Pradeep. A. R., M.Sc., Ph. D; PDF (Texas A&M)

arpradeepnair@gmail.com

Present Research interest: Molecular mechanism of host – parasite interaction

Proteome analysis of larval tissues of the mulberry silkworm, *Bombyx mori* after infection through the dipteran parasitoid, *Exorista bombycis*, leads us to identify functional genes that are differentially expressed after the infection. Notedly, it includes genes encoding components of melanisation pathway, toll pathway, signalling, NF-kappa B transcription factors and its inhibitor, insect cytokines, proteolysis, induction of autophagy and apoptosis (Pradeep et al 2012). These genes will be used to identify immuno - competent strains of *B. mori*.

The larval integumental epithelium exhibited autophagy, ER stress, nuclear condensation and DNA fragmentation leading to apoptosis induced by the parasitoid infection. These cellular events are accompanied by activation of associated genes that showed the induction of immune events in the epithelium. The gene expression patterns were co-regulated in normal larvae compared to deregulated gene activity after the infection (Anitha et al 2013).

The infection has induced upregulation of three apoptosis-associated genes, autophagy 5 like (Atg5), apoptosis – inducing factor and caspase in synchronization with cellular autophagy and apoptosis in integumental epithelium (Anitha et al 2014).

Cited publications

Anitha J, Pradeep A.R and Sivaprasad V (2014) Upregulation of Atg5 and AIF gene expression in synchronization with programmed cellular death events in integumental epithelium of *Bombyx mori* induced by a dipteran parasitoid infection. Bulletin of Entomological Research, doi:10.1017/S0007485314000686.

Anitha J, Pradeep A R, Awasthi AK , Murthy GN, Ponnuvel KM, Sasibhushan S and Rao GCP (2013) Coregulation of host–response genes in integument: switchover of gene expression correlation pattern and impaired immune responses induced by dipteran parasite infection in the silkworm, *Bombyx mori*. Journal of Applied Genetics. DOI 10.1007/s13353-013-0183-8.

Pradeep AN, Anitha J, Awasthi AK, Babu MA, Geetha MN, Arun HK, Chandrashekhar S, Rao GC and Vijayaprakash NB (2012) Activation of autophagic programmed cell death and innate immune gene expression reveals immuno-competence of integumental epithelium in *Bombyx mori* infected by a dipteran parasitoid. Cell & Tissue Research. doi:10.1007/s00441-012-1520-7

Research report (2008 – 14)

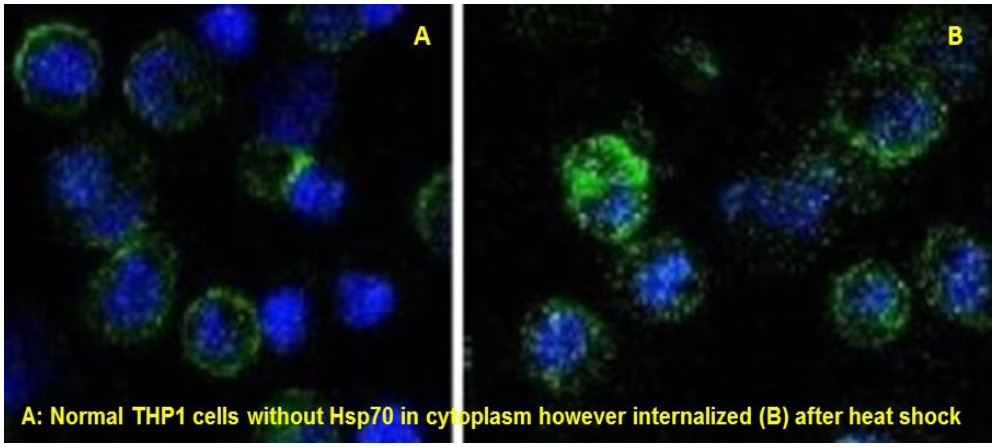
In insects, the integument forms the primary barrier between the environment and internal milieu. We elucidated cellular and immune responses of the integumental epithelium induced through infection by a dipteran endoparasitoid, *Exorista bombycis* in the economically important silkworm *Bombyx mori*. Degradative autophagic vacuoles, lamella-like bodies, a network of cytoplasmic channels with cellular cargo, and an RER network that opened to vacuoles were observed sequentially with increase in age after infection. This temporal sequence culminated in apoptosis, accompanied by the upregulation of the caspase gene and fragmentation of DNA. The infection significantly enhanced the tyrosine level and phenol oxidase activity in the integument. Proteomic analysis revealed enhanced expression of innate immunity components of toll and melanization pathways, cytokines, signaling molecules, chaperones, and proteolytic enzymes demonstrating diverse host responses. qPCR analysis revealed the upregulation of spatzle, BmToll, and NF kappa B transcription factors Dorsal and BmRel. NF kappa B inhibitor cactus showed diminished expression when Dorsal and BmRel were upregulated, revealing a negative correlation. During melanization, prophenol oxidase 2 was expressed, a novel finding in integumental epithelium. The integument showed a low level of melanin metabolism and localized melanism in order to prevent the spreading of cytotoxic quinones. The gene-encoding proteolytic enzyme, beta-N-acetylglucosaminidase, was activated at 24 h post-infection, whereas chitinase, was activated at 96 h post-infection; however, most of the immune genes enhanced their expression in the early stages of infection. Thus the integument contributes to humoral immune responses that enhance resistance against macroparasite invasion.

