

Female mate choice and modulation of oviposition preference in the moth *Spodoptera littoralis*

Amanda Karlström



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Honligt partnerval och modulering av äggleggningspreferens hos nattflyet *Spodoptera littoralis*

Amanda Karlström

Supervisor: Peter Anderson, Chemical Ecology, Department of Plant Protection Biology, SLU

Assistant Supervisor: David Carrasco, Chemical Ecology, Department of Plant Protection Biology, SLU

Examiner: Mattias Larsson, Chemical Ecology, Department of Plant Protection Biology, SLU

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Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Planning, Horticulture and Agricultural Sciences
Department of Biosystems and Technology

Abstract

In this thesis, two aspects of reproductive behaviour in the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera:Noctuidae) were studied. The first was whether female moths make a mate choice based on precopulatory assessments of male mating partners, and the second whether larval food plant experience of an ectoparasite modulates the female choice of oviposition site. The ejaculates of Lepidopteran males contribute with not only sperm, but also proteins and lipids. Certain characteristics such as body size and mating history have been shown to affect male ejaculate size in Lepidoptera. Female moths which can discriminate among males, and select mating partners providing larger ejaculates (spermatophores) would minimize mating costs associated with a high mating frequency. To assess if females make active mate choices, two-choice mating experiments were conducted. The female's mate choice in the three following cases was evaluated; 1) virgin vs. recently mated males, 2) small vs. large males, 3) males reared on the same or different host plant as the female. Females were not shown to selectively mate with males based on any of the three studied characteristics. They did, however, mate with the most actively courting male. Male spermatophore size in relation to body size and mating status were also studied, and it was shown that large and virgin males contributed with larger spermatophores than small or once mated males. In host plant selection, the use of experience could be a way for generalist species to shorten decision time when presented with several host options. Adult females of *S.littoralis* normally show an induced oviposition preference for their larval host plant. In this study it was investigated if larvae reared on host plants in the presence of the mosquito *Aedes aegypti* instead would avoid oviposition on the larval food plant. This was examined in two-choice plant oviposition experiments. Female moths with mosquito experience did not show any avoidance of oviposition on the larval host plant.

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Introduction

Female mate choice

Sexual selection was first proposed as an evolutionary agent by Darwin (1871) who suggested that it strongly affects the behaviour, morphology, mating system and life history of all species. Darwin recognized two aspects of sexual selection: 1) competition between individuals of one sex in order to gain mating access to the other sex, and 2) preferred mating choices by one sex, among the individuals of the opposite sex. When considering preferred mating choices Trivers (1972) reasoned that the sex with most parental investment will be the 'choosiest', and that individuals of the sex investing less will compete among themselves in order to be chosen. Among most insect species, it is the female that makes the highest initial investment (with the production of eggs) and therefore the more selective mate. Several studies in insects have shown female mate choices based on different male characters, such as size, song, and wing pattern (Lehmann & Lehmann, 2008; Savalli & Fox, 1998; Sheppard 1952; Wiernasz, 1989). Male attractiveness to females is in some Lepidopteran species correlated with the male larval host plant, because male scent and courtship pheromones are often derived directly from ingested plant compounds (Birch et al 1990). One example is the moth *Utetheisa ornatrix* (Lepidoptera: Arctiidae), where females select males based on the intensity of the male courting pheromone. The pheromone, derived from protective alkaloids ingested as larvae, provides the female with a measure of the male's alkaloid load as well as size, two factors which both are important in the female's reproductive outcome (Iyengar et al, 2001). Krasnoff & Roelofs (1989) demonstrated how such courting pheromones vary in composition depending on male larval diet. The nutritious quality of the male larval diet can also impact female mate choice. Delisle and Hardy (1997) demonstrated that males in the tortricid, *Choristoneura fumiferana*, were more successful in acquiring a mate, if they had been reared on a more nutritious diet as larvae. It has been suggested that polyandrous female mating choice is not only exerted by choice of

mating partner, but also 'cryptic' postcopulatory events concerning female mating behaviour and internal selection of the most viable sperm (Eberhard, 1996). In their review, Eberhard and Cordero (1995) propose that females can evaluate the vigor of the male based on quantity, and/or quality of seminal products (i.e. sperm or nutritious/protective gifts). The females would then exercise a choice by manipulating the refractory period, and the onset or rate of oogenesis and oviposition. Sperm competition has also been presented as a female's postcopulatory selection mechanism in polyandrous species. By mating repeatedly, and thereby mixing sperm from several males, females can ensure that they are fertilized by the most competent sperm (Simmons, 1987).

There are several insect species where not only the female, but also the male contributes with a large, paternal investment in the offspring. This is often given as a nutritional, 'nuptial gift' to the female before, during or after copulation (Thornhill, 1976). These investments can be divided into the three categories. The first are glandular products produced by the male. This category includes spermatophores, mating plugs, salivary secretions or secretions from dorsal glands that are received and eventually ingested or absorbed in the female. The second category comprises nutritional investments in the form of food collected or captured by the male and given to the female before copula. The third category of nuptial gifts is the one where the male himself is eaten by the female during, or after mating has occurred (Thornhill, 1976).

A spermatophore is the container in which sperm is stored during the transfer from male to female. It does not only contain sperm, but also proteins and lipids that when received by the female may be used in her egg-production and somatic maintenance (Boggs & Gilbert, 1979). In the green-veined white butterfly species *Pieris napi* a virgin male can transfer an ejaculate with an amount of nitrogen equivalent to that of ~70 eggs (Karlsson, 1998). In most insects, one mating is enough to fertilize all the female eggs (e.g. Cook, 1999; Kehat & Gordon, 1975; Unnithan & Paye, 1990). Despite this, there are many polyandrous insect species. One incentive for multiple female mating in polyandrous species with nuptial gifts has been hypothesized to be the increase in fecundity, longevity and egg-production that the male ejaculates offer (Fox, 1993; Karlsson, 1998; Sadek, 2001; Watanabe, 1988; Wiklund et al, 1993). However, increased female fecundity and longevity does not occur with multiple

mating in all species (Cook, 1999), and female life-time is generally reduced by repeated mating in species without nuptial gifts (Arnqvist & Nilsson, 2000). Females must therefore make a trade-off between the increase in fecundity and decreased longevity that multiple mating incurs. The actual act of copulation is also associated with costs, such as time and energy and an increased risk of predation.

