INDUCTION OF FEEDING PREFERENCE IN LARVAE OF THE PATCH BUTTERFLY, CHLOSyne LACINIA

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Induction of preference, a phenomenon first described by Tibor Jermy, was demonstrated in larvae of the patch butterfly, Chlosyne lacinia. Using choice tests pioneered by Jermy (the “disc test”) to assay preferences, a strong induction was shown with three plant pairs. Several factors affecting induction were investigated: critical time windows, switching food plants, the amount of feeding necessary to produce a change in preference, and the effect of feeding just prior to the choice test. No early critical window (“imprinting”) was found; both the amount and recency of feeding were found to be significant factors for induction and may interact. The importance of the induction of preference is discussed.

Key words: feeding behaviour, induction of preference, phytophagous insects, Nymphalidae, Chlosyne lacinia

INTRODUCTION

Phytophagous feeders comprise approximately 50% of all living insect species (Dethier 1954) and close to 100% of Lepidoptera (Schoonhoven et al. 1998). Most of these are specialists and have strong feeding preferences that result in various degrees of host specificity. Some of these feed on agriculturally important crops with significant economic consequences in both crop loss and the costs of insect control; the latter is often compounded by concomitant environmental degradation. Thus, given the economic and environmental importance of the feeding behaviour of phytophagous insects, the need to acquire a basic understanding of its mechanisms seems compelling.

One of the interesting aspects of insect feeding behaviour is that feeding preference shows plasticity. Behavioural plasticity is not something that is usually associated with insects, and so there has been some reluctance to accept it as part of the insect behavioural repertoire. Early evidence of preference alteration due to switches in larval food plants at first appeared in brief notes in the literature (reviewed by Jermy et al. 1968, Jermy 1987). As the bulk of evidence became substantial, Tibor Jermy questioned whether these results were due to plasticity (individuals actually changing their preference), or to stringent selection (survival of...
a group of animals having a wider range of food tolerance). Therefore he proposed that future experiments monitor post-switch mortality to ensure that selection was not occurring. On research leave in VINCE DETHIER’s laboratory in 1966 at the University of Pennsylvania in Philadelphia, he clearly demonstrated plasticity: Feeding experience by a caterpillar on a plant species resulted in increased preference for that plant (JERMY et al. 1968). The authors found this in two species of Lepidoptera, Manduca sexta and Heliothis zea (now Helicoverpa zea). These workers termed this phenomenon induction of preference to distinguish it from synonymous terms (e.g., conditioning) which may have special meaning in the behavioural literature.

Induction of preference has since been described in other species (see review by JERMY 1987) and possible mechanisms have been proposed (STÄDLER & HANSON 1976, 1978, DEBOER & HANSON 1984, 1988, DEBOER 1992, DEL CAMPO & RENWICK 2000, DEL CAMPO et al. 2001). However, many aspects of the phenomenon have not been fully explored. For example, is there a critical time window for exposure, as there is in vertebrate imprinting? Is there a threshold amount of feeding on an inducing plant that is required for the manifestation of induction? Does induction increase with exposure (cumulative effect)? Is recency of feeding a factor in induction, or only quantity of feeding? Experiments designed to test hypotheses generated by these and other questions were performed on larvae of the patch butterfly, Chlosyne lacinia GEYER, using a modification of the behavioural choice test pioneered by TIBOR JERMY (JERMY 1961).

METHODS AND MATERIALS

Animals and culturing

The experiments were performed with caterpillars of the nymphalid patch butterfly Chlosyne lacinia GEYER, native to Texas, Central and South America. Larvae were collected from sunflower (Helianthus annuus) in and around Austin, Texas, and reared in continuous culture in the laboratory under controlled conditions of light (L:D 16:8) and temperature (ca. 20–23°C) during spring, summer and early fall (NECK 1977). Both the cultures and the larvae undergoing induction were reared on leaves. Growth and mortality rates were found to be comparable among the four host plants.

In rearing larvae for experiments, care was taken to circumvent brood effects. Egg masses were collected from oviposition cages, allowed to hatch in the absence of plants and distributed in equal numbers to the rearing plants. Thus if adverse effects of inbreeding, previous generation diet or other effects did occur, each culture would be equally affected.
Plants

The plants for the culture and experiments were locally collected host plants (Compositae): *Helianthus annuus* (henceforth abbreviated “H”), the common sunflower; *Ambrosia trifida* (abbreviated “A”), the common ragweed; *Zexmenia hispida* (abbreviated “Z”); and occasionally *Ximenia enceloides* (abbreviated “X”).

