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Genomic Organization of Heat Shock Protein Genes of Silkworm *Bombyx mori*

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The Hsp 20.8 and Hsp 90 cDNA sequence retrieved from NCBI database and consists of 764 bp and 2582 bp lengths respectively. The corresponding cDNA homologus sequences were BLAST searched in *Bombyx mori* genomic DNA database and two genomic contigs viz., BAAB01120347 and AADK01011786 showed maximum homology. In *B. mori* Hsp 20.8 and Hsp 90 is encoded by single gene without intron. Specific primers were used to amplify the Hsp 20.8 gene and Hsp 90 variable region from genomic DNA by using the PCR. Obtained products were 216 bp in Hsp 20.8 and 437 bp in Hsp 90. There was no variation found in the six silkworm races PCR products size of contrasting response to thermal tolerance. The comparison of the sequenced nucleotide variations through multiple sequence alignment analysis of Hsp 90 variable region products of three races not showed any differences respect to their thermotolerance and formed the clusters among the voltinism. The comparison of aminoacid sequences of *B. mori* Hsps with dipteran and other insect taxa revealed high percentage of identity growing with phylogenetic relatedness of species. The conserved domains of *B. mori* Hsps predicted, in which the Hsp 20.8 possesses α-crystallin domain and Hsp 90 holds HATPase and Hsp 90 domains.

Key words: Genomic organization, Hsp, *Bombyx mori,* Domains, Phylogeny, Exon

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Introduction

Silkworm *B. mori* is a lepidopteron, piokilothermic and monophagous insect. The insect is reared in both tropical and temperate climates. The temperature, an abiotic factor affects its growth and silk yield. The heat shock response of organism's habitating tropical climate is likely to be different from those of temperate climate. The native Indian polyvoltine races of *B. mori* exhibit more tolerance to high temperature, than the exotic bivoltine races of temperate origin (Krishnaswami *et al.,* 1977; Pershad *et al.,* 1986).

The heat shock response is a universal physiological phenomenon exhibited by both prokaryotes and eukaryotes (Glover 1982). Ritossa (1962) studied the heat induced a characteristic pattern of puffing in salivary gland chromosome of *Drosophila* larvae. It is normally manifested by the appearance of a set of new proteins or an increase in the quantity of certain specific pre-existing proteins in response to exposure of an organism or a cell to thermal stress and other form of stresses (Lindquist 1986; Nover 1991).

Heat shock response was studied in different cell types of *Drosophila* and other model systems (Tissiers *et al*., 1974; Dean and Atkinson 1983; Nath and Lakhotia 1989; Joplin and Denlinger 1990). The Hsp are presumed to ensure survival under stressful conditions by involvement in damage protection or damage repair due to their action as molecular chaperons (Hightower 1991). Generally heat shock response depends on the magnitude at temperature elevation and duration of exposure, and is relative to the environmental temperature at which the organism normally survives (Nath and Lakhotia 1989). Cells that have been pre-exposed to thermal stress and such a thermo tolerance is caused by an enhanced resistance of proteins against thermal denaturation and aggregation (Kampinga 1993).

The Hsp 90 family is a very highly conserved group displaying higher than 50% identity among eukaryotes and ubiquitously expressed in prokaryotes to eukaryotes (Lindquist and Craig, 1988). The presence of Hsp 90, Hsp 70 and other heat shock proteins are required for protecting the functional domains of the denatured protein. The protection provided by the heat shock protein binding helps refolding of denatured protein (Becker and Carig, 1994). The Hsp 90 function as folding of non native proteins (Weich *et al.,* 1992), peptide translocation (Brugge *et al.,* 1981), regulation of nuclear receptors and kinases (Holley and Yamamoto, 1995; Pratt and Toft, 1997), centrosome structure (Lange *et al.,* 2000), membrane biogenesis (Deshaies *et al.,* 1988) and anti apoptic pathway (Pandey *et al.,* 2000). Landais *et al.,* (2001) identified the Hsp 90 is a unique gene in both *S.frugiperda* and *B.mori* genomes.

