Salivary gland proteins in *Drosophila nasuta nasuta* during pupariation.

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In *Drosophila* the larval salivary glands consist of two types of cells: secretory cells and duct cells, which develop from the lateroventral plates of anterior ectoderm. During the larval stage the secretory cells synthesize glue proteins, which are extruded out during the onset of pupal stage. These secretions are believed to help the pupae to attach to the substrate (Fraenkel and Brookes, 1953; Korge, 1977; Riddiford, 1993). After the glue has been discharged from the salivary glands, the gland tissue continues biosynthetic activity until autolysis of the salivary glands is induced following pupal formation (Loveriza and Mitchell, 1982). The prepupal salivary glands in *D. melanogaster* continue to function as a secretory organ throughout the prepupal stage and after pupation (Mitchell *et al*., 1977). There is evidence to show the accumulation of these secretory proteins in the pupation fluid (Sarmiento *et al*., 1982). In *D. gibberosa* the rate of protein synthesis continued to increase through the late larval stage and pupariation. It has been proposed that the secretory proteins of the pre-pupal salivary glands have a functional role in early pupation of *D. gibberosa* (Shirk *et al.*, 1988). A kunitz type protease inhibitor related protein is synthesized in *Drosophila* prepupal salivary glands and released into the moulting fluid during pupation (Kress *et al.*, 2004). Although ample information is available on salivary gland secretions and its function in *D.* melanogaster, very little is known regarding the salivary gland products of the evolutionarily closely related *D. nasuta* subgroup.

The *nasuta* subgroup of *Drosophila*, which belongs to *immigrans* group, includes an assemblage of morphologically almost similar species/subspecies. The members of this subgroup have been categorized based on their morphophenotypic complex (Nirmala and Krishnamurthy, 1973). *D. n. nasuta* belongs to the frontal sheen complex. Studies on larval glue proteins in various members of *D. n. nasuta* subgroup have revealed that the glue is produced in copious amounts, and most protein fractions are X-chromosomal in origin (Ramesh and Kalisch, 1988, 1989). But the nature and pattern of protein synthetic activity beyond the larval stage has not been studied so far. Hence, a preliminary study was undertaken to get an insight on the salivary gland proteins synthesized during the prepupal stage in *D. n. nasuta.*

For the present study the *D. n. nasuta* stocks obtained from Drosophila Stock Centre, University of Mysore were maintained and amplified at 22±1°C. Synchronized eggs were collected by following the modified Delcour method and were allowed to hatch. The prepupae which were white and with everted spiracles were collected. These white prepupae just after eversion of spiracles were referred to as 0 hr prepupae.

Salivary glands from various prepupal stages were dissected, and the secretions were precipitated in absolute alcohol. Further they were processed in chloroform methanol (1:1) according to the protocol established by Ramesh and Kalisch (1989). The precipitate was dissolved in SDS-PAGE sample buffer pH 6.8 (62.5 mM Tris, 5% β-mercaptoethanol, 2% SDS, 10% glycerol, 0.001% bromophenol blue) and subjected for 13.7% SDS PAGE initially at 40 V and later at 70 V. Staining was carried out using CBB R-250, and the gel was documented using Molecular analyst Software from Bio Rad of Gel Doc 1000 (Bio Rad, USA).

The glands were dissected at different hours to check for documenting changes in the morphology. The bloated glands filled with secretions appear transparent. The 0 hr prepupal salivary glands were sticky and devoid of secretions. The 3 hr prepupal salivary glands seem to regain their rigidity with no sign of any histolytic activity. Following a quiescent phase, the glands again appear to secrete proteins, which are transported into the lumen observable by 5-6 hrs. There was a steady increase in protein content by 8 hr, but the secretions do not bloat the glands as it happens in the late larval stage. The secretions, however, are no longer observable by 9 - 10 hr. By 12 hr the lumen was not observed. The glands seem to look quite fragile at this time point.

