O'Grady, 2006). Thus, data from both our population genetic analysis and haplotype networks support the notion that regional populations may be isolated from each other by environmental barriers. Sampling additional parts of the species range would reveal whether low mt COI variability is characteristic of these species as well as whether differentiation among regions exists.

Table 2. Table of sequences used for population genetic summary and haplotype network.

Species and/or line #	n	Collection Location	Genbank accession #
D. emarginata			
Emarginata 1	1	Gamboa, Panama	HQ696949
Emarginata 2	17	Gamboa, Panama	HQ696950
Emarginata 3	1	Gamboa, Panama	HQ696951
14042-0841.0	N/A	Turrialba, Costa Rica	AF045108
14042-0841.4	N/A	La Palma, El Salvador	AF045110
14042-0841.7	N/A	Quito, Ecuador	AF045109
D. sturtevanti			
Sturtevanti 1	22	Gamboa, Panama	HQ689648
Sturtevanti 2	5	Gamboa, Panama	HQ689649
Sturtevanti 3	1	Gamboa, Panama	HQ689650
Sturtevanti 4	1	Gamboa, Panama	HQ689651
14043-0871.0	N/A	Turrialba, Costa Rica	AF045098
14043-0871.2	N/A	Volcan, Soufriere, Lesser Antilles	AF045099
14043-0871.9	N/A	Martinique, West Indies	AF045100

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Preliminary studies on isolation and characterization of major glue protein genes in *Drosophila nasuta nasuta*.

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Abstract: The *Drosophila nasuta* salivary gland secretion protein gene/s are coordinately expressed in salivary glands of third instar larvae. The secretion consists of glycoproteins that could

be electrophoretically separated into at least seven bands. We have focused our attention and to isolate and develop the probes to localize the X-linked glue protein gene/s (encoding 43 kD proteins in *D. nasuta nasuta*). The RNA was isolated from the larval salivary glands of *D. nasuta nasuta* by standard protocol using Qiagen RNeasy kit. We obtained good yield of RNA (972 µg /ml). Degenerate primers were designed based on sequence information (Roth, *et al*., 1999, NCBI Sequence viewer Accession NM-057369) and used in RT-PCR for the synthesis of cDNA. Preliminary RT-PCR experiments were carried out to obtain cDNA. Alterations in different steps in the RT-PCR are being worked out to obtain better yield of cDNA. An alternative approach to understand genetic differentiation in the X-linked fraction in *nasuta*, subgroup polyclonal antibodies against 43 kD glue protein fraction of *D.n.nasuta* have been raised for the purpose of N-terminal sequencing. Key words: *D. nasuta nasuta*, salivary gland, glue protein gene/s, RT-PCR

Introduction

The *nasuta* subgroup of *Drosophila,* which belongs to immigrans group, includes a number of species. The species of this subgroup have been excellent material for analysis of the patterns and process of differentiation in closely related species. In the last three decades the data generated by the analysis of protein and isozymes polymorphism of a number of loci by gel electrophoretic techniques in various species and populations of *Drosophila* has helped us to understand the patterns and process of genic variations and population/species differentiation (Ayala *et al.,* 1972; Ramesh and Rajasekarasetty, 1980; Aruna and Ranganath, 2004; Aruna and Ranganath, 2006). Many results obtained by glue protein analysis in *Drosophila nasuta* subgroup have provided enough circumstantial evidence to propose that the secretions of the larval salivary glands in *Drosophila* probably have more functions, in addition to helping the pupa to fix to a solid surface (Beckendrof and Kafatos, 1976; Velissarious and Ashburner, 1980).

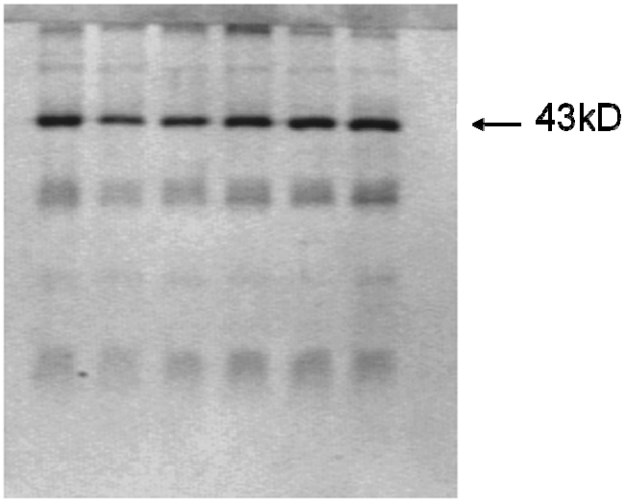

Figure 1. SDS-PAGE pattern carried out to analyze the glue protein patterns on 13.7% gels. One sample from a pair of larval salivary glands of a single individual was prepared and used for SDS-PAGE.