Small heat shock proteins (sHsps) were 15-30-kDa heat-shock proteins monomers, consist of a conserved α-crystallin domain of approximately 90-100 amino acid residues bordered by variable amino and carboxy terminal extensions (de Jong *et al.,* 1993; Jakob *et al*., 1993). Small heat shock proteins (sHSPs) were associated with nuclei, cytoskeleton and membranes, and as molecular chaperones. They bind partially denatured proteins, there by preventing irreversible protein aggregation during stress (Sun and Mac Rae 2005). The multiple sHsps occur in living cells and number of genes encoding sHsps is not identical (Narberhaus et al., 2002; Haslbeck *et al*., 2005). The six putatively encoding small heat shock protein (sHsp 19.9, 20.1, 20.4, 20.8, 21.4 and 23.7) genes was characterized in silkworm *B. mori* (Sakano *et al*., 2006). Small Hsps and Hsp 20.8 having a similar domain to that of α-crystallin proteins and occur ubiquitously in a variety of organisms and involved in various phenomenon such as apoptosis protect against heat stress. In this paper, we report the genomic organization and phylogenetic relationship of heat shock protein genes (Hsp 90 and Hsp 20.8) in silkworm *B. mori.* These sequences differences could be used to discriminate racial differences existing in the silkworm races. The information generated from the present study would be useful to clone, characterize and to analyze polymorphic patterns of the genes in silkworm germplasm, and the polymorphic studies could be correlated with thermotolerance level of silkworm.

Materials and Methods

Silkworm strains selected

A total of six silkworm strains *viz.,* Pure Mysore, Nistari, PMX, NB4D2, CSR 18 and CSR 19 were utilized in the present work. The first three strains are polyvoltine in nature and the other three strains are bivoltine silkworm strains. Among the polyvoltine silkworm races, Pure Mysore and Nistari were found to be hardy races, tolerant to thermal stress, while PMX is a susceptible breed. CSR 18 and 19 showed higher thermo tolerance than NB4D2 among bivoltine strains.

Identification of Hsp 90 and Hsp 20.8 gene and genomic contig

The cDNA of Hsp 20.8 (AF315317) and Hsp 90 (AB060275) genes was already identified and deposited by Sakano *et al.,* 2006 and Landais *et al.,* 2005 respectively. The gene sequence was BLAST searched with *B. mori* genomic DNA database for identification genomic contig homologous sequence to corresponding gene sequence. The genomic contig DNA sequences showing homologous sequence to *B. mori* Hsp 20.8 and Hsp 90 gene was identified and subsequently translated to determine putative amino acid sequence. The amino acid sequence was further analyzed through conserved domain search for the presence of domain in Hsp 20.8 and Hsp 90 gene using the programme conserved domain search (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml).

Selection of primers

The up and down gene specific primers were designed for Hsp 20.8 and Hsp 90 genes in the *B. mori* genomic contigs using the software programme of primer3 (http://frodo.wi.mit.edu/cgibin/primer3). Based on the software programme, the primer binding site and the PCR product size were determined.

PCR amplification and analysis of amplified product

The genomic DNA isolated from silk moths using standard protocol (Nagaraja and Nagaraju 1995) and used as template in PCR reactions. The PCR reaction was performed for Hsp 20.8 and Hsp 90 variable region reaction in a 20 µl final volume, each reaction mixture containing 50-100 ng of genomic DNA as template, 2.0 µl of 10X PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 66 ng of gene specific (each forward and reverse primer) and 0.3 U of *Taq* DNA polymerase (MBI fermentas). The PCR schedule was 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 2 min and a final extension of 7 min at 72°C.

The PCR products were resolved on 1.5% agarose gel in Tris-Acetic acid/EDTA buffer with a constant voltage of 80V in parallel with standard markers. Gel was stained with ethidium bromide (0.5 µg/ml) and photographed with gel documentation. The PCR amplified products were purified through Gel-spin column (Bangalore

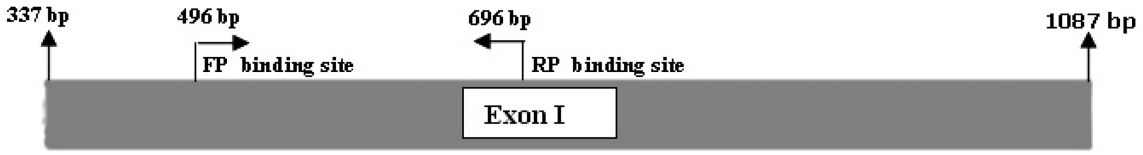

Fig. 1. Schematic diagram of exon and primer binding site for *Bombyx mori* Hsp 20.8 gene based on spidey analysis. The arrow position 337 and 1087 on contig (BAAB01120347) indicates the first and last position of Hsp 20.8 cDNA sequence (AF315317). Position 496 bp and 696 bpdenotes the primer binding site on contig.