Many species show an increase in spermatophore size with increased male body weight. It is also true for numerous insect species that ejaculate size decreases with number of previous matings (Kaitala & Wiklund, 1995; Seth et al 2002; Svård & Wiklund, 1989). In *Spodoptera litura*, males that mated a second time also showed a lower fertility than virgin males (Seth et al, 2002) Female mating behaviour in polyandrous species is affected by the received spermatophore size, a female receiving a small spermatophore will remate sooner than one receiving a large ejaculate. (Savalli & Fox, 1998; Sugawara, 1979; Wiklund and Kaitala, 1995). Hence, polyandrous females paired with virgins or large males may mate with fewer males during their life-time than females mated with small or non-virgin males.

Females that can discriminate against males with smaller spermatophores would gain fitness, not only because of the more sperm and the larger nutritious ejaculates they would receive but also because they would reduce the number of lifetime matings and thereby associated costs.

Host plant selection

An important part of the reproductive success of herbivorous insect species lies in the adult female's choice of oviposition-site. It is a result of that offspring survival and development is largely dependent on the suitability of the chosen host plant. Female oviposition choice has been described as especially important in Lepidoptera, because the offspring is highly immobile compared to the adults (Renwick, 1989). When selecting an appropriate plant for oviposition the female, to a large extent, relies on chemical cues emitted from the plant (Dicke, 2000). Generalist herbivores suffer a disadvantage compared to specialist species when it comes to processing these plant cues, due to limitations of the neural system (Bernay, 2001). In studies

comparing generalist and specialist Lepidopteran's host-choice, the generalist species often made sub-optimal choices (see Bernay, 2001 and references therein). It is possible that generalist species use earlier experience in order to enhance plant-cue processing and shorten decision time (Cunningham & West, 2008). Studies have demonstrated how larval diet affects adult female preferences (Akthar & Isman, 2003; Chow et al, 2005; Hershberger & Smith, 1967). One example is female moths of *Spodoptera littoralis* which are deterred from oviposition by extracts of a potato-based diet and larval frass from that diet, unless they were reared on the potato-diet themselves as larvae (Anderson et al, 1995). Thöming et al. (2013) showed an existence of plant preference hierarchies among ovipositing females of *S. littoralis*. However, plant preference hierarchy in this species is modified in such a way that the plant experienced by the larvae becomes of the highest preference for ovipositing females. Mechanisms for such effects have been proposed to be either exposure to chemicals from the larval environment (postimaginal conditioning), or retention of memory through metamorphosis into adulthood (preimaginal conditioning). The possibility of retention of larval experience into adult stage has been heavily debated (Barron, 2001). However, retention of associative learning through metamorphosis has been shown in both the fly *Drosophila melanogaster* (Tully et al, 1994) and in the moth *Manduca sexta* (Blackiston et al, 2008).

A female about to make a host plant decision not only needs to assess the host suitability, but also potential competition and predation. Denno et al. (1995) found that out of 193 pair-wise interactions in phytophagous insects 76 % showed evidence of competition. By choosing host plants where no other herbivores are present, the female can reduce such competition and also avoid plants where an induced systemic defense is already triggered. *Spodoptera littoralis* females are able to distinguish between volatiles from plants damaged by herbivores and non-damaged plants (Jönsson & Anderson, 1999). Behavioural implications of this differentiation include avoiding competition by showing a lower preference to oviposit on leaves damaged by conspecific larvae than undamaged leaves (Anderson & Alborn, 1999; Zakir et al, 2013) The female moths also alter their calling behaviour in response to herbivore-induced changes in host plant by spending more time calling around non-damaged cotton leaves compared to leaves damaged by larvae (Zakir, 2012). Avoidance of oviposition sites with high predation risk is an important factor in the

relatively immobile offspring's survival. Hence, females able to assess predation risk and avoid ovipositing on high-risk host-plants should be favored. Several studies show how female insects avoid egg-laying where the risk of parasitism and predation is higher (e.g. Brodin et al, 2006; Carrasco & Kaitala, 2009; Grostal & Dicke, 1999; Mokany & Shine, 2003; Resetarits, 2001). It is also found that females of both mosquitos (*Anopheles punctipennis*) and phantom midges (*Chaoborus albatus*) avoid ovipositing in pools with predators (Petranka & Fakhoury, 1991). Whiteflies of *Bemisia tabaci* avoid laying eggs on host-plants with predatory mites, especially if they have earlier experience of the predator as adults (Nomikou et al, 2003).

Study organisms

Spodoptera littoralis

The Egyptian cotton leafworm, *S. littoralis* (Lepidoptera, Noctuidae) is a highly polyphagous species with a recorded 88 species of host plants in a wide range of families (Brown & Dewhurst, 1975). *Spodoptera littoralis* is distributed throughout Africa and the Mediterranean region where it is highly destructive on crops such as cotton (*Gossypium hirsutum*, Malvaceae), groundnut (*Arachis hypogaea*, Fabaceae) and cowpea (*Vigna unguiculata*, Fabaceae).

Spodoptera littoralis is a polyandrous species with an average of 2,3 female matings in laboratory conditions (Kehat & Gordon, 1975). Courtship in the species is initiated when the male moths are attracted to sex pheromones emitted by calling females. A calling female expose her pheromone gland, which is situated on the ventral side, at the end of the abdomen. The female may also hold her wings slightly raised over the abdomen (Ellis & Brimacombe, 1980). Ellis and Brimacombe (1980) identified several behavioural patterns in the courtship of *S. littoralis* males. When activated, the male flies towards the calling female and hovers over her with his hair-pencils fully extended. The female then responds by lifting her wings, curving the abdomen and withdrawing the pheromone gland. The male positions himself beside the female while clasping the female's abdomen with his pencils, after which copulation is initiated. Rutowski (1982) identified several rejection behaviours common in Lepidopteran females in response to courting males: 1) moving away, 2) initiating

flight movements, or patterns, 3) assuming postures or displays that physically prevent copulation, 4) or doing nothing.