The larval salivary gland in *Drosophila* consists the first glue protein. This tissue specific protein is expectorated to the exterior at the time of pupation and is said to be involved in the process of cementing the puparium to a solid surface. The *Drosophila nasuta* salivary gland secretion protein gene/s are coordinately expressed in salivary glands of third instar larvae. Glue protein electrophoresis patterns in *D. nasuta* subgroup members are simple, and major glue fractions follow X-linked pattern of inheritance in a codominant fashion. Studies on glue proteins in various members of *D. nasuta* subgroup during the last two decades (Ramesh and Kalisch, 1988, 1989a, 1989b; Shivanna *et al.,* 1996; Zajonz *et al.,* 1996a) have revealed that the glue in *D. nasuta* and its related members is produced in copious amounts, which on SDS-PAGE separates into 5 domains consisting

of at least 6 fractions in total. Ramesh and Kalisch (1988) working with *D. n. nasuta* have shown that at least four of the seven glue protein fractions are synthesized by genes in the X- chromosome and have suggested that these genes may be located in the puff at division of X- chromosome. Further, Zajonz *et al.* (1996a) working with the members of *nasuta* subgroup have shown that all fractions except 14 kD are synthesized by genes in the X- chromosome. We have focused our attention to isolate and develop the probes to localize the X-linked glue protein gene/s encoding 43 kD proteins in *D. nasuta nasuta* (Figure 1).

Materials and Methods

a) Stocks of *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans* were obtained from the *Drosophila* stock centre, University of Mysore. The stocks were built that were necessary for the experimental work. Glue protein samples from salivary glands of *Drosophila nasuta albomicans* larvae were prepared for quantitative analysis. The micromethod procedure as described by Neuhoff (1985) was followed for determination of protein quantity in the samples.

b) *SDS PAGE Electrophoresis*: Glue protein plugs were prepared by treating isolated salivary glands in ethanol, further 13.7% SDS PAGE was performed and stained using Coomassie Brilliant blue for staining the bands.

c) *RNA isolation from salivary glands of* Drosophila nasuta nasuta: Total RNA was isolated according to the protocol specified (Qiagen total RNA isolation kit, Germany). We got good yield of total RNA (872 µg/ml). The concentration of RNA was measured at the absorbance of 260 nm (A_{260}) in a spectrophotometer**.** Concentration (µg/ml) = $A_{260} \times 40 \times 200$ (dilution factor). Total RNA was electrophoresed. The gel was visualized in Transilluminator and the results were documented using UVP IT^{TM} system. We got two clear bands of total RNA (28S and 18S) (Figure 2). To construct cDNA, 2 µg of total RNA was used and standard protocol was followed for amplification, according to procedure given in the Qiagen One-Step RT-PCR Kit Handbook.

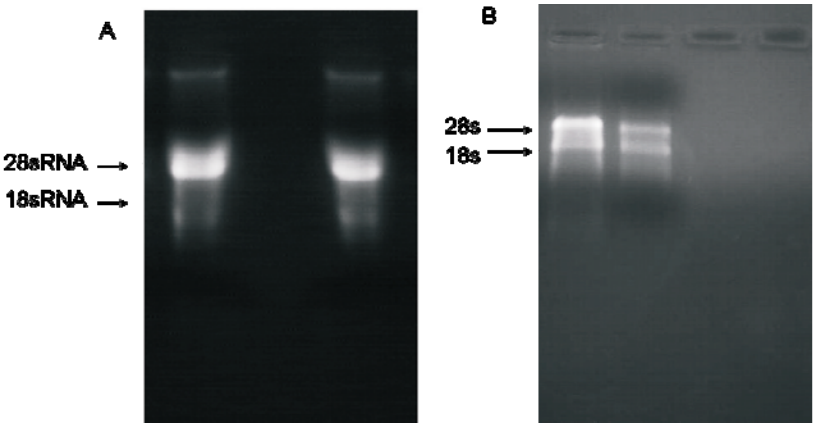

Figure 2. The total RNA was isolated from the larval salivary glands of *D. nasuta nasuta* by conventional (A) and by standard protocol using Quagen RNeasy kit (B). The RNA was run on FA gel (1%). Two distinct bands of RNA are seen in the figure.

d) *Raising of antibodies against 43 kD glue protein fraction of* D. n. nasuta: As an alternative approach to understand genetic differentiation in the X-linked protein fractions in *D. n. nasuta* and *D. n. albomicans*, polyclonal antibodies against 43 kD glue protein fraction of *D. n. nasuta* have been raised for the purpose of N-terminal sequencing. Ouchterlony double diffusion (ODD) was performed to check the specificity of the antibodies (Figure 3).