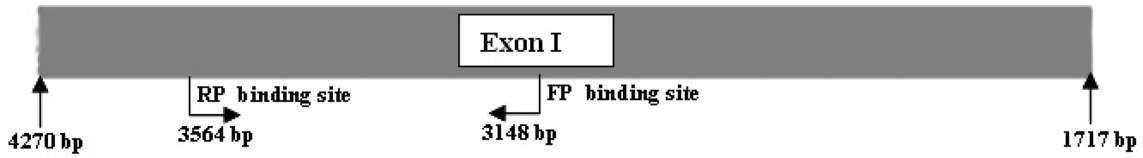

Fig. 2. Schematic diagram of exon and primer binding site for *Bombyx mori* Hsp 90 gene based on spidey analysis. The arrow position 1717 bp and 4270 bp on contig (AADK01011786) indicates the first and last position of Hsp 90 cDNA sequence (AB060275) Position 3148 bp and 3564 bp denotes the primer binding site on complementary strand of the contig.

Genei). In sequencing reaction, the gene specific positive sense primer was used.

Identification of exons and introns

Spidey programme, a specific tool for mRNA and genomic alignment, was used in the present study to elucidate the details on the number of exons, introns, donor, acceptor sites, the gaps, the length of exons in the heat shock protein genes Hsp 20.8 and Hsp 90 of *B. mori.* In this analysis, all parameters are default unless otherwise specified. Spidey takes as input a single genomic sequence and a set of mRNA accession numbers or FASTA sequences.

Phylogenetic tree analysis

The sequence of the each genomic DNA was determined using ABI automatic sequencer and the sequences were compared using the BLAST programme provided by NCBI (http://www.ncbi.nlm.nih.gov/blast). The gene bank, EMBL and DDBJ databases were searched for aminoacid sequence homology using a BLAST algorithm programme. The amino sequence of Hsp 90 and Hsp 20.8 gene with other insects and vertebrate Hsp 90 and Hsp 20.8 database sequences were aligned using ClustalW and phylogenetic analysis was performed using the neighbor-joining method with pair-wise deletion of gaps using MEGA3 software (Kumar et al., 2004). The support for each node was assessed using 1000 bootstrap replicates.

Results

Organization of Hsp genes

In the present study, the cDNA sequences of Hsp 20.8 and Hsp 90 were retrieved from NCBI database. The Hsp 20.8 cDNA of 764 bp gene sequence was compared with *B. mori* genomic contig by BLAST (Basic local alignment search tool). It was found that a single contig with the length of 1918 bp had maximum homology (Accession No. BAAB01120347). Based on spidey analysis, the single genomic contig of Hsp 20.8 gene has one exon (Fig. 1) without any intron. Maximum length 751 bp exon was found in the region of 337-1087 bp of genomic contig and there were no mismatches and gaps found in the genomic DNA.

Similarly, the available cDNA sequence (2582 bp) of Hsp 90 gene was compared with genomic contig of *B. mori* through BLAST. It was found that a single contig (Accession number AADK01011786) length of 10338 bp had maximum homology of 98% with cDNA of Hsp 90 gene. Based on spidey analysis, the contig of Hsp 90 has only one exon (Fig. 2) and the alignment was in the reverse orientation. The length of exon 2582 bp was found in the region of 4270-1717 genomic contig. There were 29 mismatches and 10 gaps found in the genomic DNA. The polyA tail length is about 25 bp. It indicates that the above contig of genomic DNA transcribed the corresponding mRNA of the Hsp gene.

The Hsp 90 cDNA sequences were analyzed for the presence of conserved and variable region based on the available information (Landais *et al.*, 2001). The primers sequences were designed in the variable region of Hsp 90 gene and primers were also designed for the Hsp 20.8 gene sequence. In Hsp 20.8, the forward primer binds at 496 bp and reverse primer at 696 bp position of the contig. In contrary, the Hsp 90 was aligned in the complementary strand of the contig AADK01011786. The forward primer binds at the location of 3148 and reverse

Table 1. Particulars of primers of heat shock protein genes of silkworm *Bombyx mori*

Gene	cDNA accession number	Contig accession number	5' → 3' primer sequence	Contig binding location	Product size
Hsp 20.8	AF315317	BAAB01120347	Forward primer CTAACCCCGAACGACATGCT	496 bp	216 bp
			Reverse primer GATGTACCCATCGGCAGTCT	696 bp	
Hsp 90	AB060275	AADK01011786	Forward primer TTCCCAGTTCATTGGCTACC	3564 bp	437 bp
			Reverse primer TCTTGCGCTTCTTGTTTTCA	3148 bp	

primer at 3564 of the contig (Table 1). The predicted amplicon size for the Hsp 20.8 and Hsp 90 are 216 bp and 437 bp respectively. It was found that the amplified product showed similar size with predicted amplicon size. The sequenced data of PCR product confirmed the Hsp 20.8 and Hsp 90 gene.