Testing procedures and calculations

The procedure for testing food preference was a modification of the JERMY disc test (JERMY 1961, JERMY et al. 1968). Two-choice tests were set up in plastic petri dishes of nine cm diameter for testing fifth instar larvae. Smaller dishes were used for testing for the lower instars. Four leaf discs of 8.5 mm diameter were punched from leaves of each of the two plant species to be examined. Smaller punch-outs were used with lower instars. The leaves destined for leaf discs were carefully selected from the top 1/3 of the plant to eliminate senescent leaves.

The leaf discs were arranged with the two plant species alternating around the inside circumference of the petri dish. This arrangement ensures that an active larva has an equal chance of encountering each leaf species. The small size of the leaf discs required multiple encounters with each leaf species to reach criterion, thus measuring choice and not feeding duration. In the center of each dish was placed a small circle of moist filter paper to prevent desiccation of the leaf discs.

Insects to be tested were removed from the culture at the end of the last rearing instar in the non-feeding premoult stage. They were placed in isolation without food and tested soon after moulting into the next instar. To start the test, a single larva was placed in the center of the dish, and leaf consumption was observed at approximately hourly intervals thereafter. At the time when one of the plant species was about 50% consumed (the equivalent of two discs) by a given larva, its test was terminated and the amount eaten of each plant species was visually estimated and recorded. These were summed on each plant, and the percentage of total consumption of each plant was calculated for each animal as an indirect measure of its choice, as follows:

\[
\text{Percent total consumption of plant #1} = \frac{\text{consumption of plant #1}}{\text{consumption of plants #1 plus #2}}.
\]

A similar calculation was done for consumption of plant #2. Means and standard errors were calculated for N animals. The “Choice Index” is the difference between the two means and has a range of −100 to +100. The “Induction Index” is the absolute value of the difference between Choice Indices of the two larval cohorts and has a range of 0 to 200. Statistical evaluations used the Wilcoxon non-parametric tests (signed rank test for P-choice, rank-sum test for P-induction) since the data distributions were similar but generally not normal.

Experiments

The basic induction of preference experiment tested the preferences of two cohorts of insects each reared on separate host plant species. The rearing plants were also the test plants. Five variations of the basic experiment were performed, as explained in the Results. All trials to be compared within each variation were performed within a few weeks of each other to avoid seasonal variation in plant quality.
RESULTS

Can a preference be induced in the patch butterfly, Chlosyne lacinia? Larvae reared for four instars on sunflower (Helianthus annuus, H) or ragweed (Ambrosia trifida, A) demonstrated a classic induction of preference response when given a choice test on this plant pair in early fifth instar: Larvae reared on H strongly preferred H whereas those reared on A strongly preferred A (Fig. 1). There was a large difference in feeding scores (Induction Index) for the two cohorts indicating a significant induction of preference (P<0.001; Table 1). Naïve control animals reared on another host plant, Ximenesia enceloides, X, but tested on the same plant pair showed a preference intermediate to that of H and A (Fig. 1, right pair of columns). Similar results also were seen with plant pairs Z:H and A:Z (Z is Zexmenia hispida) with large Induction Indices (P<0.001; Table 1); naïve controls also showed intermediate preferences in these experiments.

![Graph showing consumption preferences](image)

**Fig. 1.** Induction of preference in larvae of Chlosyne lacinia. Graphs depict the consumption of the two test plants (each expressed as percent of total consumption) during the choice test administered to a cohort of larvae raised on the designated host plant. Columns represent means for N larvae ±SE. Legend: Raised on = the plant on which that cohort of animals was reared for four instars. N = number of larvae tested. Choice Index = difference in feeding scores on the plant pair tested (range: −100 to +100). P choice = significance of difference in choice between test plants. See Materials and Methods for plant species abbreviations. All larvae were tested in early fifth instar. Note that rearing larvae on a plant increases its feeding preference for that plant.
A separate set of experiments sought to determine whether earlier instars could also manifest an induction of preference. Larvae were reared on an inducing plant for two instars and tested in early third instar. These experiments also showed strong inductions of preference for $H:A$ ($P<0.001$, $N=66$) and $H:Z$ ($P<0.001$, $N=79$).

