult female butterflies use chemoreception of a specific chemical cue to locate the host plant on which to leave their eggs. It can then be difficult to determine if and how larvae also use chemoreception to identify the host plant on which they begin their life cycle (Miles et al., 2004). An understanding of chemosensory behavior in lepidopterans requires examination of each stage of the life cycle.

Pieris rapae caterpillars are sensitive to the sugar compound glucosinolate gluconasturtiin (phenylethylglucosinolate), which is found in the family Brassicaceae (Cruciferae). The common name of the cabbage white butterfly is due to its affinity for agricultural species Brassica oleracea (cauliflower, kale, and broccoli). P. rapae larvae and gravid butterflies use glucosinolates to recognize cues to locate food sources or an ovipository source, respectively (Miles et al., 2004). This sugar compound allows the P. rapae larvae to find host plants in an efficient manner despite a noisy

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environment. A set of taste sensilla styloconica located on the *P. rapae* larvae mouthparts are especially sensitive to the gluconasturtiin. *P. rapae* larvae have been observed to react behaviorally and neurophysiologically to this specific compound (Miles *et al.*, 2004).

Improved and increased efficiency in finding and locating the proper food allows for rapid decision-making (Schäpers *et al.*, 2015). Being able to quickly filter stimuli to detect a specific food source greatly helps the caterpillar’s ability to gain the necessary nutrition and energy requirements before metamorphosis. This specificity also reduces the chance of the caterpillar selecting the “wrong” food source and risking the chance of death via starvation, toxicity, or predation (Miles *et al.*, 2004). When the host-specific *P. rapae* larvae were oviposited upon a noncruciferous plant, nasturtium (*Tropaeolum majus*, Tropaeolaceae), the larvae did not consume it and ended up starving to death (Ma, 1972; Renwick and Huang, 1995; Miles *et al.*, 2004).

Like other insects, *P. rapae* have developed decision-making skills via generations of adaptive anatomical and physiological changes in their chemosensory organs (Reuven, 2008). However, the search behavior manifested by insects is still unclear because there are so many search modalities in each ecological context that allows the insect to locate its target (Schäpers *et al.*, 2015). When selective pressures change, a shift in host plant specificity may also be observed. For example, monarch butterflies, *Danaus plexippus* are not native to the Hawaiian Islands. Via accidental transport or release by humans, a transported population adapted to the new environment. Monarchs are highly plant-specific during their larval stage and only eat milkweed, *Asclepias syriaca*. Although Hawai’i does not have milkweed, plasticity in the new population allowed for the consumption of a similar plant (crown flower, *Calotropis gigantea*) that provides relatively the same nutritional and survival value as the original host plant. This behavioral adaptation allowed for *D. plexippus* to use a plant that contained a similar sensory cue and nutritional value as *A. syrica* and allowed the survival of the Hawaiian *D. plexippus* population (Comstock, 1966; Zalucki and Clarke, 2004). Monarchs typify the crucial life history trade offs faced by animals. Specialists are more efficient in finding their particular food source in a noisy environment, but their survival may be constrained if that food source becomes limited or unavailable. Over evolutionary time in a reasonably stable environment, innate preferences are expected to be the optimal strategy. If there is a drastic change to the environment and phenotypic plasticity allowing for the learning of new cues is lacking, then the population would not be able to adapt and survive.

Learning therefore may also be relevant to understanding why an animal chooses one food over another, rather than wholly relying on innate cues. Herbaceous insect food preferences can change after a feeding experience, and the repeated selection of previously chosen plants increases significantly (Bernays and Weiss, 1996). Two main types of learning can influence preference. Imprinting occurs when an organism experiences a sensory stimulus at a crucial point in development, and demonstrates a positive long-lasting response in absence of any pre-existing neural mechanisms or continued stimulus. Associative learning occurs when a stimulus repeatedly becomes associated with a previously unrelated stimulus (Bernays and Weiss, 1996). These two learning concepts may influence food preference when *P. rapae* larvae are reared on an artificial food source containing the same nutritional compounds found in cruciferous plants.