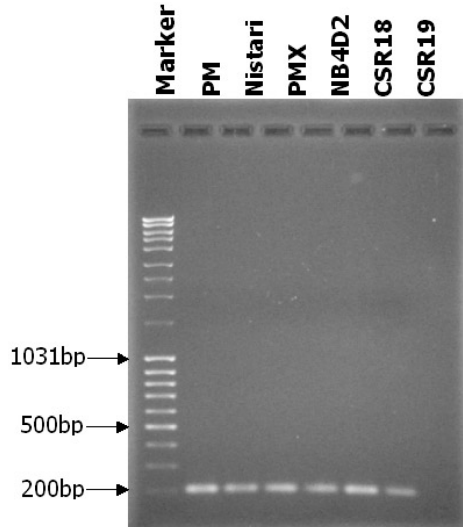

Fig. 3. PCR amplification of Hsp 20.8 genes in silkworm *Bombyx mori.*

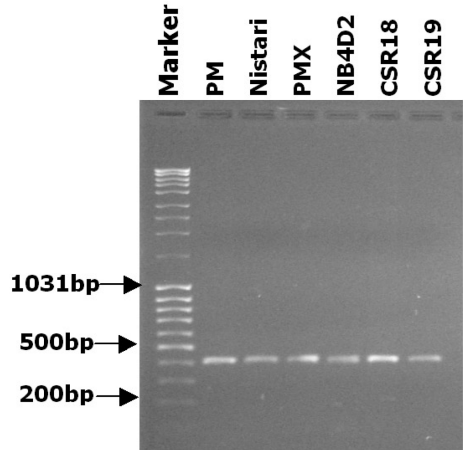

Fig. 4. PCR amplification of Hsp 90 genes in silkworm *Bombyx mori.*

The Hsp genes were amplified in all the six races of contrasting response to thermotolerance. The amplified PCR product size of Hsp 90 was 437 bp and 216 bp for Hsp 20.8 gene in all these races (Fig. 3 and 4). The multiple alignement data of three sequences revealed the results indicate that polyvoltine breeds viz. Pure Mysore and PMX clustered as single group. PMX a thermosusceptible race compared to Pure Mysore, was grouped in a single cluster (Fig. 5). While the bivoltine breed CSR 19 formed a unique cluster.

Hsp 20.8 aminoacid sequences were analyzed through conserved domain search (NCBI Conserved Domain Search) and sequences showed α-crystallin domain, which was located in the region of 65 to 150 aminoacids regions in length of 186 amino acids (Fig. 6). The Hsp 90 gene full-length cDNA sequences of 716 amino acids had two domains in the N terminal and as well as in the C terminal region (Fig. 7). One third of the total amino acids sequence length was covered with HATPase domain, which presumed to be involved in ATP binding site. Two third of the Hsp 90 amino acid sequence was covered with the Hsp 90 domain from 275 to 716 amino acids.

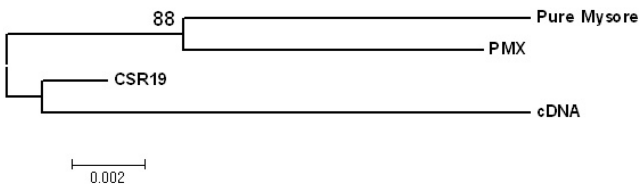

Fig. 5. Phylogenetic tree based upon PCR product sequences of Hsp 90 gene of Pure Mysore, PMX, CSR 19 silkworm races and compared with the cDNA Hsp 90 gene (AB060275). The Neighbor joining tree was constructed with 1000 bootstrap replicates in Mega 3.1 programme. Branch numbers refers to bootstrap value (percentage of 1000 replicates).

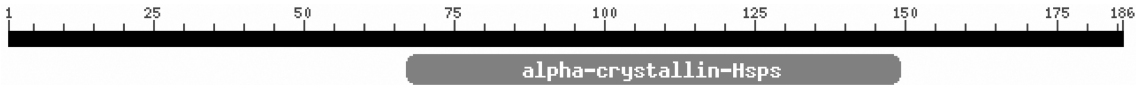

Fig. 6. Prediction of Putative conserved domain α crystallin in HSP 20.8 gene of *Bombyx mori* through the conserved domain analysis programme.

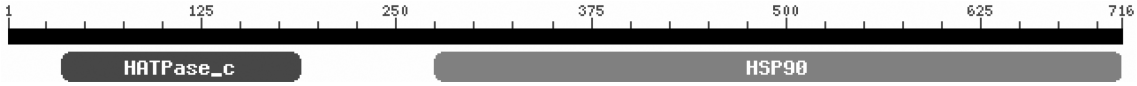

Fig. 7. Prediction of Putative conserved domain HATPase and HSP90 domain in HSP 90 gene of *Bombyx mori* through conserved domain analysis programme.