Host plant preferences in *S. littoralis* were studied by Thöming et al (2013) who showed a female preference hierarchy for oviposition site. Females preferred ovipositing on clover, followed by cowpea, maize, cotton and cabbage.

Aedes aegypti

Females of the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae) are ectoparasites that feed on the blood of several victims during their lifetime. Martel et al. (2011) observed *A.aegypti* feeding on the haemolymph of larvae of *S. littoralis*, and the presence of mosquitos was shown to affect both the behaviour and development of the larvae. Larvae reared with mosquitos developed slower, had a lower pupal weight and also emigrated from the host plant to a higher extent than the control group.

Objective

Female mate choice

The aim of the study is to determine 1) if females of *S.littoralis* make a selective mating choice, with the hypothesis that females favour male characteristics such as a) virginity, b) large body size and c) having the same larval rearing host as the female; and, 2) if male spermatophore size is affected by a) size and b) previous mating. The hypothesis is that large, virgin males produce bigger spermatophores than small males which have mated once.

Modulation of host plant preference

The aim of this part of the study is to establish whether oviposition preference in *S. littoralis* is modulated by larval experience of attacks by *Aedes aegypti* (Diptera: Culicidae) on host plant. The hypothesis is that females from the control group will have a higher preference to oviposit on the larval host plant, while females reared in the presence of mosquitos will avoid ovipositing on the host.

Material and method

The Egyptian cotton leafworms used in the experiments were all from the laboratory colony at SLU, Alnarp. The colony originates from individuals collected in Alexandria, Egypt and is replenished at least once a year with wild moths from the same area. Plants used as larval food as well as for experiments were grown in greenhouses (25 ± 2 °C, $70 \pm 5\%$ RH) in 1,5 l pots filled with a commercial substrate (Kronmull, Weibull Trädgård AB, Hammerhög, Sweden).

Female mate choice

In order to assess whether females prefer a certain male over another when choosing a mating-partner, two-choice mating experiments were conducted. The female's mate choice in the three following cases was evaluated; 1) virgin vs. recently mated males, 2) small vs. large males, 3) males reared on the same or different host plant as the female.

Laboratory rearing conditions

During larval stage the moths were reared in a climate chamber at, 25 ± 2 °C, $70 \pm 2\%$ RH and 17:7h L:D. Batches of larvae were reared on either; potato-based artificial diet, cotton (*Gossypium hirsutum*, Malvaceae) or cabbage plants (*Brassica oleracea* v. *capitata*, Brassicaceae). Only the foliage of the plants was fed to the larvae. After pupation, each individual was sexed, weighed and individually transferred into a marked plastic cup with a lid. Both pupae and adult moths were kept in a climate chamber at 25 ± 2 °C, $70 \pm 2\%$ RH and 16:8h L:D. Because female and large, male pupae eclosed earlier than others, they were kept colder (17 ± 2 °C, 24:0 L:D) during the first 1-2 days of pupal stage in order to delay eclosion. On the day of eclosion the adult moths were moved to 250 ml plastic jars with perforated lids and provided with two cotton-balls soaked in either honey water or water.

Table 1. Content of artificial diet provided to *S. littoralis*

Artificial diet
Potato, yeast mixture (wheat germ, dried yeast flakes, methyl-4- hydroxybenzoate, sorbic acid, asorbic acid, cholesterol), vitamin mix (nicotinamidae (Fluka 72340, cas 98-92-0), d-panthothenic acid calcium salt, riboflavin (Vit. B2), thiamine (Vit. B1), pyridoxolhydrochlorid (Vit. B 6- hydrochlorid), folic acid, D-biotin (Vit. H), cyanocobalanin (Vit. B12), sodium benzoate, distilled water, plant agar, DL- alpha- tocopherol acetate (Vit. E), oil, 96% ethanol

Mating experiments

The males and females used for the virgin vs. non-virgin (exp. 1) tests had been reared on artificial diet and were from several batches. The non-virgin males in the experiment had mated once before, with a virgin female. The first copulation took place one day before the test. The non-virgin was matched with a virgin male of the same age and weight for the mating test.

Moths used in experiment 2, small vs. large males were also reared on artificial diet. The males that were chosen for these tests significantly differed in pupal weights (paired t-test, T-Value =12,6 P-Value<0,001). Mean weight and standard error for large males were $300 \pm 7,36$ mg and $206 \pm 5,34$ mg for the small males.

Mating choice between males reared on the same or different host plant was tested on females reared on cabbage or cotton. The females were coupled with one cotton and one cabbage male of equal weights. All males and females used in experiments were of an age of either 2 or 3 days. Females were always virgins.

Table 2 Scheme of the two-choice mating tests

Female	Male 1	Male 2
Artificial diet	Small	Large
Artificial diet	Mated once	Virgin
Cotton diet	Cotton diet	Cabbage diet
Cabbage diet	Cotton diet	Cabbage diet

Mating experiments were conducted in the dark room under red light at 25 ± 2 °C, 61 ± 5 % RH, in plastic jars (height:16.5 cm, diameter:10 cm). One to three hours into the scotophase the two males were put into a plastic jar, in order to settle down before the experiment. One hour later a female was added (see fig. 1). To distinguish the two males they were painted on different wings, using water-soluble finger paint (Fingerfärg, Egmont Kärnan AB, Malmö, Sweden. In all experiments it was recorded which male that was chosen for mating as well as the copulation time. Female and male behaviors were also recorded in mating experiments with virgin/non-virgin males. Recorded female behaviors were; calling time and female active rejections to the courting male (flying away from or walking away when being approached by the male). No other rejection behaviours were recorded.