Our goal was to determine if the behavior and food preference of *P. rapae* larvae is determined by innate or learned cues. We hypothesized that innate cues have a stronger influence on *P. rapae* caterpillar food selection behavior due to the evolutionary pressure of host-plant specificity. We predicted that if *P. rapae* larvae reared on an artificial food source are presented a choice between that artificial food and a cruciferous plant as a food source, then the caterpillars would demonstrate higher affinity to the cruciferous plant (innate cue) than the artificial food (learned cue) in which they were reared.

**Methods**

**Study organisms.** All *Pieris rapae* larvae were supplied by Carolina Biological Supply Company®. These larvae were hatched and reared on Carolina® caterpillar food. Prior to experimentation, larvae were housed in the containers in which they were shipped, which included a stock of the artificial food upon which they had been reared. Immediately prior to experimentation, larvae were deprived of food for one hour in a clean container. This was to ensure hunger and to eliminate prior alternative volatile scent cues that could influence choice in the trials. Following experimentation, larvae were placed into clean containers containing the Carolina® caterpillar food.

**Initial group trials.** We first wanted to determine if it would be appropriate to test caterpillar food preference in groups or individually and to establish an appropriate
time for the experimental testing period. We engaged in an initial set of trials, in which pairs of caterpillars were presented a choice of food and observed for 60 minutes.

This first set of trials and all subsequent trials consisted of experimental arenas crafted from Tupperware® brand 32 quart tubs. These experimental containers measured 58.4 cm long x 41.3 cm wide x 15.2 cm deep. Food choices were placed at each end of the tub, 3.5 cm from the end and 10.5 cm from each side. We massed 1.05 grams of kale (innate cue) and artificial food (learned cue) for this first set of trials. The artificial food used in each trial was taken from the container in which the caterpillar had been housed to ensure the consistency of the learned scents to the cue offered. Caterpillars were placed in the middle of the tub, 16.5 cm from each food choice (Figure 1). Each caterpillar was oriented so that its anterior end faced 90 degrees away from either food choice to reduce the influence of food volatiles in odor plumes.

After 60 minutes of food deprivation, pairs of caterpillars who had been housed together were placed into the testing arena and observed continuously for 60 minutes. The time it took for the caterpillars to reach a food source was recorded. If a caterpillar had not reached a stimulus after 60 minutes, we recorded the orientation of the caterpillar to a food source when the anterior end of the caterpillar was pointing to that food source at an angle of less than 90 degrees and it was on the same side of the arena as that food source. We recorded contact if a caterpillar physically touched the other caterpillar, and following if a caterpillar was within 5 centimeters and its anterior end was pointed at the other caterpillar at angle of less than 90 degrees.

Following each trial, the caterpillars were placed in a clean container containing artificial food. Each experimental container was wiped with soap and water prior to the next trial to eliminate scent cues from the previous trials.

**Individual Choice Trials.** We began with a new set of naïve caterpillars to start the individual choice trials. These individual trials eliminated the potential for behavioral interactions that might influence food choice when more than one caterpillar was present. These trials also used caterpillars acquired from Carolina Biological Supply Company®. Each individual caterpillar was first placed in a clean empty container for 60 minutes previous to its experimental trial to allow it to acclimate, become hungry and to eliminate prior alternative volatiles from its housing that might influence choice in the trials.

We used the same experimental set up established in the initial group trials, but food items were massed to 1.50 grams, which was an easier measurement to make precisely. The artificial food used in each trial was taken from the container in which the caterpillar had been housed. Individual caterpillars were placed in the middle of the tub, 16.5 cm from each food choice. Each caterpillar was oriented 90 degrees away from either food choice.

Each caterpillar was observed continuously for 30 minutes (the observational period was determined by previous group effects trials; once caterpillars chose a food source they did not change preference during a one-hour trial). The time it took the caterpillar to reach a food source was recorded. If the caterpillar did not select a food choice during the course of the thirty-minute period, then orientation was determined and measured in the same way as in the initial group trials.