Phylogenetic analysis

A vast number of ubiquitous and highly conserved genes have been used in molecular taxonomy, as they provide useful models for phylogenetic analysis. In order to verify the phylogeny of insects with a higher number of taxonomical orders, we constructed Hsp 90 gene phylogenetic tree using 20 different eukaryotic organisms representing different taxonomical group. Among different taxonomical group 17 species were derived from insect taxa and three organisms represented from mammalian, which was included as an out group member. The results showed that all insects form an out group from the mammalian. The Lepidopterans and dipterans insects formed a separate cluster. Similarly hymenoptera and orthoptera members clustered as one group. Interestingly, *Tribolium castaneum,* a red flour beetle belonging to the coleopteran family formed a cluster with Lepidoptera (Fig. 9). The *Bombyx mori* Hsp 20.8 gene was also analyzed for the phylogenetic relationship with other organisms (Fig. 8). The results indicate that the lepidopteron insects formed separate clusters and *venturia canescens* hymenoptera formed group with diptera. The coleoptera and orthoptera grouped with Lepidoptera.

Discussion

Heat shock proteins play a major role in protection and maintenance of many fundamental cellular functions (Fink 1999; Nadeau *et al*., 2001). They play a vital role in embryonic development (Ali *et al*., 1996), cell differentiation, cell cycles and hormonal stimulation (Schlesinger 1990). In addition, they act like chaperonins to maintain regular proteins in a folded state (Currie and Tufts 1997; Lewis *et al.,* 1999), allow newly formed proteins to translocate across cell membranes (Sejerkilde *et al.,* 2003),

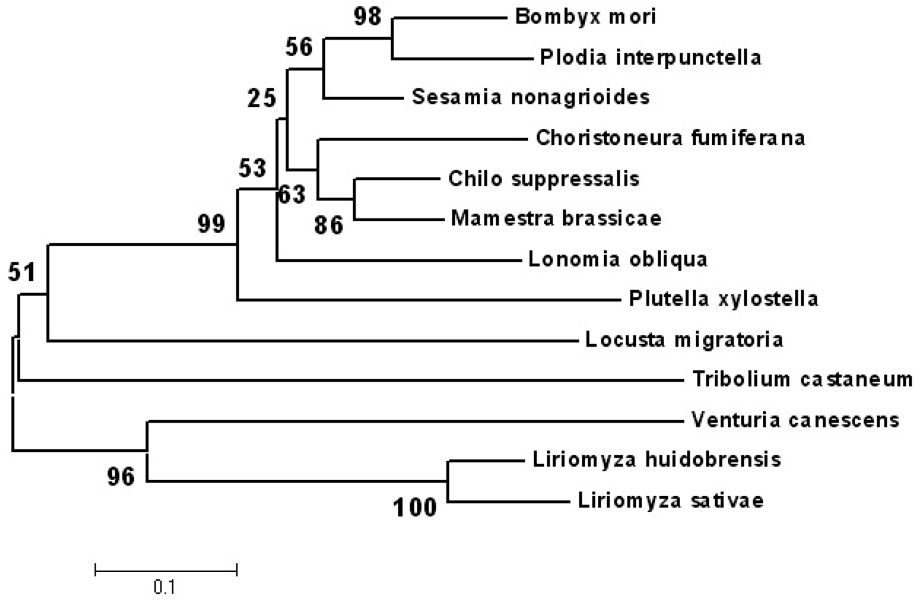

Fig. 8. Phylogenetic tree based upon amino acid sequences of the *Bombyx mori* Hsp 20.8 gene and twelve known Hsp 20.8 sequences of insects. The Neighbor joining tree was constructed with 1000 bootstrap replicates in Mega 3.1 programme. Branch numbers refers to bootstrap value (percentage of 1000 bootstrap value).

