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Larval host plant experience modulates both mate finding and oviposition choice in a moth

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Host plant choice in polyphagous insects can be connected with costs of being naïve when confronted with several potential host plants, for example through slower decision making. Utilization of earlier experience could be one way to overcome some of these limitations. We studied whether larval feeding experience influences mate finding by males and female choice of oviposition site in the moth *Spodoptera littoralis*. Larvae were reared on either artificial diet or the host plants, cotton, clover or alfalfa, and we recorded the behaviour of adults from each diet. In two sets of experiments, in both the laboratory and the field, we investigated the female's oviposition choice and the male's response to female pheromone with different plant odour backgrounds. We found that experience with cotton, clover or alfalfa during the larval period induced female oviposition on the corresponding plant both in the laboratory and in the field. Furthermore, males were more attracted to female sex pheromone combined with odour from a host plant species that they had experienced as larvae than to sex pheromone combined with odour from host plant species they had not experienced. The results show convergent modulation of male and female responses to plant odour depending on their larval food plant. The influence of larval experience during the first-to-fifth instars on host preference was also stronger than the influence of experience acquired in the late larval, pupal and early adult stages. Consequences for host plant choice efficiency and fitness effects for males and females are discussed.

Host plant choice is crucial for herbivorous insects. This is certainly true for specialists, but generalist herbivorous insect species also face a difficult task in finding suitable host plants. Even though generalists can utilize a large number of plant species, many species or individual plants occurring in their environment are unsuitable or suboptimal for larval development. In the process of host plant selection specialist insects can canalize their sensory system to a limited range of cues from a small selection of host plants, while generalist species have to be able to detect and interpret a wider range of cues. Thus, neural limitations may be more pronounced in generalist species and are likely to represent a significant problem for fast and accurate choice of feeding and oviposition sites (Bernays 2001).

Exploiting experiences from previous life stages could be one way for polyphagous species to facilitate information processing. Experiences in the natal habitat have been reported to affect selection of the postdispersal habitat in a broad range of animal taxa (Davis & Stamps 2004). In holometabolous insects several studies have examined how larval diet affects female choice of host plant. This phenomenon has been debated and questioned (e.g. Van Emden et al. 1996; Barron 2001; Rietdorf & Steidle 2002; Janz et al. 2009). However, recent examples suggest that larval experiences may influence adult host choice behaviour in several insect species (e.g. Rietdorf & Steidle 2002; Akhtar & Isman 2003; Gandolfi et al. 2003; Hora et al. 2005; Olsson et al. 2006; Moreau et al. 2008). It has also been shown that both male and female fruit flies remain on or return to the larval host plant, thus showing an experience-based host fidelity (Feder et al. 1994; Funk et al. 2002).

In many insects mating occurs on the host plant and it is important for both males and females to find the mating site. Thus, there can also be pressure on males to find suitable plants on which females are present. In Lepidoptera males use female-produced sex pheromones to locate females ready for mating. The response to sex pheromone has been shown to be influenced by plant odours (Landolt & Phillips 1997; Ochieng et al. 2002; Deng et al. 2004; Trona et al. 2010) which means that the general background of plant odours has the potential to affect male detection of females. However, it has not been generally shown whether males exhibit adaptive behavioural plasticity to a host plant environment in the same way as females do.

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The Egyptian cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) is a polyphagous moth that has several generations per year in its natural habitat in Egypt (Amin & Gergis 2006). Thus, the species represents a model of a generalist in which phenotypic behavioural plasticity could be required to optimize host plant choice for every generation and to make it better adapted to the seasonal availability of potential host plants. In earlier studies we have found that larval diet can affect adult oviposition behaviour (Anderson et al. 1995) and that early adult experience can change behavioural and neural sensitivity to olfactory cues (Anderson et al. 2007; Anton et al. 2011). The aim of this study was thus to address questions regarding the effect of larval experience on adult behaviour in this generalist moth. In laboratory and field experiments we investigated: (1) whether female oviposition preference is affected by larval experience; (2) whether male mate search using female sex pheromone is influenced by host plant background; and (3) the relative influence of early larval experience versus late larval or early adult experience on host plant preferences in a field situation.

METHODS

Laboratory Experiments

Insects and plants

The insects used in the laboratory experiments originated from a strain founded from insects collected in the Alexandria region in Egypt. The culture has been supplemented at least once annually with wild moths from Egypt. The larvae were reared on a semi-artificial diet (Hinks & Byers 1976). Egg batches were collected from the laboratory culture, randomly divided into three groups and transferred to boxes with cotton leaves, clover leaves and artificial diet as a neutral food source, respectively. On these three different diets larvae were reared until pupation at 25 ± 2 °C, 70 ± 2% relative humidity (RH) and 16:8 h light:dark (L:D) in a climate chamber. Fresh plant leaves or artificial diet were provided daily. The pupae were collected and sexed, and the males and females were kept separately in different climate chambers (25 ± 2 °C, 70 ± 2% RH, 16:8 h L:D) until adult emergence. Newly eclosed males and females were isolated daily.