Following each trial, the arena was wiped with soap and water to eliminate scent cues. Each caterpillar was returned to a clean container containing artificial food. As trials continued, experienced caterpillars were added to this container and maintained together. After all caterpillars had been tested once, we began a second set of trials to determine if experience might influence choice. Ultimately, three sets of trials with the same caterpillars were conducted to determine if experience over time influenced choice preference.

**Statistical analyses.** $X^2$ tests were used to test the distribution of caterpillars on the food choices in each trial. We assumed a null distribution of evenly assorted caterpillars for all choice options. Because in each trial there were some individuals that did not choose food, we
examined choice using several different \( \chi^2 \) tests: (1) \( \chi^2 \) test of caterpillars selecting each food source; (2) \( \chi^2 \) test of caterpillars selecting each food or undecided (if they did not arrive at a choice by the end of the testing period); and (3) \( \chi^2 \) test of caterpillars selecting each food choice with positive taxis included as indicative of selection.

For the initial group trials, we also compared the time it took caterpillars to select either the innate choice of kale or the learned choice of artificial food using an unpaired, two-tailed t-test. To contrast the food selection behavior with increasing experience in the individual trials, we considered repeated measure of Analysis of Variance (ANOVA), but as we did not maintain individual caterpillar identification we determined this test would not be appropriate. We did compare the mean time it took caterpillars to select the host food in all three trials within a 95% confidence interval to examine variation with experience. We used mean time to selection of kale as the measurement for this analysis, as our hypothesis predicted \( P. \) rapae larvae would select kale as their preferred food.

### Results

#### Initial group trials. Caterpillars that were grouped together in pairs did interact with one another in three out of six trials and in all but one trial they asserted themselves to opposite ends of the testing arena. \( \chi^2 \) results did not show a significant difference \((p = 0.11)\) between the innate cues offered by kale and the learned cues offered by the artificial food (Table 1). In the five trials in which both caterpillars selected food, kale was always the first food selected. The average time to selection of kale was 7 minutes and 44 seconds and the average time to selection of artificial food was 18 minutes and 42 seconds. This difference was not significant (unpaired two-tailed t-test \( n = 8, t = 1.78, p = 0.12 \)). In one trial, neither caterpillar selected food, but they were observed moving to opposite ends of the testing arena.

<table>
<thead>
<tr>
<th>Statistical comparison</th>
<th>Choice</th>
<th>Initial group trial</th>
<th>Individual trial 1</th>
<th>Individual trial 2</th>
<th>Individual trial 3</th>
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<tr>
<td>Innate, learned, or undecided</td>
<td>Kale</td>
<td>4</td>
<td>15</td>
<td>14</td>
<td>13</td>
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<tr>
<td></td>
<td>Artificial food</td>
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<td>2</td>
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<td>5</td>
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<td>14</td>
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<tr>
<td></td>
<td>Artificial food</td>
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<td>15</td>
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<tr>
<td></td>
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<td>4</td>
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<td>0.0028</td>
<td>0.0046</td>
<td>0.0495</td>
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</table>

Table 1. Results of \( \chi^2 \) tests of distribution for caterpillars in grouped trials and individual trials. Null hypotheses assumed equivalent distribution between both choices, or between both choices and indecision. Caterpillars in individual trial 1 were not previously experienced with testing conditions. Caterpillars in individual trial 2 and 3 had increasing levels of experience with testing conditions. One caterpillar died between Individual trial 1 and Individual trial 2.

#### Individual trials. Because caterpillars were observed to interact with one another and to assert to opposite ends of the testing arena, we were concerned that social interactions could influence prey choice. We decided to examine their prey choice individually. Individual trials were significant in all three methods of choice determination (Table 1). In naïve caterpillars, kale was chosen significantly over artificial food or no choice made \((p = 0.0018)\). When only caterpillars choosing a food were compared, the choice of kale was still significantly higher \((p = 0.0016)\) as it was when caterpillars exhibiting taxis were included as definitive measures of choice \((p = 0.0028)\) in the naïve caterpillars. Caterpillars with increasing amounts of experience demonstrated the same significant choice of kale over artificial food in all tests (Figure 2). Results of the three trials with increasing experience were equivalent within a 95% confidence interval (Figure 3).
Figure 2. Number of caterpillars choosing kale (innate cue), artificial food (learned cue) or undecided with increasing experience. n = 22 in trial 1; n = 21 in trials 2 and 3 (due to death of one caterpillar).