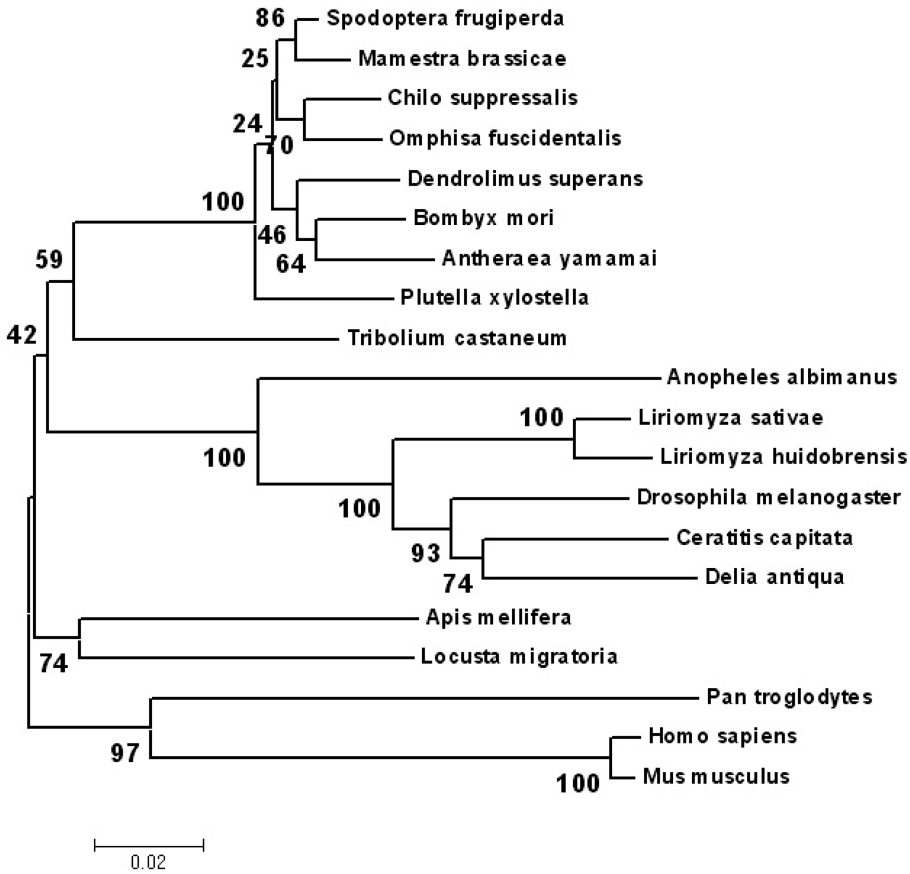

Fig. 9. Phylogenetic tree based upon amino acid sequences of the *Bombyx mori* Hsp 90 gene and sixteen Hsp 90 sequences of known insects and three of mammalia. The Neighbor joining tree was constructed with 1000 bootstrap replicates in Mega 3.1 programme. Branch numbers refers to bootstrap value (percentage of 1000 bootstrap value).

fold and assemble polypeptides (Schlesinger 1990), minimize the aggregation of non-native proteins and target non-native or aggregated proteins for degradation and removal from cells (Feder and Hofmann 1999). Minimizing the aggregation and removal of denatured proteins are presumably the most important functions in coping with environmental stresses.

This study indicates that the Hsp 90 and Hsp 20 are organized in the genome with out any intronic region. The most obvious characteristic of Hsp gene locus is the presence of long exon without any intron. The thermal stress related protein usually lacks splicing mechanism as splicing process is inhibited by the heat shock (Yost and Lindquist 1986). This present report confirms the earlier repot of absence of introns in heat shock proteins. The similar observation was also reported in the lepidopteran insect, *Spodoptera frugiperda.* In several *Drosophila* species, a short intron located within the 5' UTR was described (Konstantopoulou and Scouras, 1998). In *S. frugiperda,* the Hsp 90 genomic organization studies indicate no intron in 2.7 Kb genomic DNA sequence (Landais *et al.*, 2001). In our study, the complete sequence obtained from the PCR product did not revealed no difference with the cDNA sequence and allowing us to conclude that there is no intron in the Hsp 90 genomic sequence.

The results of multiple sequence alignment analysis of three races clearly indicate that the Hsp 90 sequences formed groups based on the voltinism rather than the thermotolerance. But still this hypothesis may be confirmed by analyzing upstream regulatory region of the Hsp 90 genes of different silkworm races and these sequences variation could be correlated with thermo tolerance status of different silkworm races. Goldsmith *et al.,* (2005) reported that the UTR possessed conserved regions and microsatellite domains, which are useful to determine the genetic variability in the functional genes of silkworm germplasm stocks. Therefore, this study would help in identification of variation in distribution of variable regions in the non-coding and coding regions of stress related genes of silkworm germplasm stocks.

The Hsp 20.8 gene showed the α-crystallin domain in the full length aminoacids. This domain is the common

characteristic of the α-crystallin/small heat-shock protein (Hsp 20.8) family is the presence of a conserved homologous sequence of 90-100 residues (de Jong *et al.,* 1993). Unlike the other heat-shock proteins, small heat-shock proteins function as molecular chaperones, preventing undesired protein-protein interactions and assisting in refolding of denatured proteins (Jakob *et al.,* 1993). Many of the small heat-shock proteins are differentially expressed during normal development, involved in cytomorphological reorganizations and in degenerative diseases (Hayens *et al.,* 1996; den Engelsman *et al.,* 2005) and signal transduction. In Hsp 90, both N and C terminal region possess HATPase and Hsp 90 domains and these domains meant for the biological process involved in the thermal stress and also involved in the cell function (Minami *et al.,* 2001).