The infection enhanced host–response gene expression in integument early in the infection and was lowered asymptotically. Principal component analysis (PCA) showed heterogeneity while explaining ~80 % variance among expression timings. PCA showed positive and negative correlation with gene expression and differentiated transcriptional timings, and revealed cross talk within the immune system. Pearson correlation analysis showed significant linear correlation (mean R^2=>0.7; *P* <0.004) between the expression of 16 pairs of genes in control, while the relation switched over to curvilinear due to parasitism. The genes showed pleiotropic

interaction among them, with four genes each for prophenoloxidase activating enzyme (PPAE) and caspase. Besides, after parasitism, exclusive correlation of five gene pairs including PPAE–Spatzle pair (R^2=0.9; *P* <0.011) was observed in the integument. Switchover from linear to curvilinear correlation and the appearance of new gene correlations in parasitized integument revealed deviation from gene coregulation, leading to impaired immune responses, characterized by lowered gene expression and varied phenotypic consequences.

Infection of the commercially important silkworm, *Bombyx mori* by a tachinid parasitoid, *Exorista bombycis* induced activation of genes and cellular responses associated with apoptosis in integumental epithelial cells. Composite cellular profile showed initial autophagy, intermediate endoplasmic reticulum degranulation and deformed nucleus as well as later DNA fragmentation indicating apoptosis. Two cell death-associated proteins, autophagy 5-like (Atg5L) and apoptosis-inducing factor (AIF), in addition to caspase, are identified from the infected integumental epithelium through mass spectrometric analysis. Genes encoding these proteins showed age-dependent activation after the infection as revealed by quantitative expression analysis. Atg5 showed early upregulation in association with signs of autophagy whereas AIF showed late upregulation in association with DNA condensation and fragmentation. Activation of Atg5, AIF and caspase genes in close association with different cell death events revealed the synchronized differential expression of apoptosis-associated genes in response to the macroparasitism indicate parasitism-induced activation of genetic machinery to modulate cell death events in the epithelium, which was hither to unknown in invertebrate systems.

The exact mechanism by which Hsp70 gains access to the extracellular milieu remains unknown. Our study demonstrated that the plasma membrane of cells functions as a reservoir for Hsp70 and thermal stress induces redistribution of plasma membrane - bound Hsp70 into subcellular cytosolic components. From there, the nucleolin-mediated transport system carries the Hsp 70 to the plasma membrane for its relocalization and final release into the extracellular milieu, thus nucleolin acts as a transporter of intracellular Hsp 70 from cytoplasm to plasma membrane (Kaur et al 2012)



A: Normal THP1 cells without Hsp70 in cytoplasm however internalized (B) after heat shock

Genetic variability among silkworm populations

Deforestation and exploitation has led to the fragmentation of habitats and scattering of populations of the economically important eri silkworm, *Samia cynthia ricini*, in north-east India. Genetic analysis of 15 eri populations, using dominant ISSR markers, showed 98% inter-population, and 23% to 58% intra-population polymorphism. Nei's genetic distance between populations increased significantly with altitude (R^2 = 0.71) and geographic distance (R^2 = 0.78). On the dendrogram, the lower and upper Assam populations were clustered separately, with intermediate grouping of those from Barpathar and Chuchuyimlang, consistent with geographical distribution. The Nei's gene diversity index was 0.350 in total populations and 0.121 in subpopulations. The genetic differentiation estimate (Gst) was 0.276 among scattered populations. Neutrality tests showed deviation of 118 loci from Hardy-Weinberg equilibrium. The number of loci that deviated from neutrality increased with altitude (R^2 = 0.63). Test of linkage disequilibrium showed greater contribution of variance among eri subpopulations to total variance. D'2IS exceeded D'2ST, showed significant contribution of random genetic drift to the increase in variance of disequilibrium in subpopulations. In the Lakhimpur population, the peripheral part was separated from the core by a genetic distance of 0.260. Patchy habitats promoted low genetic variability, high linkage disequilibrium and colonization by new subpopulations. Increased gene flow and habitat-area expansion are required to maintain higher genetic variability and conservation of the original *S. c. ricini* gene pool.