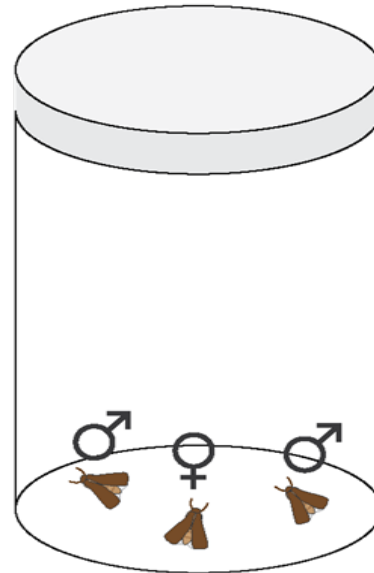


Figure 1. Plastic jars containing one female and two males were used for testing female mate choice. (Petter Lundahl, 2013)

In the limited light conditions it was difficult to detect the pheromone glands of the calling females. Calling behavior was therefore determined as when the female was wind fanning or held her wings slightly raised. Male activity was recorded for each individual male by counting number of mating attempts and take-offs towards the female. Time elapsed between female insertion into the jar and male activation (wind fanning) was also recorded for the individual males. The behavior of the animals was recorded until mating was initiated. The triplet of moths was discarded if a male was not activated, or copulation had not occurred within 60 minutes. In experiments where no behavior was recorded (i.e. small vs. large males and cabbage vs. cotton) the moths were discarded if they had failed to mate after 120 minutes.

Spermatophore size

Male spermatophore size was measured, in order to relate male characteristics such as size and previous mating with reproductive quality. The female and male moths utilized for this were the same as in the mating experiments described above, therefore the pupal weight was recorded and used as a measure of body size. Spermatophore size was examined for both first and second matings of males. The second copulation took place one day after the first mating. When copulation was completed, the mated female was frozen within one hour. The female was then dissected, the spermatophore removed and then dried for one day in a desiccator containing silica-gel. When dry, the spermatophores were weighed. The scale used had a graduation of 0.0001 g.

Modulation of host plant preference

A two choice test was conducted to examine if females of *Spodoptera littoralis*, attacked by mosquitos on the larval host plant, have a lower adult oviposition preference for this specific plant.

Larval rearing conditions

During their first week, larvae were reared in a climate chamber (25 ± 2 °C, 70 ± 2 % RH, 17:7h L:D) and fed with an artificial diet. They were then moved to cages (93 x 47.5 x 47.5 cm, mesh size < 1 mm, BugDorm-4D, Mega View Science Co., Ltd., Taichung, Taiwan) with twenty larvae in each cage, and kept in a greenhouse. All cages were set with one potted cowpea plant, *Vigna unguiculata* subsp. *unguiculata*, (Fabaceae), which was changed to a new one when it no longer suited as larval food. In half of the cages, 20 adult females of *Aedes aegypti*, (age: 4-6 days) were added together with the larvae. The mosquitos were provided with 10 % sucrose solution, and did not have any blood meal before the experiment. An additional number of 20 mosquitos were added to each cage a some days after. At pupation, the moths were collected from the cages and the females separated from the males. They were then kept in a climate chamber at 25 ± 2 °C, 70 ± 2 % RH, 16:8h L:D until experiments started.

Oviposition experiment

The two-choice oviposition tests started 1-3 days after female eclosion. They were performed in plastic cages, 30×30×30 cm (BugDorm-1, Mega View Science Co., Ltd., Taichung, Taiwan), in a dark room at $25 \pm 2^\circ\text{C}$, $61 \pm 5\%$ RH. One cotton leaf and one cowpea leaf

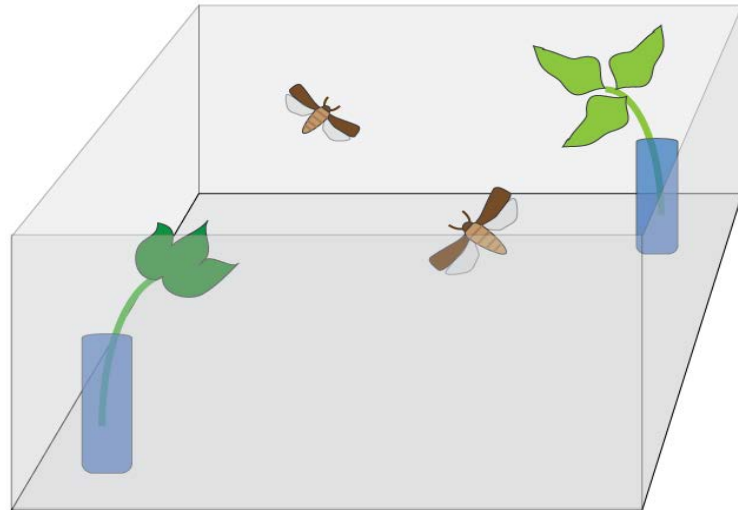


Figure 2. Experimental set-up to test oviposition preference in females of *S.littoralis*, the cage contained one female and one male moth. (Petter Lundahl, 2013)

were placed in each cage, in plastic tubes (2.5 cm diameter, 8 cm high), filled with water. The tubes were placed diagonally opposite each other (see fig. 2) in a random manner to avoid positional effects. The leaves were selected to be similar in size.

Each female was together with a male placed in a cage for three days to mate and oviposit. The moths were provided with honey-water for feeding. Cages and leaves were checked each day for egg-batches and leaves that held eggs were replaced by new. All egg-batches were recorded, then removed from their surface and weighed. The scale had a graduation of 0.1 mg.

Statistical analyses

All statistical analyses, except Wilcoxon signed rank test, were conducted in Minitab 16.1.1 Statistical Software. The Wilcoxon signed rank tests were performed in R (R Core Team, 2013)

Female mating choices were analyzed with a 1 sample, binominal test (null-hypothesis: $p=0.5$). Differences in duration of mating between large/small males and virgin/non-virgin males were analyzed with Student's *t*-test. For the virgin vs. non-virgin experiments where behaviour was studied, Wilcoxon signed rank test was used

to analyze differences in male activity. The analyses were done on difference in activity between virgin and non-virgin males, and chosen and non-chosen males. The activity for each male was calculated as the sum of his mating attempts and take-offs towards the female. The Wilcoxon signed rank test was also used to analyze differences in female rejection between virgin and non-virgin males. In order to do so, a ratio was calculated by dividing female rejections with the number of male mating attempts. The ratios for virgin and non-virgin males were then analyzed. The trials, in which one of the males failed to make any attempts, were not included in the analysis.

The difference between spermatophore size from first and second mating of the same male was analyzed with a paired *t*-test. Existence of correlation between male body size and spermatophore size was analyzed with Pearson correlation.