The phylogenetic analysis results showed the lepidoptera members were evolved from the common ancestor and possessing similar molecular mechanism to over come the undesirable stress conditions. The phylogenetic analysis of variable region of Hsp genes revealed that like conserved region, these regions may also be used to analyze the phylogenetic relatedness.

References

Ali, A., P.H Krone, D. S. Pearson and J. J. Heikkila (1996) Evaluation of stress inducible Hsp 90 gene expression as a potential molecular biomarker in *Xenopus laevis*. *Cell Stress Chaperones* **1**, 62-69.

Becker, J. and E. A. Craig (1994) Heat-shock proteins as molecular chaperones. *Eur. J. Biochem.* **219**, 11-23.

Brugge, J. S., E. Erikson and R. L. Erikson (1981) The specific interaction of the rous sarcoma virus transforming protein, pp60src, with two cellular proteins. *Cell* **25**, 363-372.

Currie, S. and B. Tufts (1997). Synthesis of stress protein 70 (Hsp 70) in rainbow trout (*Oncorhynchus mykiss*) red blood cells. *J. Exp. Biol.* **200**, 607-614.

Den Engelsman, J., D. Gerrits, W. W. de Jong, J. Robbins, K. Kato and W. C. Boelens (2005) Nuclear import of αB-crystallin is phosphorylation-dependent and hampered by the myopathy-related mutant R120G. *J. Biol. Chem.* **280**, 37139-37148.

De Jong, W. W., J. A. M. Leunissen and C. E. M. Voorter (1993) Evolution of the a-Crystallin / Small Heat-Shock Protein Family. *Mol. Biol. Evol.* **10**, 103-126.

Dean, R. L. and B. G. Atkinson (1983) The acquisition of thermal tolerance in larvae of *Calpodes ethlius* (Lepidoptera) and the *in situ* and *in vitro* synthesis of the heat shock proteins. *Can. J. Biochem. Cell Biol.* **61**, 472-479.

Deshaies, R. J., B. D. Koch and R. Schekman (1988) The role of stress proteins in membrane biogenesis. *Trends Biochem. Sci.* **13**, 384-388.

Feder, M.E. and G. E. Hofmann (1999) Heat shock proteins, molecular chaperones and the stress response evolutionary and ecological physiology. *Annu. Rev. Physiol.* **61**, 243-282.

Fink, A. F. (1999) Chaperone-mediated protein folding. *Physiol. Rev.* **79**, 425-449.

Glover, C. V. C. (1982) Heat shock effects on protein dephosphorylation in *Drosophila;* Heat Shock from Bacteria to Man. Schlessinger, M. J., M. Ashburner and Tissieres A. (ed.), Cold Spring Harbor Laboratory, NY.

Goldsmith, M. R., T. Shimada and H. Abe (2005) Genetics and genomics of the silkworm *Bombyx mori*. *Ann. Rev. Entomol.* **50**, 71-100.

Haselback, M., T. Franzmann, D. Weinfurtner and J. Buchner (2005) Some like it hot: structure and function of small heat shock proteins. *Nat. Struct. Mol. Biol.* **12**, 842-846.

Hayens, J. I., M. K. Duncan and J. Piatiogorsky (1996) Spatial and temporal activity of the alpha B-crystallin/small heat shock protein gene promoter in transegenic mice. *Dev. Dyn.* **207**, 75-88.

Hightower, L. E. (1991) Heat shock stress protein, chaperones and proteotoxicity. *Cell* **66**, 191-197.

Holley, S. J. and K. R. Yamamoto (1995) A role fore Hsp 90 in retionoid receptor signal transduction. *Mol. Biol. Cell* **6**, 1833-1842.

Jakob, U., M. Gaestel, K. Engel and J. Buchner (1993) Small heat shock proteins are molecular chaperones. *J. Biol. Chem.* **268**, 1517-1520.

Joplin, K. H. and D. L. Denlinger (1990) Developmental and tissue specific control of the heat shock induced 70 kDa related proteins in the flesh fly *Sarcophaga crassipalpis*. *J. Insect Physiol.* **36**, 239-249.

Kampinga, H. H. (1993) Thermotolerance in mammalian cells: Protein denaturation and aggregation and stress proteins. *J. Cell Sci.* **104**, 11-17.

Konstantopoulou, I. and Z. G. Scouras (1998) The heat shock gene Hsp 83 of *Drosophila auraria*: genomic organization, nucleotide sequences and long antiparallel coupled ORFs (LAC ORFs). *J. Mol.Evol.* **46**, 334-343.