One ISSR locus each specific to high and low altitude eri silkworm population was identified. The locus from high altitude showed deviation from Hardy-Weinberg equilibrium but that from low altitude was in neutrality suggests that the high altitude loci could be under pressure from the altitudinal variations. In association with different yield traits, 18 loci were identified. Of which, three markers showed association with more than one trait indicative of pleiotropic influence. Stepwise addition of markers enhanced the correlation between markers and the associated trait pointed to polygenic influence. Association of markers with altitude and yield traits suggests an imperative relation of rare genetic loci with gene-environment interaction and phenotypic variability in *S. c. ricini*.

The Indian golden saturniid silkmoth (*Antheraea assama*), popularly known as muga silkmoth, is a semidomesticated silk producing insect confined to a narrow habitat range of the northeastern region of India. We observed significant genetic diversity in one of the populations (WWS-1, a population derived from West Garo Hills region of Meghalaya state). Analysis of the remaining five populations (excluding WWS-1) showed a marked reduction in the number of alleles at all the employed loci. Structure analysis showed the presence of only two clusters: one formed by WWS-1 population and the other included the remaining five populations, inferring that there is no significant genetic diversity within and between these five populations, and suggesting that these five populations are probably derived from a single population. The information generated will be very useful in conservation of dwindling muga culture in Northeast India.

Simple sequence repeats (SSRs) and interSSR (ISSR) marker systems were used to reveal genetic changes induced by artificial selection for short/long larval duration in the tropical strain Nistari of the silkworm *Bombyx mori*. Artificial selection separated longer larval duration (LLD) (29.428 ± 0.723 days) and shorter larval duration (SLD) (22.573 ± 0.839 days) lines from a base, inbred population of Nistari (larval span of 23.143 ± 0.35 days). SSR polymorphism was observed between the LLD and SLD lines at one microsatellite locus, Bmsat106 (CA7) and at two loci of 1074 bp and 823 bp generated with the ISSR primer UBC873. Each of these loci was present only in the LLD line. The loci segregated in the third generation of selection and were fixed in opposite directions. In the F2 generation of the LLD × SLD lines, the alleles of Bmsat106 and UBC8731074bp segregated in a 1:1 ratio and the loci were present only in the LLD individuals. UBC873823bp was homozygous. Single factor ANOVA showed a significant association between the segregating loci and longer larval duration. Together, the two alleles contributed to an 18% increase in larval duration. The nucleotide sequences of the UBC8731074bp and UBC873823bp loci had 67% A/T content and consisted of direct, reverse, complementary and palindromic repeats. The repeats appeared to be "nested" (59%) in larger repeats or as clustered elements adjacent to other repeats. Of 203 microsatellites identified, dinucleotides (67.8%) predominated and were rich in A/T and T/A motifs. The sequences of the UBC8731074bp and UBC873823bp loci showed similarity (E = 0.0) to contigs located in Scaffold 010774 and Scaffold 000139, respectively, of the B. mori genome. BLASTN analysis of the UBC8731074bp sequence showed significant homology of (nt.) 45–122 with upstream region of three exons from *Bombyx*. The complete sequence of this locus showed ~49% nucleotide conservation with transposon 412 of *Drosophila melanogaster* and the Ikirara insertions of *Anopheles gambiae*. The A + T richness and lack of coding potential of these small loci, and their absence in the SLD line, reflect the active process of genetic change associated with the switch to short larval duration as an adaptation to the tropics.

Translational Technology information developed

1. Long larval duration line with high silk and fecundity

Long larval duration (LLD) line of the mulberry silkworm *Bombyx mori* Nistari was developed by artificial directional selection. The LLD lines were characterized by the presence of exclusive DNA markers. The LLD line showed significantly higher production of silk in cocoon and fecundity which can be used to produce high yielding Nistari strain.

2. Immunocompetence among germplasm

Host-response proteins and immune proteins were identified from parasitized *B. mori* larvae. Genes encoding these proteins showed differential expression. This information will be used to identify immunocompetent strains among germplasm accessions of the silkworm, *B. mori*.

