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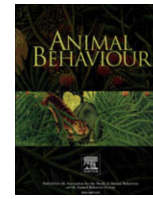

A sensitive period for larval gustatory learning influences subsequent oviposition choice by the cabbage looper moth

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We examined the effects of larval feeding experience with a deterrent plant latex on subsequent adult oviposition behaviour in the cabbage looper moth, *Trichoplusia ni*. Larvae were exposed to the latex of *Hoodia gordonii* at 100 ppm in an artificial diet. This plant material strongly deters oviposition in naïve moths in laboratory choice tests. Larvae that were exposed to the latex either for their entire final instar or throughout larval development showed no oviposition deterrence. This effect persisted when larval exposure to *H. gordonii* latex was suspended for the final 2 days immediately prior to pupation. However, when exposure to latex was suspended for 1 or more days in the beginning of the final instar or for 3 or more days prior to pupation, oviposition behaviour was deterred in subsequent adult moths (i.e. the adults behaved as naïve moths). Our observation that adult oviposition choice can be influenced by a change in diet in the final larval instar indicates the presence of a sensitive period whereby larval feeding memory can be lost or maintained in adulthood.

Development of food preference is often influenced by learning and experience of a food early in development. Genetic predispositions to accept or reject foods work jointly with these experiences to shape food preferences. Induced food preferences have been observed in the dietary preference of young rat pups for the diet of their mothers as a result of ingesting their mother's mammary secretions (Galef & Henderson 1972). Human infants and young children have also shown developed preferences for tastes that they have previously experienced (Beauchamp & Moran 1982; Sullivan & Birch 1990). Several studies on the induction of food preference have focused on imprinting during a sensitive period. This typically occurs shortly after birth or hatching and is believed to persist into adulthood (Immelmann 1975). Food imprinting has been shown in turtles (Burghardt & Hess 1966), spiders (Punzo 2002) and cuttlefish (Darmaillacq et al. 2006).

Similar to the induction of food preference by imprinting, deterrence to an unfavourable food can be reduced or eliminated by habituation. Habituation is a type of nonassociative learning in which there is a progressive reduction of a behavioural response with repeated exposure to a stimulus. In the field of insect–plant interactions, it is generally accepted that the secondary chemistry of plants provides a deterrent stimulus that makes most plants unpalatable to most insects. However, if situated on a putatively unsuitable host plant, many insects will tend towards wider polyphagy when threatened by starvation (Dethier 1954). Therefore, these deterrent substances can be harmless (Bernays & Graham 1988) as well as adaptive at nontoxic concentrations (Glendinning & Gonzalez 1995). Gustatory rejection response to deterrent compounds in phytophagous insects decreases noticeably after repeated or continuous dietary exposure for several hours or days, thus showing habituation to the compound (Akhtar & Isman 2004). For example, repeated or continuous exposure to extracts of *Melia azedarach*, *M. volkensii, Origanum vulgare*, or the pure allelochemicals, thymol and xanthotoxin applied to cabbage leaves decreases feeding deterrence in *Trichoplusia ni* (Akhtar et al. 2003).

The gustatory habituation caused by repeated or continuous dietary exposure has been theorized to carry over through pupation to the adult stage and consequently, affect oviposition behaviour. This is the Hopkins' host selection principle (HHSP), which proposes that the feeding and oviposition behaviour of phytophagous insects is influenced not only by experience of the adult insect (Cassidy 1978; Rausher 1978; Prokopy et al. 1982; Jaenike 1982, 1983; Hoffman 1985) on a specific food, but also by larval

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experience for that food (Hopkins 1917). Evidence for this effect includes studies with the plant allelochemicals *trans*-anethole and toosendanin (Akhtar & Isman 2003), and with a plant latex of *Hoodia gordonii* (Chow et al. 2005) on the cabbage looper (*T. ni*).

The chemical constituents of host plants play a critical role in directing oviposition choice (Renwick 2001). Determining the suitability of a host plant is not always based on a few key stimuli, but often on a combination of many stimulatory and inhibitory plant chemicals acting together (Schoni et al. 1987). Plant chemistry can be highly variable, as can insect responses to plant chemicals as a result of genetic variation, developmental characteristics and/or environmental factors (Renwick 2001).

The oviposition choice of phytophagous insects depends on their evaluation of host plant suitability for the development of their offspring (Singer 1986). The probability of acceptance of a plant for oviposition is influenced by both innate tendencies and prior experience. These influences may operate synchronously, increasing or decreasing the probability of acceptance (Miller & Strickler 1984). The ability of adults to locate and oviposit on suitable host plants is particularly important in insects such as Lepidoptera because of the relative immobility of their offspring (Feeny et al. 1983).