For the oviposition tests two indexes were made for each female:

$$1) \frac{\text{weight of eggbatches, cowpea} - \text{weight of eggbatches, cotton}}{\text{total weight of eggbatches on cowpea + cotton}}$$

$$2) \frac{\text{number of eggbatches, cowpea} - \text{number of eggbatches, cotton}}{\text{total number of eggbatches on cowpea and cotton}}$$

To determine whether there was a preference for any of the plants within each treatment, a 1-sample sign test was performed on both indexes and both treatments (H_0 : median = 0). To conclude whether there was any difference in egg-laying preferences between the two treatments, index-values (for both number of eggs and weight of eggs) were analyzed with a Mann-Whitney test. In the results all mean values are presented with standard error of the mean.

Results

Pupal weight

Male pupae reared on the artificial diet had a mean weight of 247 ± 2.67 mg, and female pupae a mean weight of 265 ± 4.01 mg. The mean weight for male cotton pupae was 199 ± 4.91 mg and 216 ± 7.72 mg for female. Cabbage pupae had a mean weight of 203 ± 4.93 mg for males and 179 ± 6.09 mg for females.

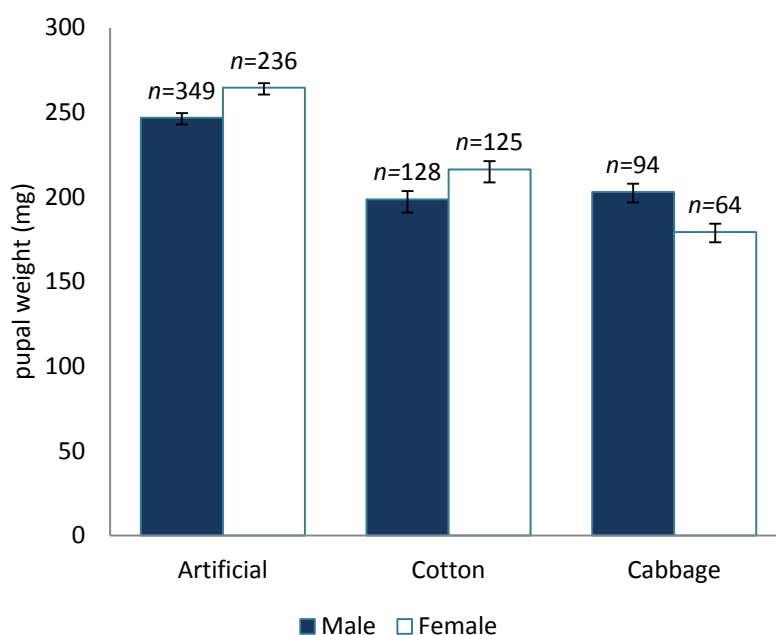


Figure 3. Mean of weight and standard error for pupae reared on the three different substrates; artificial diet, cotton and cabbage.

Female mate choice

Large vs. small male

53 % of the females mated with the large male (fig. 3a). The probability of the female to mate with either of the two males, was not significantly different from 50 % ($n=15$, $P=1.000$). There was no difference in mating time between large and small males (T -Value=0.60, $P=0.569$, $DF=7$). Mean mating time for large males was $60.9 (\pm 3.79)$ minutes ($n=8$), and for small males $68.9 (\pm 12.8)$ minutes ($n=7$).

Cotton vs. cabbage male

Females reared on cabbage chose cabbage males in 43.8 % of the mating experiments (fig. 3c). The statistical analysis showed that there was no significant preference among the cabbage females to mate with any certain male ($n=16$, $P=0.804$). Females reared on cotton mated with cotton males in 64.3 % of the experiments (fig. 3d), they had no significant preference between the males ($n=14$, $P=0.424$).