Does the strength of induction increase with duration of feeding on the inducing plant? Larvae reared on $H$ show an increased preference for $H$ with each additional instar of feeding on it (Fig. 2a). Thus, either induction increases with each instar of rearing on the inducing food, or else a specific increase in preference for $H$ is a normal developmental phenomenon. Sibs reared on $A$, however, did not show an increased preference for $H$ during development (Fig. 2b); thus, we conclude that the increase in preference for $H$ in Fig. 2a is due to an increased induction with each additional instar of rearing on the inducing plant. Presumably this also would hold true for $A$ but is not manifested in Fig. 2b because $A$ is already so highly preferred to $H$ in the early instars that no further increase in preference for $A$ could take place.

Can a preference be changed by switching host plants? The original observations in the literature indicated that switching host plants results in a preference change towards the new host. To further investigate these reports and to determine if there is a requisite time period or quantity of feeding, we switched host plants after each instar and tested in the fifth instar. Our results confirm these early reports: Switching from $A$ to $H$ and vice versa does indeed change the preference (Fig. 3a,b: compare left pair of columns with right pair). These data also show that the feeding experience in the fourth instar accounts for all of the observed induction. Similar results from other plant pairs are reported in Table 2.

Occasionally we observed failure of induction or manifestation thereof. This occurred only when one member of the plant pair is very highly preferred over the other. For example, in the plant pair $H:Z$ depicted in Fig. 3c, no induction was seen,
Fig. 2. Strength of induction depends on the number of instars of feeding on inducing plant. Larvae were tested after rearing on one plant for the indicated number of instars (0 = neonates tested; H = fed for one instar on H and tested in early second; HH = fed for two instars on H and tested in early third, etc.). Legend as in Fig. 1; also, P induction = significance of difference in feeding preference between the indicated cohorts (0 vs. HHHH, or 0 vs. AAAA). (2a) Note that the preference for H increases with the number of instars reared on H. (2b) Since A is already maximally preferred by neonates, the preference for A could not be increased further.
presumably because $Z$ was so much preferred to $H$ in the test that very little $H$ was selected.

Is there a critical time window for induction? This question was answered by substituting a second plant during one instar only, but varying the instar in which the substitution occurred. Results show that the larvae preferred the plant species they consumed in the fourth instar; switching to an alternate plant during one of the other instars had no discernible effect (Fig. 4a, b).

Is the important induction factor the total amount of inducing food, or the most recent food? The above data show that stronger induction is seen in later instars when most of the larval feeding occurs, but do not distinguish between the quantity of inducing food vs. the most recent food prior to the test. To attempt to discriminate between these two alternatives, larvae were reared for four instars in the normal fashion and thus had consumed a large quantity of inducing food. Early in the fifth instar just prior to testing, they were fed an additional small amount (four leaf discs) of either their inducing plant or the paired plant. The amount fed, four discs of the type offered in the test, is a small fraction of the quantity of food

Table 2. P-induction for five cohorts of larvae that were switched from host plant #1 to host plant #2 at first, second, third or fourth instars and tested in the fifth instar

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Table 3. Amount vs. recency of feeding as important factors in induction. For each of three pairs of plants, the first control cohorts of larvae were fed only on plant #1, including four discs in the fifth instar just prior to testing. In contrast, the first experimental cohorts were reared on plant #1 for four instars and then fed four discs of plant #2 just prior to testing. The procedure was then reversed for the second control and experimental cohorts. Note that slight but significant induction occurred in most plant pair comparisons

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Fig. 3. Host plant switching. Animals were reared on plant #1 for a variable number of instars, then switched to plant #2 until tested in early fifth instar. Legend as in Fig. 2; also, Induction Index (range: 0 to 200) is the absolute value of the difference between choice indices of the above two cohorts, and P induction is the significance of that induction index. (3a, 3b) Note that the plant on which the animal fed during its fourth instar is the plant the animal prefers when tested in the fifth. (3c) Induction is not manifested when one plant, Z, is so highly preferred that little or no feeding occurs on the other plant, H, in the test.
they had consumed during their first four instars. The results show that this amount of feeding on the paired plant just prior to the test does indeed produce a slight but significant increase in preference for it in most cases (see Fig. 5 for A:Z; other plant pairs are reported in Table 3). This suggests that recency of feeding on a different species may also be a factor in determining preference.