Cotton plants, *Gossypium hirsutum*, and Egyptian clover plants, *Trifolium alexandrinum*, were cultivated for 6 weeks at 25 ± 2 °C, 70 ± 2% RH and 12:12 h L:D in a greenhouse until they were used for experiments. The plants were potted in 1.5-litre pots in a commercial substrate (Kronmull, Weibull Trädgård AB, Hammenhög, Sweden). Six-week-old plants were used for experiments; at that age the cotton plants had approximately eight true leaves.

Oviposition preference in the laboratory

Two-choice oviposition experiments were performed in plastic cages (30 × 30 × 30 cm; BugDorm-1, Mega View Science Co., Ltd., Taichung, Taiwan) placed in a climate chamber (25 ± 2 °C, 70 ± 2% RH and 12:12 h L:D). Cotton leaves and branches of clover, equal in fresh weight, were offered to ovipositing female moths. The branches of clover and the petioles of cotton leaves were inserted into plastic tubes (2.5 cm diameter, 8 cm high) filled with water and placed in the plastic cage. To avoid a positional effect, two leaves of cotton and two groups of clover branches were arranged opposite to each other in a cage, resulting in two cotton and two clover tubes diagonally situated in each cage. One male and one female, 3–4 days old, both virgins, were placed in each cage. The animals were supplied with 10% honey water solution for feeding, and were left in the cage until mating and oviposition. The egg batches on each host plant were recorded and removed daily for 3 days and used for the analyses. In each experiment oviposition of 30 females from each diet, artificial diet, cotton leaves and clover leaves, was recorded.

Attraction to sex pheromone and plant odour in the laboratory

We conducted two-choice wind tunnel experiments testing simultaneous exposure to female-produced sex pheromone and plant odour. We used a Plexiglas wind tunnel (250 × 100 cm and 100 cm high) with an air flow of 20 cm/s. The experiments were performed at 2 lx, 25 ± 2 °C and 40–60% RH. At the upwind end of the wind tunnel one Egyptian clover plant and one cotton plant were placed side by side separated by 40 cm. A filter paper (0.5 × 1.0 cm) was fixed a few centimetres in front of each of the two plants 50 cm above the floor. Each of the filter papers contained female gland extract as a sex pheromone source in a concentration equivalent to half a female gland. Thus, the equivalent of the averaged pheromone amount produced by one female was used in each experiment. New gland extract filter papers were used and the positions of the two plants were switched every 12 min. The sex pheromone extraction from the female pheromone glands was conducted according to the protocol of Anderson et al. (2007). The extracts were checked by gas chromatography to measure the concentration of the pheromone compounds. At the downwind end of the wind tunnel 3–4-day-old naïve and virgin male *S. littoralis* were placed singly in glass tubes (12.5 × 2.5 cm) 50 cm from the floor and 200 cm downwind from the odour sources. The males were tested 3–5 h into the scotophase. For 6 min the moths were allowed to respond and the time of activation, take off and landing on the filter paper of one of the two plants were recorded. Twenty-five males from each of the three diets were tested.

Field Experiments

Plants and field description

Cotton and alfalfa, *Medicago sativa*, were grown in a field for 2 successive years. As shown below, some of the experiments carried out in the first year were repeated in the second year. The experimental field was part of a vast agricultural area near the city of Assiut, Egypt (27°10′58″N, 31°10′58″E). The field area was about 1400 m^2, divided equally between the two crops. The borderline between the two plants was set parallel to the wind direction, which was determined using a small flag, although wind in this region of Egypt is very light, if any. Throughout the experiments the field area was surrounded by maize plants on three sides, and a main road on the fourth. The plants were not grown for corn; they were cut repeatedly to be used as animal fodder. Thus, effects of neighbouring plants on the tested insects were assumed to be roughly even.

Owing to active use of insecticides, the natural population of *S. littoralis* was low in the experimental field and we considered its influence on the experiments to be minimal. Furthermore, before the experiments the plants were thoroughly checked for egg batches, which we gently removed using a fine brush that did not cause visible damage to the plant.

Oviposition preference in the field

Three groups of *S. littoralis* larvae were reared in the laboratory from hatching until pupation in the rearing conditions mentioned above. The three groups were taken from eggs of the same generation of adults that were reared as larvae on the artificial diet. However, the eggs were kept at 4 °C to maintain their viability, as the three groups of animals were not reared simultaneously, but with a lag of about 2 weeks between the starting points. This was done to have each group of animals ready for field tests at the time when the previous group had been eliminated from the field. The first group was reared on cotton, the second on alfalfa and the third on artificial diet.

Seven days after pupation, 82 female and 68 male pupae were taken from the first group and placed at several points along the