Figure 3. Mean time to host food selection for each trial with increasing experience. Error bars represent 95% confidence interval. Due to the large overlap of the 95% confidence interval, it is reasonable to state that there is no difference between the samples. n = 22 in trial 1; n = 21 in trials 2 and 3 (due to death of one caterpillar).

**Discussion**

The results of the initial group trials did not support our hypothesis that caterpillars would select food based primarily on innate cues. There was not a significant difference in the amount of time it took caterpillars to select food, even though the first food selected was always kale. This lack of significance may be due in part to low sample size, but the selection of both foods in a statistically equivalent amount of time could indicate that both foods are acceptable choices for *Pieris rapae* larvae.

In five of six trials, the first caterpillar to choose a food selected kale with the second selecting artificial food. This may be indicative of an optimal foraging strategy, in which the early choosing caterpillars gain the preferred food source of kale with late choosing caterpillars selecting the artificial food for themselves rather than sharing kale. The goal of this study was not to determine optimality patterns in *P. rapae* caterpillars, but the apparent variation between caterpillars selecting kale and artificial food in paired settings (no significant difference) and individually (significant difference) is intriguing. Group trial results indicated that there are social interactions between caterpillars, who made contact with one another in 50% of the trials. These interactions could influence food preference.

As predicted by our hypothesis, the results of the individual trials provided evidence that caterpillars prefer a food source that is recognized via innate cues, instead of learned cues (Figure 2). Naïve and experienced caterpillars consistently chose kale (innate cue) over artificial food (learned cue), which indicates that caterpillars make decisions based on their evolutionary history. Even though caterpillars were reared and maintained on artificial food that contained all the appropriate nutrients to survive, the caterpillars still demonstrated a higher preference for the kale. Caterpillars did exhibit some plasticity in food choice as a small number of them did select the artificial food. Coupled with the results of the initial group trials, in which equivalent numbers of caterpillars selected the artificial food (although always after kale had already been selected by another caterpillar), this is evidence of some plasticity in food preference. We did not track individual caterpillars and were not able to determine if there were consistent individual preferences for artificial food in some caterpillars, which might also indicate a genetic or innate aspect to phenotypic plasticity in using learned cues over innate ones (Nylin and Gotthard 1998).

The results of this small study support that *P. rapae* larvae are host specific towards cruciferous plants and, in individual settings, rely primarily on innate cues to select food. In this set of experiments, the preferred food was also a novel choice for these larvae, who had been reared on the Carolina ® caterpillar food. We
would not expect novel food choice to be an evolutionary stable strategy particularly in an organism known for host specificity, and reliance on innate cues is the most likely explanation for the behavior of *P. rapae* larvae. An interesting line of future study would be to examine caterpillar prey choice in groups of larvae reared upon both food options. The evolutionary pressure of host-plant specificity in *P. rapae* influenced food choice in the individual trials, even when an appropriate alternative food source with which they had experience was available. Relying on specific innate cues allows *P. rapae* caterpillars to make food choices efficiently within a noisy environment in order to optimize survival. Small rates of learned cue choice by individual caterpillars and higher rates of learned cue selection by caterpillars in groups indicate flexibility in food choice, could be a potential optimal foraging strategy. Potential avenues for future investigation include a more structured comparison of caterpillar food choice behavior in groups and individually to determine optimal foraging strategies. The examination of consistency of choices made by individually identified caterpillars could also help determine persistence of preference and possible genetic foundations for rates at which caterpillars select innate and learned cues.

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**Literature Cited**