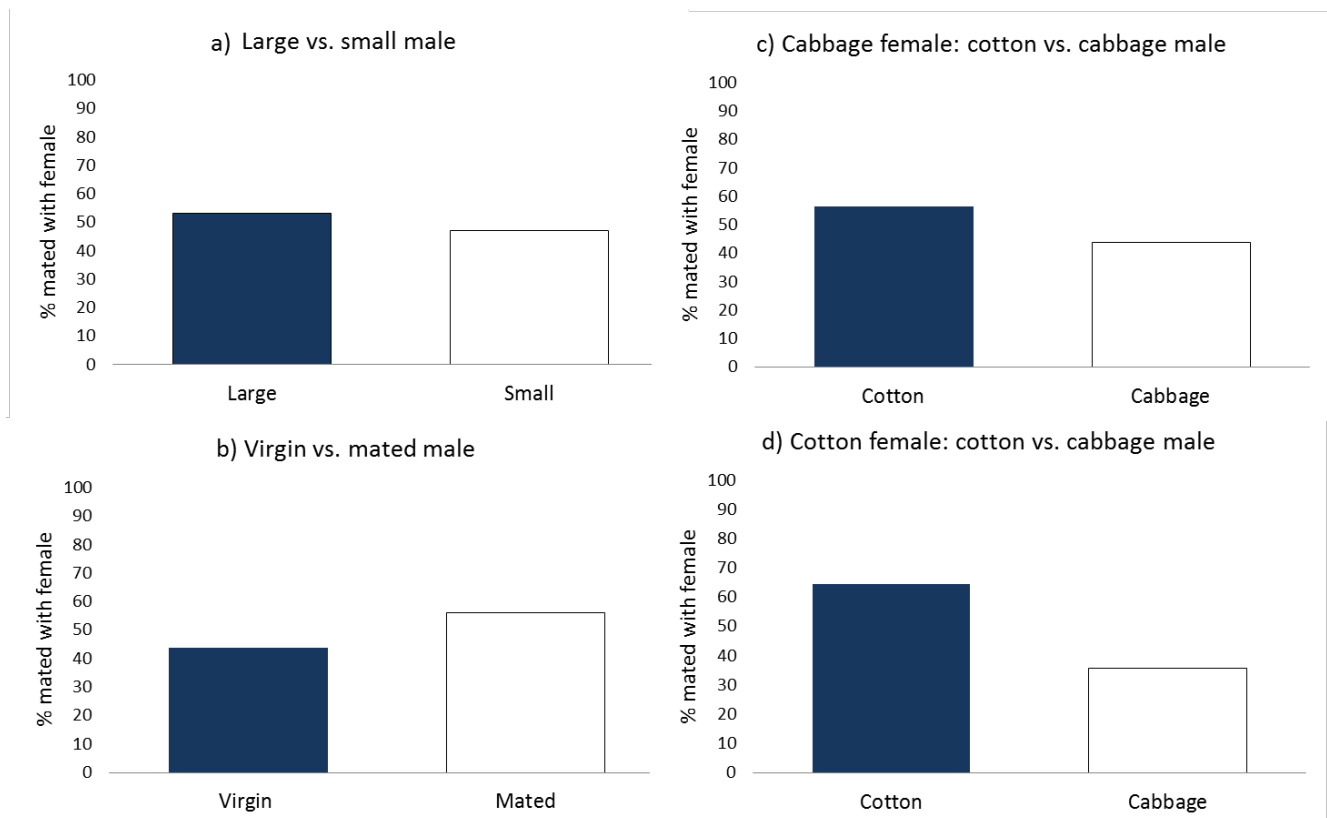


Figure 4. Chosen mating partner by females of *S. littoralis* in the following two-choice tests; a) large vs. small male, b) virgin vs. mated male, c) cabbage female choosing between cotton and cabbage male d) cotton female choosing between cotton and cabbage male. Results are shown as the percentage of males that successfully mated with the female. The females showed no significant preference, in any of the two-choice experiments, to mate with males of certain characteristics.

Virgin vs. mated male

Females called on an average of 2.80 (± 2.38) min before copulation was initiated. Virgin males were on an average activated after 0.47 (± 0.27) minutes, while non-virgins were activated 0.07 (± 0.07) minutes after the female was added.

Females mated with virgin males in 43.8 % of the trials (fig. 3b). There was no significant difference in which male the female finally mated with ($n=16$, $P=0.804$). Nor was there any difference between virgins and non-virgins in number of female rejections per male mating attempt ($n=9$, $P=0.834$). Mean and standard error for virgin male ratio (female rejections /male mating attempts) were $0.32 (\pm 0.22)$, and for non-virgin males $0.51 (\pm 0.19)$ (fig. 4).

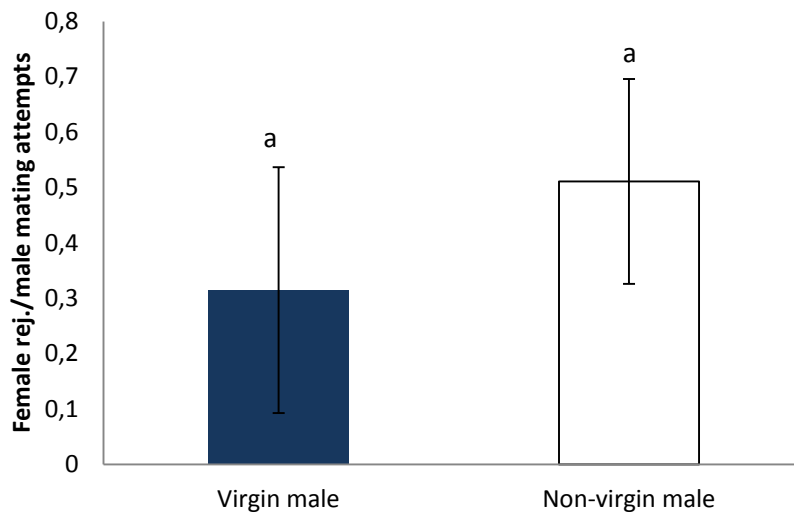


Figure 5. Mean and standard error of the ratios between female rejections and male mating attempts are shown for virgin and non-virgin males

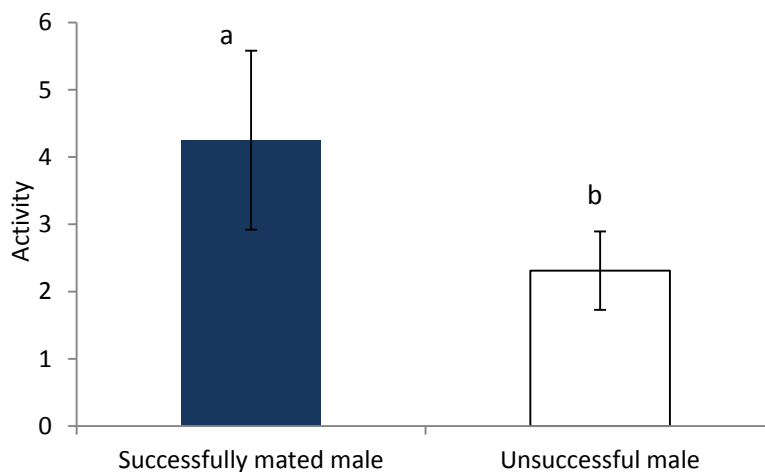


Figure 6. Mean and standard error of male activity (number of mating attempts and take-offs towards female) for the males that succeeded to mate with a female, and the males that did not. Male activity was only recorded in the virgin vs. non-virgin two-choice experiments.

There was a difference in activity (i.e. take-offs and mating attempts) between the male that successfully mated with the female and the unsuccessful male ($n=16$, $P=0.029$). Mean of activity and standard error for successful males were 4.25 ± 1.33 ,

and for unsuccessful males 2.31 ± 0.58 (see fig. 5). Virgin and non-virgins did not exhibit any difference in activity ($n=16$, $P=0.290$).

Mating times in virgin and already mated males did not significantly differ (t -Value=1.21, $P=0.272$ d.f=6). Mean and standard error of mating-times for virgin males were 85.4 ± 19 ($n=7$) minutes, and for mated males 62.3 ± 3.9 ($n=9$).