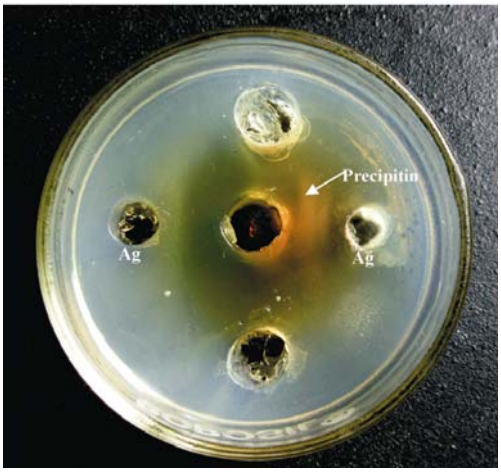

Figure 3. Ouchterlony double diffusion (ODD). The 43 kD protein band was extracted from the gel and raised antibodies against 43kD glue protein fraction of *D. n. nasuta*. Ouchterlony double diffusion (ODD) was performed to check the specificity of the antibodies. Arrow indicated the precipitin band.

Results and Discussion

The synthesis of most of the glue proteins has been shown to cease abruptly from the time of puparium formation (Beckendorf and Kafatos, 1976), and it may even be repressed by rising levels of ecdysone (Poels, 1970, 1972). We used 20-Hydroxy ecdysone as it is the physiologically active form of the hormone, and we could show the regression of the puff in division 10 of those glands, which were treated *in vitro* with 20-hydroxy ecdysone for 30 min. These results clearly indicate that there is a positive correlation between the duration of activity of the puff and the phase of larval glue synthesis there by suggesting that the puff at division 10 of X chromosome harbors glue protein gene(s) in case of *D. n .nasuta.* In *Drosophila melanogaster*, only one glue protein gene, *i.e*., *sgs4*, is found to be located on the X chromosome. This gene shows dosage compensation in males. But this compensation is not complete (Korge, 1975). Moreover, there are some X-linked genes in *D. melanogaster* like X-linked alpha chain gene of larval serum protein, which do not show dosage compensation and also the white-eosin mutant gene where there is no complete dosage compensation (Muller, 1932; Robert and Evans-Roberts, 1979). In view of this, major X-linked glue protein fraction in *D. nasuta nasuta* was analyzed through "volume analysis" mode of molecular analyst of Bio-Rad Gel Doc 1000, by which individual fractions on an SDS-PAGE could be quantified. The results revealed that there is dosage compensation by elevated expression of X-linked gene that produces major glue protein fraction.

The mRNA template was isolated by standard protocol using Stratagene RNeasy kit. Amplification of DNA fragments requires sequence information of homologous genes at the amino acid level or at nucleic acid level. Degenerate primers were designed based on sequence information (Roth *et al*., 1999; NCBI sequence viewer Accession NM_057369) and used in RT-PCR for the synthesis of cDNA (Figure 4).

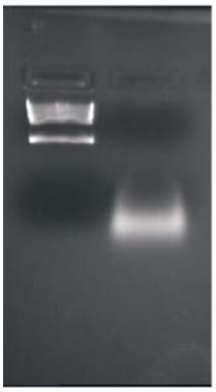

Figure 4. RT PCR Analysis- RT-PCR was done using the total RNA isolated and cDNA was synthesized using the primers

The primer sequence was -5' ATGAGTCGAGACTCCATAAGCGG CCGCTTTACG 3', 3' ATGAGTCGAGACTCCATAAGCGGCCGCTTTACG 5'. Preliminary RT-PCR experiments were carried out to obtain cDNA. Alteration in different steps in the RT-PCR are being worked out to obtain better yield of cDNA. Alternatively, samples have been collected from both *D. n. nasuta* and *D. n. albomicans* which will be subjected to separation on the SDS-PAGE, and the

protein fractions of interest, that is 43 kD from the *D. n. nasuta* and 35 kD from *D. n. albomicans*, will be transfered to polyvinlidene difluoride (PVDF) membrane, that will be used for N-terminal amino acid sequencing.

Conclusion

It is an important genetic approach to understand the X-chromosomal glue protein genes in *Drosophila nasuta nasuta.*

Acknowledgment: The authors thank Department of Science & Technology, Government of India for financial support.

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Male age influence on sons mating success in low and high larval densities in *Drosophila bipectinata.*

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A great diversity of male traits is known to influence on female mating decisions. One male characteristic that has received a lot of attention as a possible cue for female mate preference is age (Beck *et al.,* 2002). The success of males in achieving mating is often linked to the reproductive benefits which females derive (Bussiere *et al.,* 2005). Males typically vary in their ability to provide benefits and determining how females detect which males have to offer has revealed much about the processes that drive the evolution of mate choice. Females often use phenotypic cues that serve as indicators of mate choice benefits (Jacot *et al.,* 2007).

Different models were proposed for female preference for males of different age classes. Both theoretical and empirical evidence of female preference for male age has demonstrated preferences for old, young, and even middle aged males in a variety of species (Jones *et al.,* 2000; Brooks and Kemp, 2001), and much research has been devoted to understand the benefits females derive from mating with males of particular age. Trivers (1972) and Halliday (1978) offered the first verbal arguments of age as an important factor affecting female mate choice. They stated that all