Fig. 4. Critical time window for induction. (4a) Animals were reared on Z except for one (variable) instar on A and tested in early fifth instar on A and Z. (4b) Same as 4a, but the plants were reversed. Legend as in Fig. 3, except that the Induction Index and P induction always compare responses of the indicated cohort against those of the first cohort. Note that the larvae always prefer the plant they fed on during the fourth instar
DISCUSSION

A classic induction of feeding preference was clearly demonstrated for most of the tested plant pairs by larvae of the nymphalid butterfly, *Chlosyne lacinia*. Preference for a plant is significantly increased by feeding on that plant. Most reports in the literature also show an increased preference for the rearing plant, although a few studies report reverse induction (e.g., WASSERMAN 1982, PORTILLO *et al.* 1996) or failures on certain plant pairs (e.g., JERMY *et al.* 1968, HANSON 1976, CHEW 1980). An explanation for failure has been suggested by DEBOER and HANSON (1984), namely that the two members of the experimental plant pair are too close taxonomically and thus chemically too similar. In his review of the role of experience in host selection, JERMY (1987) lists 21 lepidopteran species in which this phenomenon was shown, and others have since been added (e.g., *Spodoptera frugiperda* and *S. latifascia* by PORTILLO *et al.* 1996). Induction has been seen in other orders as well (JERMY 1987, LU & LOGAN 1993). A phenomenon this widespread must be important for insects and deserves the attention of experimental and evolutionary biologists to provide a basic understanding of its proximate and ultimate mechanisms.

![Fig. 5. Recency and amount of feeding affects preference. Larvae were reared on plant #1 for four entire instars; in the fifth instar just prior to testing, the control larvae were fed four more discs of the same plant (first pair of columns) whereas the experimental larvae were fed four discs of plant #2 (second pair of columns). Legend as in Fig. 2. Note that a slight but significant increase in preference results from recent feeding on plant #2](image-url)
One of the characteristics of induction is that it begins early in the larval stage. Tests on *C. lacinia* in the beginning of the third instar show that highly significant induction has already taken place due to feeding in the first two instars. This supports the observations of Wiklund (1973) who reported that larvae of *Papilio machaon* developed a preference for their rearing plants by the third instar. Larvae of the gypsy moth, *Lymantria dispar*, have been shown to have experience-dependent preference changes by the second instar that intensify with further feeding (Barbosa et al. 1979). Our experiments on *C. lacinia* also show a comparable increase in the strength of induction with each instar of feeding on the inducing plant (Fig. 2). A similar result was reported in *Manduca sexta* by Yamamoto (1974) using a different bioassay (attraction test).

The host plant switching experiments patterned after the classic studies cited earlier showed that preferences induced in *C. lacinia* are moderately labile. Literature reports indicate that even though a strong preference can be demonstrated in a choice test, most species are still capable of switching to another host plant. Exceptions are noted by Ma (1972) who reported that after induction, *Pieris brassicae* could not be successfully switched to certain other host plants, and Hanson (1983) who reported a similar result for the promethia moth, *Callosamia promethea*. Presumably, as yet undiscovered incompatible switches exist for other insect species as well.

In the present paper, the host plant switching experiments on *C. lacinia* provided an example of an inconsistency sometimes encountered in these types of experiments: inductions successfully demonstrated at one time fails at another. For example, larvae show significant induction on *H:Z* in Tables 1 and 3, but clearly not in Fig. 3c and Table 2. Perhaps the explanation for this anomaly is that the plants vary due to season or may have had an unseen contaminant or disease. Our experimental protocol sought to minimize such problems by scheduling trials of one experiment within as narrow a time window as possible, and we place more confidence in comparisons within than across experiments.