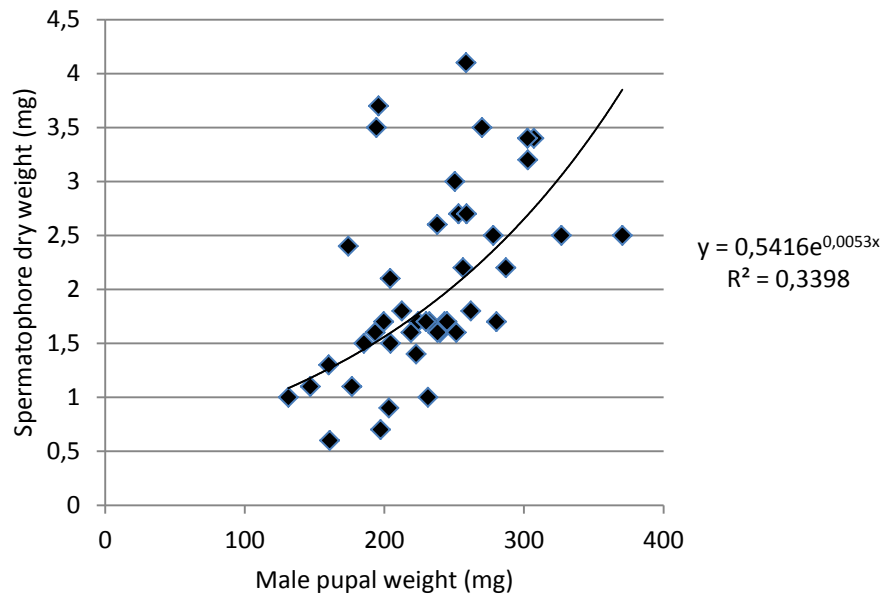


Figure 7. Spermatophore dry weight plotted against pupal weight of males of *S. littoralis*. Included in the graph are regression-line with equation and R-square.

Spermatophore size

A correlation ($r=0.531$, $P < 0.001$) between male body weight and weight of spermatophore was found. The spermatophore size differed significantly between a male's first mating and the mating performed the day after ($n=13$, t -Value=4.59, $P=0.001$). Mean spermatophore weight after the first mating was $1.93 (\pm 0.175)$ mg dry weight, and after the second $0.99 (\pm 0.21)$ mg dry weight.

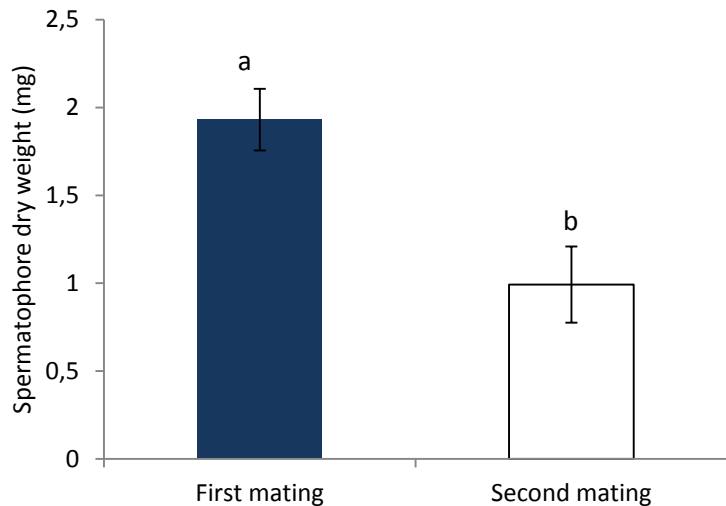


Figure 8. Mean and standard error of spermatophore weights from the first and second mating of male *S. littoralis*.

Modulation of host plant preference

Female moths reared in the presence of *Aedes aegypti* laid 45.5 % of their eggs on the plant-leaves, while the rest of their eggs were deposited on the cage. The same number for the control females was 54.5 %. Females within the mosquito treatment laid more eggs on cowpea than on cotton ($n=11$, $P=0.022$). In this treatment there was also a tendency to deposit a larger egg-mass on cowpea than on cotton ($n=11$, $P=0.065$). In the control treatment the females did not preferably oviposit their egg-batches on any of the plants ($n=11$, $P=0.180$), nor did they lay larger egg-masses on any of the plants ($n=10$, $P=0.109$). Indexes for females of the two treatments did not differ regarding number of egg-batches ($P=0.136$) (fig.9), or weight of egg-batches ($P=0.126$) (fig.10)

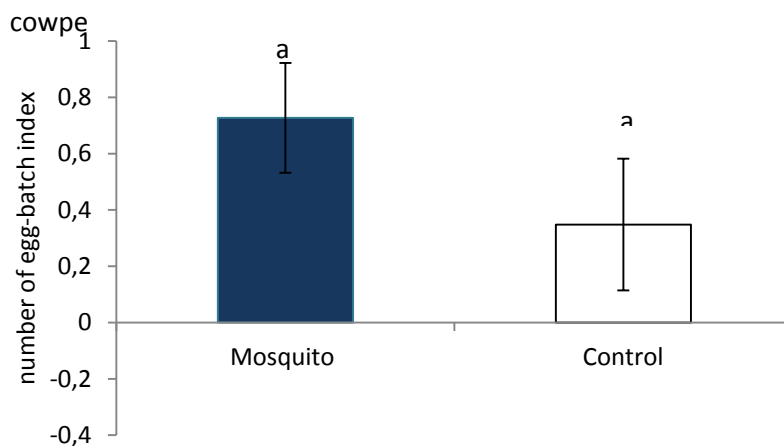


Figure 9. Index values for number of egg-batches in mosquito and control treatment. Presented as mean and standard error. 1= total number of egg-batches deposited on cowpea, -1= total number of egg-batches deposited on cotton.