Although HHSP has been examined in numerous insects, few studies have investigated the developmental stage important for the acquisition and retention of larval memory that persists into the adult stage. In the parasitic wasp *Hyssopus pallidus,* preimaginal exposure to a stimulus at the beginning of development (i.e. at the greatest time interval from the adult stage) was the most behaviourally effective. No behavioural effect was observed when *H. pallidus* was exposed at the end of preimaginal development, or around the time of adult emergence (Gandolfi et al. 2003). In contrast, exposure of *T. ni* to an allelochemical in the last larval instar was sufficient in influencing subsequent adult oviposition choice (Akhtar & Isman 2003). In the present study we investigated a specific period in the last larval instar of *T. ni* at which larval memory to an oviposition deterrent can be acquired or lost.

The cabbage looper moth, *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae), is a generalist, feeding on a relatively diverse range of host plants. It attacks crops, including lettuce, beets, peas, celery, cotton, tomatoes, ornamental plants, and many weedy species, but is a particularly important pest on cruciferous plants. *Trichoplusia ni* is suitable as a model for this study because it has a short life cycle (egg–adult, 24–33 days), lays eggs singly and is easy to rear in the laboratory.

The latex from *Hoodia gordonii,* a South African milkweed (Asclepiadaceae), was the test material used for rearing and choice experiments. It was selected based on previously determined oviposition deterrent properties (Chow et al. 2005). Since *T. ni*, a New World species, would never encounter *H. gordonii* in nature, these two species were used strictly as a model system to study the influence of plant chemistry on insect behaviour. Our main objectives in the present study were to determine (1) the minimum time required for *T. ni* in the last instar to habituate to *H. gordonii* latex and influence subsequent adult oviposition behaviour and (2) whether acquired memory of a plant extract by larvae can be lost if exposure is discontinued prior to pupation.

METHODS

Plant Material

Cabbage plants (*Brassica oleraceae* var. Stonehead) were regularly planted in plastic flats containing a mixture of sandy loam soil and peat moss (4:1) in a greenhouse at the University of British Columbia, Vancouver, BC, Canada. Leaves from 5–6-week-old cabbage plants were used.

Test Material

A crude plant latex obtained from the South African milkweed *Hoodia gordonii* (Asclepiadaceae) was a gift from Phytopharm plc (Cambridge, U.K.). The plant latex (lot LB99/0001) consisted of spray dried sap from the sliced and pressed stems of the plant grown in screen cages in South Africa. Methanol (MeOH) was used as a carrier.

Test Insect

Trichoplusia ni eggs were acquired from a laboratory colony maintained for more than 60 generations. The colony larvae were reared on Velvetbean Caterpillar Diet no. F9796 (Bio-Serv Inc., Frenchtown, NJ, U.S.A.) with 30 g of Vanderzant's vitamins and 20 g of alfalfa meal supplemented to every 300 g of diet. Larvae were reared in a growth chamber at 23 °C and 40% RH on a 16:8 h light:dark cycle. These conditions ensured that the vast majority of larvae fed for 5 full days in the fifth instar prior to pupation.

General Procedure

Two groups of *T. ni* we reared for this study: naïve and experienced. The naïve (control) group was reared on a normal artificial diet. The experienced group was reared on an artificial diet in which *H. gordonii* latex was incorporated to give a final concentration of 100 ppm (fresh weight).

The concentration used for rearing, based on preliminary experiments, did not cause significant larval growth inhibition (unpublished data). For example, to produce 200 g of treated artificial diet, 20 mg of *H. gordonii* latex was dissolved in 20 ml of carrier solvent (MeOH) and applied to 35 g of dry diet with a micropipette. Carrier solvent alone was applied for the untreated diet. After the solvent was evaporated in a fumehood, the diet was combined with a mixture of water (160 ml) and agar (5 g), which had been heated to boiling, and mixed thoroughly. The diet was evenly divided into seven Styrofoam cups, and allowed to cool and set. Approximately 20 freshly hatched larvae (<24 h old) were placed into each cup. Plastic lids were placed on the cups to prevent escape.

The oviposition choice bioassay was performed according to that described by Akhtar & Isman (2003) with minor modifications. Larvae were removed from the diet cups when they completed feeding and began preparing for pupation. Pupating larvae were placed in separate cups until pupation was complete and then separated according to sex. When subsequent moths emerged, individual male/female pairs were placed in an oviposition cage with a control and treated cabbage leaf for 48 h. The oviposition cages were made from Styrofoam containers (24 cm high, 16.5 cm diameter). Plastic mesh was placed on top and secured with thumbtacks to prevent escape. Sucrose solution (10%) was placed inside each cage to feed the moths. Treated leaves were sprayed with 0.25% *H. gordonii* latex solution, whereas the control leaves were sprayed with the carrier solvent alone. This concentration was found to deter oviposition by 50% (OD_{50}) relative to controls, in a previous study (Chow et al. 2005). After 48 h, the eggs on control and treated leaves were counted.

Larval memory acquisition experiment: naïve larvae exposed

We performed six rearing manipulations to determine the minimum length of exposure to *H. gordonii* latex required during larval development of naïve *T. ni* to influence subsequent adult oviposition behaviour in *T. ni* moths (Table 1). Naïve larvae were reared on an untreated diet from neonate (first instar) to the start of the final (fifth) instar. They were then separated into six groups. One group was kept on an untreated diet throughout the final instar