Krishnaswamy, S., M. N. Narashimanna, S. K. Suryanarayana and S. Kumaraj (1977). Silkworm rearing; in *Manual on sericulture Vol. 2*., Mysore Publ., Central Silk Board.

Kumar, S., K. Tamura and M. Nei (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* **5**, 150-163.

Landais, I., J. M. Pommet, K. Mita, J. Nohata, S. Gimenez, P. Fournier, G. Devauchelle, D.C. Martine and M. Ogliastro (2001) Characterization of the cDNA encoding the 90 kDa heat-shock protein in the Lepidoptera *Bombyx mori and Spodoptera frugiperda*. *Gene* **271**, 223-231.

Lange, B. M., A. Bachi, M. Wilm and C. Gonzalez (2000) Hsp 90 is a core centrosomal component and is required at different stages of the centrosome cycle in *Drosophila* and vertebrates. *EMBO J.* **19**, 1252-1262.

Lewis, S., R. D. Handy, B. Cordi, Z. Billinhurst and M. H. Depledge (1999). Stress proteins (HSP's): methods of detec-

tion and their use as an environmental biomarker. *Ecotoxicology* **8**, 351-368.

Lindquist, S. and E. A. Craig (1988). The heat-shock proteins. *Annu Rev Genet.* **22**, 631-677.

Lindquist, S. (1986) The heat-shock response. *Annu. Rev. Biochem.* **55**, 1151-1191.

Minami, M., M. Nakamura, Y. Emori and Y. Minami (2001) Both the N and C-terminal chaperone sites of Hsp 90 protein refolding. *Eur. J. Biochem.* **268**, 2520-2524.

Nadeau, D., S. Corneu, I. Plante, G. Morrow and R.M. Tanguay (2001) Evaluation of HSP 70 as a biomarker of effect of pollutants on the earthworm *Lumbricus terrestris. Cell Stress Chaperones* **6**, 153-163.

Nagaraja, G. M. and J. Nagaraju (1995) Genome fingerprinting of the silkworm *Bombyx mori* using random arbitrary primers. *Electrophoresis* **16**, 1633-1638.

Narberhaus, F. (2002) Á-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. Microbiol. *Mol. Biol. Rev.* **66**, 64-93.

Nath, B. B. and S. C. Lakhotia (1989) Heat shock response in ovarian nurse cells of *Anopheles stephensi. J. Biosci.* **14**, 14-152.

Nover, L. (1991) (ed.) *Heat Shock Response.* Boca Raton, FL: CRC

Pandey, P., A. Saleh, A. Nakazawa, S. Kumar, S. M. Srinivasula, V. Kumar, R. Weichselbaum, C. Nalin, E. S. Alnemri, D. Kufe and S. Kharbanda (2000) Negative regulation of cytochrome C-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. *EMBO J.* **19**, 4310-4322.

Pershad, G. D., R. K. Datta, H. V. Vijayakumar, S. K. Bhargava and M. S. Jolly (1986) Performance of some multivoltine races of *Bombyx mori* L. *Sericologia* **26**, 295-301.

Pratt, W. B. and D. O. Toft (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr. Rev.* **18**, 306-360.

Ritossa, F. M. (1962) A new puffing pattern induced by a temperature shock and DNP in *Drosophila. Experientia* **18**, 571-573.

Sakano, D., L. I. Bin, X. I. A. Qingyon, K. yamamoto, H. fujii, and A. S. O. Yoichi (2006) Genes encoding small heat shock proteins of the silkworm *Bombyx mori. Bioscience, Biotechnology and biochemistry* **70**, 2443-2450.

Schlesinger, M. J. (1990) Heat shock proteins: a mini review. *J. Biol. Chem.* **265**, 12111-12114.

Sejerkilde, M., J. G. Sorensen and V. Loeschcke (2003) Effects of cold and heat hardening on thermal resistance in *Drosophila melanogaster. J. Insect Physiol.* **49**, 719-726.

Sun, Y. and T. H. Mac Rae (2005) Small heat shock proteins: molecular structure and chaperone function. *Cellular and Mol. Life Scie.* **62**, 2460-2476.

Tissieres, A., H. K. Mitchell and U. Tracy (1974) Protein synthesis in salivary glands of *D.melanogaster.* Relation to chromosome puffs. *J. Mol Biol* **84**, 389-398.

Weich, W. J., J. Buchner, R. Zimmermann and U. Jackob (1992) Hsp 90 chaperones protein folding *in vitro. Nature* **358**, 169-170.

Yost, H. J. and Lindquist, S. (1986) RNA splicing is interrupted by heat shock protein and is rescued by heat shock protein synthesis. *Cell* **45**, 185-193.