

Life cycle of spodoptera litura



Insects have been around for more than 400 million years and it could be argued that they are the most successful and enduring life form that has ever arisen on this planet. Insects are abundant and ubiquitous. From the poles to the equator, from the surface of the sea to the highest peaks and from deserts to rain forests it is estimated that there are somewhere in the range of 1×10^{18} individuals on earth at any given time.

The described species of insects are distributed unevenly amongst the higher taxonomic groupings called orders. Five major orders stand out for their high species richness, the beetles (Coleoptera), flies (Diptera), wasps, ants and bees (Hymenoptera), butterfly flies and moths (Lepidoptera), and the true bugs (Hemiptera). The Hymenoptera have more than 115,000 described species, with the Diptera and Lepidoptera having at least 150,000 described species each, and Hemiptera, almost 100,000. Of the remaining orders of living insects, none exceed the approximately 20,000 described species of the Orthoptera.

Insects are so important to the continued working of the global ecosystem that, as long as the well-being of insects is safeguarded, the earth should remain habitable for humans (Berenbaum, 1995). On the other hand insects are also known for the damage they can cause to the agricultural crops. On average, one fifth of all crops grown around the world are eaten by insects.

Several approaches to pest management have yielded good results, however, with associated problems. Chemical control programs though gave good results initially, are posing problems in the form of environmental pollution, biomagnification, soil infertility and so on, in addition to

development of resistance by insects to these chemicals, which has worsen the problem. Resistance by insects led the farmers to increased application of chemicals or switch to another chemical which will also end up with the same fate.

Alternative to chemicals such as pheromone trapping, transgenic plants, and several biopesticides are doing rounds in farming sector. However, are not enough to control some of the pests which are notorious by being polyphagous. An alternative thinking, of the late, conceived by scientists is to use the insects own products, on the lines of pheromones, such as proteins/peptides especially the behaviour modifying peptides and or neuropeptides to control the pest insects. Use of naturally occurring behavior modifying peptides/proteins involved in the regulation of biological processes to interfere with the pest's own mechanisms resulting in its failure to successfully reproduce could be a novel approach. The advantages of the likelihood of being specific, eco-friendly and sustainable, provided, an effective method is evolved for employment of these proteins; it could turn out to be an effective, if not total replacement, an alternative for chemical pesticides. It would also be possible to artificially manipulate the physiology of the pest by designing synthetic analogs of the behavior modulators as well as cloning the responsible genes and application of genetic engineering to over-express such genes to interfere with the regulatory mechanisms of the insect pests. For the development of specific and safe insect control strategies utilizing peptides/proteins, a clear knowledge of the underlying molecular mechanisms involved is essential.

Several studies have shown the presence of such peptides in the reproductive system of male that are transferred to female at the time of mating and take a control over her post mated reproductive behaviour (Kingan et. al., 1993). To identify such proteins it is important to understand the reproductive behaviour of insects so that one can track the behaviour to biomolecule.

Successful reproduction results from a succession of interdependent steps which are often completely different in nature and take place at various times in the insect's biological cycle. Both nervous and hormonal mechanisms are important in coordinating these interdependent steps in addition to complex coordination of sensory input and motor responses. Synchronization of copulation with a number of factors is the hallmark of a successful reproduction that has been evolved by many insects over a period of time through several generations. Presence of mature or nearly mature male and female gametes, ability to produce the secretions necessary for sperm transfer in male and availability of nutrients for egg maturation in female synchronizing with copulation ensures the success of reproduction. The underlying neuronal and hormonal machinery ensures this requirement and the neuroendocrine system forms the essential link connecting the two coordinating systems.

The occurrence of a number of peptides/proteins that regulate reproduction have been studied in several insects (review/ many ref Holman et al., 1990) which are shown to be synthesized, activated or released at appropriate periods. Insects have evolved a simple mechanism to synchronize the occurrence of peptides/proteins in female with its reproductive phase by

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introducing them at the time of mating. Majority of such peptides originate in the Accessory glands associated with male reproductive system though some of them originate in ejaculatory duct and some times in testis.

In spite of their morphological diversity, the male accessory glands exhibit several commonalities, as far as basic features are concerned, between several insect groups (Chen, 1984). The accessory glands arise as discreet outpocketings of the vas deferens, seminal vesicle or Ejaculatory duct which themselves originate from the coelomic cavities of the ninth or tenth abdominal segment. These glands are considered as functional homolog of the human prostate (Bertram et al., 1992). They originate from a special set of cells in the male primordium of the genital disc (Nöthiger et al., 1977) whose developmental fate is determined by the male sex determination pathway during larval period. A single layer of secretory cells set on a basement membrane forms the gland wall, outside which is a muscle layer of varied composition. The glandular cells generally have an ultra-structure similar to cells concerned with the production of proteinaceous material and export. Rough endoplasmic reticulum and well developed Golgi complex is prominent throughout the cells. The nucleus is large and may contain a number of nucleoli. The apical plasma membrane is folded into microvilli of variable density, and the basal plasma membrane may be deeply indented into the cell (Gillott and Gaines, 1992).