List of important publications:

Proteomics

1. Anitha J, Pradeep AR and Sivaprasad V (2014) Upregulation of *Atg5* and *AIF* gene expression in synchronization with programmed cellular death events in integumental epithelium of *Bombyx mori* induced by a dipteran parasitoid infection. Bulletin of Entomological Research (Cambridge, UK). In Press.
2. Anitha J, Pradeep AR, Awasthi AK, Murthy GN et al (2013) Coregulation of host–response genes in integument: switchover of gene expression correlationpattern and impaired immune responses induced by dipteran parasite infection in the silkworm, *Bombyx mori*. J Appl. Genet DOI 10.1007/s13353-013-0183-8
3. Pradeep AR, Anitha J, Awasthi AK, Babu MA, Geetha MN, Arun HK, Chandrashekhar S, Rao GC, Vijayaprakash NB. (2013) Activation of autophagic programmed cell death and innate immune gene expression reveals immuno-competence of integumental epithelium in Bombyx mori infected by a dipteran parasitoid. Cell Tissue Res. 352: 371-85; Epub 2012 Nov 18.
4. Kaur P, Pradeep AR and Asea A (2012) Nucleolin: A novel intracellular transporter of HspA1A. Chapter 8 In: Cellular Trafficking of Cll Stress Proteins in Health and Disease – Heat Shock proteins, Volume 6;. Eds. Pockley AG, Henderson B, Dordrecht, The Netherlands, Springer, pp115-124.
5. Pradeep.A.R, Arun K.H, Anitha J, Geetha N.M, Awasthi A.K, Rao C.G.P and Vijayaprakash N.B (2011a) Immunity in insects against parasites and pathogens: Recent developments in silkworms. National Conference on Sericulture Innovations: Before and Beyond, Mysore, India, 27-29 January, 2011, pp 149-150.
6. Pradeep A R, Nagaraja G, Kaur P, Asea E, Bempong P, Lillard S, Shapiro L, Asea A (2010) Involvement of Nucleolin in Hsp72 Intracellular Trafficking, in Section: Immunology and Inflammation, Society of Thermal medicine Annual meeting, Clearwater, Florida, USA, April 23-26, 2010.

Molecular Biology

1. Arunkumar KP, Sahu AK, Mohanty AR, Awasthi AK, Pradeep AR, et al. (2012) Genetic diversity and population structure of Indian golden silkmoth (*Antheraea assama*). PLoS ONE 7(8): e43716. doi:10.1371/ journal.pone.0043716
2. Pradeep A.R, Anuradha H.J, Singh K.C, Awasthi A.K, Vikas Kumar, Rao C.G.P and Vijaya prakash N.B (2011b) Genetic analysis of scattered populations of the Indian eri silkworm, *Samia cynthia ricini* Donovan: differentiation of subpopulations. Genet. Mol. Biol. 34: 502-510.
3. Pradeep A.R, Awasthi A.K, Singh K.C, Anuradha H.J, Rao C.G.P and Vijayaprakash N.B (2011a) Genetic evaluation of eri silkworm *Samia cynthia ricini*: ISSR loci specific to high and low altitude regimes and quantitative attributes. J. Appl. Genet. 52: 345-353.

4. Pradeep, A. R., A. K. Awasthi, Raje Urs S. (2008) Association of A/T rich microstellite with response to artificial selection and differentiation of larval development duration in silkworm *Bombyx mori*. Molecules and Cells, 25:467-478.
5. Awasthi A.K, A.R.Pradeep, P.P. Srivastava, K.Vijayan, Vineet Kumar and S. Raje Urs (2008) PCR detection of densonucleosis virus isolates in silkworm (*Bombyx mori*) from India its nucleotide variability. Indian J. Biotech. 7, 56-60.
6. Awasthi A.K, P.K. Kar, P. P. Srivastava, Nidhi Rawat, K. Vijayan, A. R. Pradeep and S. Raje Urs (2008) Molecular evaluation of bivoltine and mutant silkworm (*Bombyx mori* L.) with RAPD, ISSR and RFLP-STS markers. Indian J. Biotech. 7, 188-194.
7. Pradeep A. R., Anuradha H. J and Raje Urs S. (2007) Molecular markers for Biomass Traits: Association, Interaction and Genetic Divergence in silk worm, *Bombyx mori.* Biomarker Insights, 2, 197-217.
8. Vijayan K*, H. J. Anuradha, C. V. Nair, A. R. Pradeep*, A. K. Awasthi, B. Saratchandra, S. A. S. Rahman, K. C. Singh, R. Chakraborti and S. Raje Urs (2006) Genetic diversity and differentiation among populations of Indian eri silkworm *Samia cynthia ricini* revealed by ISSR markers. J. Insect Science 6:30, available online: insectscience.org/6.30 (*equal contributors)
9. Pradeep. A. R, S.N.Chatterjee and C.V.Nair (2005) Genetic differentiation induced by selection in an inbred population of the silkworm *Bombyx mori*, revealed by RAPD and ISSR marker systems. J. Appl. Genet. 46: 291-298.
10. Pradeep A. R, S.N. Chatterjee, B. Sarathchandra and S. Raje Urs (2005). Allelic variants of a juvenile hormone responsive gene which connote genetic differentiation in strains of the silkworm *Bombyx mori.* J. Genet. & Breed. 59: 213-224
11. Chatterjee S. N and Pradeep. A.R. (2003) Molecular markers (RAPD) associated with growth, yield and origin of the silkworm, *Bombyx mori* L. in India. Rus. J. Genet. 39: 1365-1377.