The question arises as to whether there is a critical time window for induction in *C. lacinia* as there is, for example, in newborn vertebrates that imprint on moving or sound-producing objects during their first day of life. Such an early critical window was hypothesized by Yamamoto (1974) to explain his observations that in the attraction test *Manduca sexta* became less polyphagous because of foods eaten in the first instar. Using a different assay (disc test), Deboer and Hanson (1984) found no evidence of a critical window in *M. sexta*. Likewise in the present study there is no evidence for an early critical window in *C. lacinia* (Fig. 4). The importance of the fourth instar could be construed to be a late critical window, although an alternate interpretation (below) appears to be more plausible. In all of
the above studies, however, subtle differences may not have been detected due to the crudeness of the behavioural bioassay. With the discovery of a host specific phytochemical that appears to modify chemoreceptors (Del Campo et al. 2001), this question may need to be reinvestigated using more sensitive assays.

The experimental results show that induction, when present, is determined by the plant species eaten in the instar just prior to testing. Thus recency of feeding may be an important factor. Alternatively, the total amount of the inducing food consumed may be the important factor, as would be the case if the operative mechanisms involved a cumulative effect. Many studies have shown that larvae eat more in any given instar than in all the previous instars combined, so in our plant switching experiments the total amount consumed would have been greatest in the fourth instar. Since this is just prior to testing in the fifth instar, however, recency of feeding may be confounded with amount of feeding. Further experiments (Fig. 5) indicated that recency of feeding may indeed play a role: Four (but not two) leaf discs of the second plant are enough to change the preference slightly but significantly with some plant pairs. Thus it is likely that both factors play a role and that they interact. Similarly, Jermy et al. (1968) found that the most recent 24 hours of feeding on a second plant could change the preferences of Helicoverpa zea, and Ma (1972) found that the most recent four (but not two) hours of feeding on Tropaeolum majus would change the preference of Pieris brassicae.

Perhaps the ultimate importance of induction of preference is that it may contribute to the formation of biological races, which are groups of insects occurring in the same locality but having different food preferences (i.e., host races for phytophagous insects). Dethier (1954) speculated that the formation of biological races may be an early step in the process of speciation. When feeding preferences of races differ and they establish on different hosts, spatial isolation and selection could result in genetically different races if gene interchange were sufficiently restricted. Host shifts by insects must have occurred many times in the past, and reports of this have surfaced in the literature since the beginning of the last century (Schroder 1903, Pictet 1911). The mechanism often proposed is a mutation in the insect sensory system that permits feeding and oviposition on a formerly unacceptable plant, perhaps after the population has gone through a genetic bottleneck (Pictet 1911). Alternatively, some adaptability in food choice behaviour may be present that increases the acceptability of the new plant after prolonged exposure to it. This alternative raises questions about the degree of adaptability of feeding preference that is present in a normal population (i.e., one without stringent selection). Adaptability includes plasticity of preferences, which in the pre-Jermy era had been thought to be absent in insects. Plasticity is, however, alive and well, as the induction of preference data show.
Whether the changes brought by induction are sufficiently strong to affect host race formation is not clear. From our current vantage point induction appears to be less of an ultimate and more of a proximate mechanism, such as restricting the insect to the plant on which it is currently feeding. When a larva switches plants, new detoxifying enzymes may need to be induced, and this has its metabolic cost. If these enzymes are not produced quickly, mortality may follow. As an example, induction of preference protects Callosamia promethea reared on sassafras (Sassafras albidum) or spicebush (Lindera benzoin); larvae induced on either of these will generally not switch to wild cherry (Prunus serotina), another host plant. Those that do eat cherry will die, presumably from the cyanogens in the cherry leaves which they can no longer detoxify (Hanson 1983). Similarly, Pieris brassicae larvae reared on Brassicae oleracea do not survive the switch to Tropaeolum major, which is otherwise a good host plant for Pieris when reared on it from the first instar (Ma 1972).

To conclude, the induction of preference, a concept pioneered by Tibor Jermy, has been examined in larvae of a nymphalid lepidopteran, the patch butterfly, Chlosyne lacinia. Our studies have illuminated some of the factors involved in induction and changes in preference following host plant switching. But to fully understand the basis of preference change will require further investigations into the physiological mechanisms of feeding decisions. This is stated more eloquently by Tibor Jermy: “…the insect receives very detailed information from the plant; this information is in some way stored in the nervous system and is used as reference information for the decision to be made by the insect at subsequent encounters with plants. At present the neural basis of these processes is largely unknown…” (Jermy 1987). Indeed, it is still largely unknown, but thanks to pioneers like Tibor Jermy the veil of ignorance about these feeding decisions and other insect-plant interactions is beginning to lift.

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REFERENCES


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