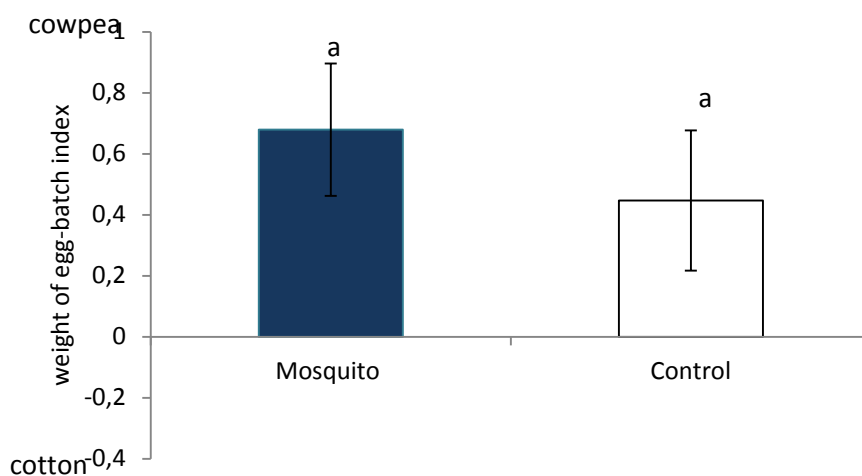


Figure 10. Mean and standard error of index values for weight of egg-batches, on mosquito and control treatment. 1= total weight of eggs oviposited on cowpea, -1= total weight of eggs oviposited on cotton.

Discussion

Female mate choice

Male body size and previous mating, was shown to affect the spermatophore size in *S. littoralis*. Large and virgin males produced larger spermatophores than small or recently mated males. Females that mate with large, virgin males would therefore gain bigger 'nutrient-packages', and can avoid costs of multiple matings. However, females of *S. littoralis* did not selectively mate with males showing these characteristics, nor did they reject already mated males more than virgins. Instead females finally mated with the most actively courting male. Hence, the females may be unable to make precopulatory assessments of male quality and make mating decisions accordingly. This is consistent with findings by Kaitala and Wiklund (1995) in *Pieris napi*, where females were unable to distinguish between virgin and mated males. Instead, as also found in this study, females of *P.napi* copulated with the most active male. Postcopulatory factors (e.g. cryptic female choice) could instead be a mechanism of sexual selection on males in *S. littoralis*. It is unlikely that sperm competition is an important selective force in *Spodoptera littoralis*, because Seth et al (2002) reported that the close relative *Spodoptera litura* showed an almost total sperm precedence for the last mating. Selection in such a species will favor males that are able to induce a longer female refractory period, i.e. males with large spermatophores (Kaitala & Wiklund, 1995; Wiklund & Kaitala, 1995), because their sperm will be available for fertilization for a longer time before being replaced by sperm from a new male. Female mating behaviour in *S.littoralis* is affected by male quality, as showed by Sadek (2001) whom found that females remated if they were paired with an already mated male, but not if they were paired with a virgin.

Females of *S.littoralis* did not selectively mate with males that had been reared on the same diet as them; hence, there is no evidence for a mechanism of reproductive isolation in the female mate choice. Such reproductive isolation could have given rise to host races and eventually speciation. The related, polyphagous moth *Spodoptera frugiperda* is a good example of a diverging population (Schöfl, 2011). The specie holds two different host strains (somewhat overlapping in hosts) which

occur sympatrically throughout South and North America, and are thought to be held separated partly through a temporal difference in mating activity during the night. In a study by Schöfl et al (2011) females of *S. frugiperda* were shown to mate more assortatively with members of the own host race, thereby contributing to the reproductive isolation more than males.

Choosiness in picking a mating partner is, compared to random mating, associated with costs for the female moth. Such costs include the increased risk of predation, risk of disease transmission, energetic costs and time loss (Pomiankowski, 1987). Female assessment and discrimination among males is more time consuming than mating with the first available male. Actively resisting male courtship can be an energetically costly behaviour for the female, especially when males are persistent. Other energetic costs associated with female choice are the specialized morphology, musculature, neurophysiological pathways or sensory capabilities that could be necessary to discriminate among males (Pomiankowski, 1987). Females of the field cricket, *Gryllus bimaculatus* removed the spermatophores of small males prematurely, in order to sooner remate and thereby selecting the paternity of the offspring (Simmons, 1986). By thus using multiple mating as a mate choice, female crickets are subjected to the costs of time loss and increased predation risk during courting and copula. However, costly choice could still be favoured, if the trait that choosy females prefer increases the fitness of the offspring, and that the increase is larger than the cost for the female to be picky (Pomiankowski, 1987).

Host plant preference

In the oviposition experiments females reared together with mosquitos of *Aedes aegypti* laid significantly more eggs on the larval host (cowpea) than on the non-experienced plant (cotton). In the control, however, females did not prefer to oviposit on any of the plants. There was no significant difference between the two treatments in preferred plant for oviposition. The results do not support the hypothesis that females which were attacked by mosquitos as larvae would have a lower preference for their larval host than the control. This can be set in relation to an earlier study, which showed how females of *S. littoralis* avoid subjecting their offspring to

competition and natural enemies by not ovipositing on herbivore-damaged plants (Anderson & Alborn, 1999). It has been proposed that predation is the largest cause of mortality in herbivorous arthropods (Feeny et al 1985; Hairston et al, 1960; Rosenheim et al, 1993) Females which make a host plant choice that minimizes the risk of predation will therefore have an increased fitness. The use of earlier experience in order to avoid natural enemies would able the polyphagous females to make quicker, and more correct oviposition decisions. Evidence supporting this has been found in the earlier mentioned study by Nomikou et al (2003), where females of the whitefly *Bemisia tabaci* which were experienced with the predator showed a much stronger preference for predator-free plants than non-experienced females. In the study with *B. tabaci*, females avoided oviposition on plants harbouring live predators. It is possible that, similarly, the females of *S.littoralis* only avoid oviposition if there are cues recounting the presence of mosquitos. A strategy of always avoiding certain host plants because of a risk of natural enemies could be suboptimal, since there is a high temporal variability in enemy distribution on plants (Scheirs & De Bruyn, 2002).

As a conclusion of this study and the above discussion the following can be said; females of *S. littoralis* do not make precopulatory mate choices, but may instead alter their mating behaviour depending on the quality of their male mates. This can have implications if Sterile Insect Technique were to be employed against *S. littoralis*. In addition, females of *S.littoralis* did not avoid a host species even though the plant was associated with mosquitos during their larval stage. The result could indicate that the moths avoid oviposition when predator cues are present, but not the associated plant species alone. Larval experience of mosquitos could, however, still have behavioural impacts when confronted with mosquito cues as an adult moth. Further research in this area would therefore be interesting.

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