The role of male accessory glands in reproduction is undoubtedly proved, by several studies, to be not just essential but critical in insect reproduction.

Elicitation of two major responses in the female: elevation of oviposition and repression of sexual receptivity have been attributed to male accessory

gland secretions which enter the hemolymph following mating and affect the nervous and/or endocrine system. The main function of male accessory glands is the production of spermatophore for sperm transfer from male to female. Even in some species that do not use a spermatophore, the glands may fulfil a variety of functions (Hinton, 1974).

Male accessory glands contents generally includes carbohydrates (both free and complexed with protein), some lipid (normally bound to protein), and small amounts of amino acids and amines (Gillott, 1988). In some species, for example, cockroaches uric acid is present in the accessory gland secretions (Roth, 1967), in several of others, prostaglandins (Lepidoptera), juvenile hormones (Lepidoptera and mosquitoes) (Borovsky et al., 1994; Park et al., 1998) and various toxic materials that serve as egg protectants following their transfer to the female (Blum and Hilker, 2002; Eisner et al., 2002) are present. However the major components of male accessory gland secretions, in both quantity and importance as modulators of female reproductive activity, are proteins (Gillott, 2003). Once they are delivered to female along with sperms, they virtually regulate almost all the post mated behaviour of that female such as calling, mating, egg maturation, egg laying and so on.

The role of peptides/proteins of male accessory glands on the female reproductive behaviour, though, has been demonstrated in a number of insects, similar information available on *Spodoptera litura* a polyphagous pest insect is very scanty.

Spodoptera litura Fabricius (Lepidoptera: Noctuidae), though, commonly known as tobacco cutworm, is not restricted to tobacco alone, but, feeds on more than 120 host plants belonging to 44 families (Qin et al., 2001). It is a serious polyphagous pest in Asia and Oceania, from the borders of North Africa to Japan and New Zealand (Armes et. al., 1997). It has a large host range of crop species such as cotton, groundnuts, jute, maize, rice, soybean, tea, tobacco, capsicum, cucurbit, potatoes and so on. Not just that, it feeds on weeds and ornamental plants too (Ramana et al., 1988). Hence, *Spodoptera litura* is an appropriate choice to explore and unravel the mechanism underlying the regulation of reproduction.

This background formed a basis for selection of the topic isolation and characterization of male derived factors modifying the physiology of oviposition in lepidopteran pest *Spodoptera litura*. The study was planned with following objectives.

1. To study the life cycle of *Spodoptera litura*.
2. Localization of proteinacious factors from the male reproductive system of *S. litura* which are responsible for modifying the physiology of egg production and egg laying.
3. Purification and Characterization of the protein
4. Determination of the amino acid sequence of the protein.

As a prelude, for effective implementation of the above objectives, following investigations were carried out.

1. Standardization of an efficient rearing method to establish insect colonies since mass rearing was necessary for studying the physiology

of the insect and also for pooling of accessory gland tissue from the male moths.

2. Study of life history characteristics of *Spodoptera litura*, as the literature available revealed that development varied when reared in different conditions.
3. Identification of parameters for quantification of the reproductive behaviour in female moth.
4. Development of an efficient bioassay technique for elucidating the influence of various male derived components on the reproductive behaviour of the female moth.

To understand the status of research at both national and international level, on accessory glands secretion and their role in reproduction and pest status of *Spodoptera litura*, an extensive literature survey was carried out which helped in identifying the objectives and also to design the experiments. An effort was also made to understand similar work carried out on other insects which may help in appreciating the present work.

Secondly, as with any other phenomenon, the diversity of insects does not permit us to extrapolate the understanding of these mechanisms in one insect to even a closely related insect. In most insects, juvenile hormone regulates the secretory activity of the male accessory glands, but in some species allatectomy appears to have no effect on the glandular function (Chen, 1984). While the sensory system, receiving stimuli both from the environment and insect's own body, plays an important role in the initiation, mediation and termination of behaviour, endogenous factors which reflect

the physiological state of the insect modulate the expression of behaviour and ensure its biological appropriateness (Bali, 1998). There are several essential points that await future experiments for clarification. (a) The male accessory secretions elicit two major responses in the female: elevation of oviposition and repression of sexual receptivity. The secretory substances enter the hemolymph following mating and affect the nervous and/or endocrine system. The precise targets, however, are unknown.

Identification of the target sites will be an initial step towards understanding the problem is exacerbated by the difficulties of rearing insects on defined diets.