Insect Physiology

1. Pradeep. A.R, M.K.Singh and S.S.Sinha 1995 Growth and its relation with prothoracicotropic hormone release during larval instars of *Antheraea mylitta* Drury (Lepidoptera : Saturniidae). Phytophaga 7: 65-72
2. Pradeep. A.R, S.K.sharan, M.K.Singh, B.R.R.P.Sinha and S.S.Sinha 1997 Endocrine mediation in termination of pupal diapause in *Antheraea mylitta* Drury (Lepidopetra : Saturniidae). Entomon 22: 173-178
3. Pradeep. A.R, S.K.sharan, B. M.K.Singh and S.S.Sinha 1997 Influence of cephalic and mating stimuli on egg laying in the tasar silkmoth, *Antheraea mylitta* Drury (Lepiodopetra : Saturniidae). Journal of Entomological Research 21: 315-319
4. Pradeep. A.R, K.Thangavelu and M.K.Singh. 1997 Endocrine events in relation to growth during larval-larval and larval-pupal development in *Antheraea mylitta* Drury (Lepidoptera:Saturniidae). Journal of Insect Science 10: 8-11
5. Pradeep. A.R, S.K.Sharan, B.M.K.Singh, A.K.Sinha and B.R.R.P.Sinha 1997 Cues determine the timing of endocrine events and moult initiation in penultimate instar larvae of *Antheraea mylitta* Drury (Lepidoptera; Saturniidae). Int. J. Wild Silkmoth & Silk 5:141-144

6. Dinesh Kumar, S.K.Sharan, P.K.Mishra, A.R.Pradeep, B.M.K.Singh and B.R.R.P. Sinha 2002 Fenoxycarb induced changes on the larval morphogenesis of tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera : Saturniidae). XIXth Congress of The International Sericultural Commission Proceedings, Bangkok, Thailand, , pp251-255.
7. Mishra P.K, Dinesh Kumar, S.K.Sharan, , B.M.K.Singh, A.R.Pradeep, and B.R.R.P. Sinha 2002 Antiecdysteroidal action of plumbagin on non-diapausing and diapausing pupae of tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). XIXth Congress of The International Sericultural Commission Proceedings, Bangkok, Thailand, pp.303-307.

Guidance to award Ph.D under University of Mysore:
1 (in progress) ; 1 (Thesis submitted)

Projects pursued/ being pursued:

1. Studies on the reproductive and hormonal physiology of *Antheraea mylitta* at CTR&TI, Ranchi. 1992-1996
2. Hormonal regulation of diapause in the Indian wild silkworm *Antheraea mylitta* at CTR&TI, Ranchi 1996-2000
3. Molecular genetics of yield potential and growth in the silkworm, *Bombyx mori* at Seribiotech RL, Bangalore 2000-05
4. Phylogeography of tasar and muga silkworm (2005-08)- funded by DBT, New Delhi.
5. Morphological and molecular characterization of eri silkworm, *Samia cynthia ricini. (2005-08)*
6. Molecular mechanism of stress in silkworms*, Bombyx mori* and *Samia cynthia ricini* – a DBT funded project. (2009-12).
7. Host-Parasite Interaction: Transcriptome responses to parasitism in the silkworm *Bombyx mori*.: DBT funded project. (2013-16).

Lab members

Ms. Anitha J, SRF, Ph D Student (2009-14)

Mr. Arun K.H, JRF (2009-10)

Ms. Bindya Joy, JRF (2010-12)

Ms. Pooja Makwana, JRF, Ph D Student (2013 – continuing)

Ms. Sameera K Reddy, JRF (2013-14)

Ms. Shambhavi P.H, JRF (2014 – continuing)

Technical Assistance: Mr. Venketashaiah

Silkworm rearing assistance: Mrs. Lakshmamma