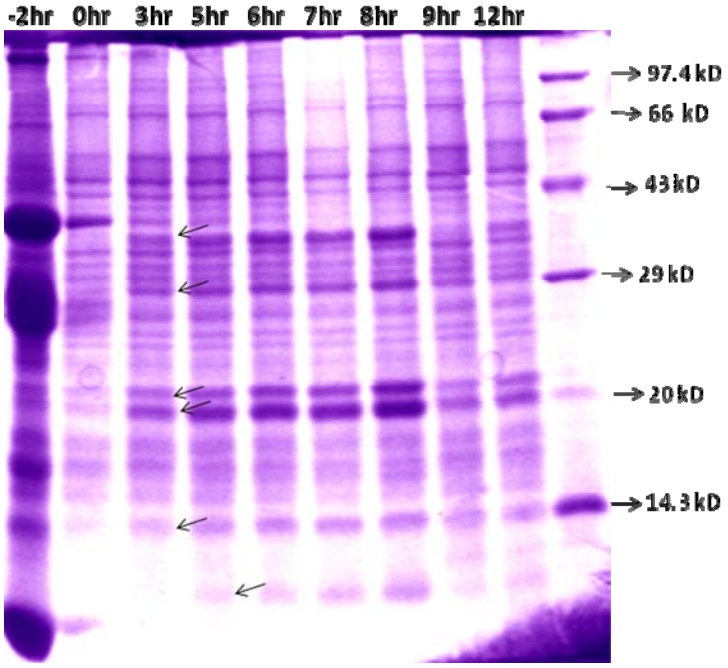

Figure 1. 13.7% SDS-PAGE protein profile of the salivary glands in *D. n. nasuta* at different time points in the prepupal stage. Arrows depict new protein fractions synthesized in the prepupal stage.

Electrophoresis of the salivary gland proteins during late larval and prepupal stage revealed that the larval salivary secretions are completely replaced by a new secretory profile in the prepupal stage. The 0 hr profile shows residual larval secretions, but by 3 hr renewal of protein synthetic activity is observed. This secretory activity consistently increases until 8 hr; but, by 9 hrs. there a sharp decrease in the secretory protein content of the glands. Between 9-12 hr there is no gross level difference seen in the protein content. This is consistent with morphological changes observed. Around 8 hr the lumen is found to contain maximum secretions. These secretions are no longer visible by 9-10 hr in the gland lumen.

The larval salivary secretions are replaced with new secretions specific to the prepupal stage. There is an increase in the secretion of low molecular weight proteins (Sarmiento *et al*, 1982). The glands continue their secretory activity until they undergo histolysis. In *D. n. nasuta* the larval salivary glands release the salivary glue proteins just prior to pupariation. After a short so-called inactive phase, which lasts for about 1-2 hr, there is a revival of protein synthetic activity of the salivary glands. This definitely confirms that the prepupal glands perform some function well beyond the larval stage. This synthetic activity continues for a few hours followed by a quiescent period again. The function of these proteins is not clear yet. By 9- 10 hr, the secretions are no longer visible in the lumen of the glands. We presume that these secretions might get discharged into the pupal case where they might serve some function. But this presumption needs to be ascertained in further experiments to understand the nature of these secretions and their functional as well as evolutionary significance.

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Raw whole *Drosophila* genome sequence traces have contaminant sequences from bacterial symbionts.

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Abstract

Many *Drosophila* genomes have recently been sequenced and assembled, and many more genome sequencing projects are in progress. *Drosophila* species have bacterial, fungal, and protozoan symbionts; therefore, DNA from these symbionts may be isolated in the course of sequencing *Drosophila* genomes. Here, we assess how much sequence is isolated from these symbionts and whether sequence contamination from symbionts affected the assembly of published *Drosophila* genomes. We find raw sequence from bacterial symbionts and humans in genome sequence traces analyzed. Surprisingly, the four most-common contaminant species were shared among the *Drosophila* genomes. However, we do not find evidence of bacterial sequences in two published *Drosophila* genome assemblies.

Introduction

The genus *Drosophila* has been a vital model system in studying adaptation, speciation, and genetics (*e.g.*, Morgan, 1910; Mustonen and Lassig, 2007; Matute *et al*., 2010;). Further investigation of such processes can now be accomplished in *Drosophila* species using bioinformatic tools and data sets, because an increasing number of *Drosophila* genomes have been assembled and annotated (Clark *et al.*, 2007). Bacterial, fungal, and protozoan symbionts, however, live in the gut and on the exterior surfaces of the *Drosophila* species (Ebbert *et al.*, 2003; Cox and Gilmore, 2007), and sequences from these symbionts may contaminate sequence from the focal organism (*e.g.*, Salzberg *et al*., 2005). If this "contamination" is not eliminated from raw genomic data sets, it has the potential to be incorporated incorrectly into a final genome assembly.

To alleviate this concern, researchers bioinformatically filter out raw sequence reads from known symbionts or rear flies in a manner that limits their bacterial content (Myers *et al*., 2000; Clark *et al.*, 2007). Filtration of all bacterial sequences may, however, be difficult when sequencing a genome *de-novo* or assembling a genome without knowledge of all potential symbionts. Whole adult