

Report

Molecular and Population Genetics of Tarnished Plant Bug: Current Status and Future Needs

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Abstract: Tarnished plant bug (TPB) continues to be the most important pest of cotton in the midsouth, but management of this insect has not been successful because of the development of resistance to insecticides. Although a large number of studies have been conducted on TPB ecology, host utilization, distribution, and insecticide resistance, there is a general paucity of data on genetics of TPB. A large array of nucleotide sequence data is available in public databases to facilitate studies on genetics, population genomics, and physiology of TPB. In this manuscript we present a brief review of past work on TPB, and discuss what could be accomplished in the near future using currently available genetics resources.

Keywords: *Lygus*, tarnished plant bug, population genetics

Introduction

Tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a widely distributed insect pest of vegetable and field crops. A succession of commonly occurring wild host plants support reproduction and development of TPB in the southern USA throughout the growing season (Robbins et al., 2000; Snodgrass et al., 2010; Snodgrass et al., 2011; Snodgrass et al., 1984). TPB is currently the most important pest of cotton, *Gossypium hirsutum* L., in the midsouth. In 2012 alone, cotton yield losses caused by TPB infestations in Mississippi were estimated at 64.7% of the total losses due to all insect pests, costing farmers approximately \$28 million (Williams, 2013). Control of TPB is achieved almost exclusively through insecticide sprays. However, resistance to certain classes of insecticides has been recorded in the Mississippi Delta since the mid-1990s (Scott & Snodgrass, 2000; Snodgrass, 1996; Snodgrass & Elzen, 1995; Snodgrass & Scott, 2000; Snodgrass & Scott, 2002). Widespread resistance to a previously highly effective insecticide, acephate, has been reported from the Mississippi River Delta (Gore et al., 2012; Snodgrass et al., 2009). TPB populations with high levels of resistance to acephate were first found in the Mississippi Delta in the fall of 2005. These resistant populations were found throughout the Deltas of Arkansas, Louisiana, and Mississippi by the fall of 2007 (Snodgrass et al., 2009). Inheritance of acephate resistance appeared to be semi-dominant which made it fairly stable and easy to select for in a population (Snodgrass et al. 2009).

Resistance of TPB to insecticides may develop by one or more of the following means: breakdown of insecticides by increased levels or enhanced activity of detoxifying enzymes (metabolic resistance), limited binding/action of the chemical through genetically modified target sites (target-site resistance), alteration of properties of exoskeleton that reduce rate of penetration of contact insecticides (penetration resistance), and through behavioural resistance where ability to detect insecticides and avoid exposure evolves (Guedes et al., 2009; Li et al., 2007; Roush & McKenzie, 1987). These adaptations reflect shifts in frequencies of alleles associated with changing environmental conditions over time (Black

et al., 2001; Fenton et al., 2010). Shifts in allele frequencies of genes responding to environmental factors can be identified by monitoring populations using genetic markers. In addition, genetic markers can be used to estimate effective population size, gene flow, and dispersal rates between populations as well as to detect genetic structure in natural populations. Data on gene flow and dispersal rates are useful in understanding spread of insecticide resistance in natural populations.

Population genetic studies commonly use naturally occurring polymorphisms in enzymes (allozyme), simple sequence repeats (microsatellites), random amplified polymorphic DNA (RAPD), and restriction fragment length polymorphism (RFLP) to identify genetic variation at population level. Well characterized molecular markers also have been used to identify and characterize modes of insecticide resistance (Aronstein et al., 1994; Ffrench-Constant et al., 2000; Ffrench-Constant et al., 1994; Steichen & Ffrench-Constant, 1994) and are useful in monitoring insecticide resistance in natural populations, implementing effective management decisions (Black et al., 2001; Ffrench-Constant et al., 2000). They are also used to map specific genetic traits, for example Wondji et al. (2009) and develop genetic maps of chromosomes (Elshire et al., 2011). Therefore, validated molecular markers developed from expressed genes and genomic DNA are tools essential for conducting population genetic and other molecular genetic studies.

Current status of genetics studies on TPB

Only a limited number of nucleotide sequence reads from expressed genes of TPB were available until recently (Allen, 2007; Allen & Mertens, 2008; Allen & Walker, 2012; Dickens et al., 1998a; Dickens et al., 1998b). With the advent of affordable high-throughput sequencing platforms, a large number of reads from TPB expressed genes have been deposited in public databases [e.g. Accessions: SRP005464, SRP005942, & SRP005944, (OPP, unpublished data); SRP010091, (Magalhaes et al., 2013)]. In addition, several hundred validated sequences of xenobiotics and secondary metabolite processing related genes, digestion related genes [Accessions: JI412110-JI412368, KC702729-KC702778, & KF208689-KF208739, (OPP, unpublished data)], and odorant binding genes (Hull et al., 2014) are available in public databases. Approximately 357,000 high-throughput nucleotide sequence reads obtained from expressed genes of adult western tarnished plant bug (WTPB), *Lygus hesperus* Knight, are also available [Accession: SRP026312, (Hull et al., 2013)]. Although the WTPB genome is estimated to be about 30 Mbp larger than the 900 Mbp genome of TPB (OPP, unpublished), high nucleotide sequence similarity in a limited number of expressed genes (Allen & Mertens, 2008; Celorio-Mancera et al., 2009; Hull et al., 2012) was observed between these two species. Therefore, it may be possible, although in limited scope, to use genetic tools developed for WTPB to study TPB. For example, some microsatellites characterized for TPB were also validated for WTPB and vice-versa (Perera et al., 2007; Shrestha et al., 2007).

The olfactory system is an essential component in host selection, mating, and aggregation responses of invertebrates. See, for example, Akers and Oconnell (1991); Anton and Hansson (1994); Chinta et al. (1994); Judd and Borden (1991); Krieger and Breer (1999); Li et al. (1992); (Mayer, 1993); Pantle and Feir (1976); Sant'Ana et al. (1999); Wang et al. (1991). Initial molecular studies of TPB olfaction included identification and characterization of an odorant binding protein from the antennae of adults (Dickens et al., 1998a; Dickens et al., 1998b; Vogt et al., 1999). Recently, olfactory co-receptors of WTPB and TPB and 32 novel odorant binding proteins from TPB were characterized (Hull et al., 2012). This study used quantitative real-time PCR and microarrays to study expression patterns of odorant binding proteins in chemosensory appendages (antennae, proboscis, and legs) of adult male and female TPB (Hull et al., 2014). Knowledge gained by this study is being extended by additional studies on the antennal transcriptome (Perera, unpublished data).

Tarnished plant bug, like most *Lygus* species, is a generalist that can feed and reproduce on many different host plants including greater than 50% of cultivated plant species in the USA (Capinera, 2001; Esquivel & Mowery, 2007; Snodgrass et al., 1984; Young, 1986). *Lygus* species are also known to be facultative predators or cannibals (Wheeler Jr., 1976). In order to efficiently utilize such a diverse array of food, digestive and metabolic systems of TPB must be highly resilient and adaptive. Examination of TPB and WTPB salivary glands and midgut digestive enzyme profiles revealed presence of trypsin-

and elastin-like proteases, α -amylases, α -glucosidases, pectinases, and phospholipase-c in one or both tissues. Very similar activity profiles were found in both plant bug species (Agusti & Cohen, 2000). Habibi et al. (2001) examined effects of diet on expression profiles of WTPB salivary gland proteins. Although salivary gland protein profiles of artificial diet-fed insects were different from those in insects fed on cotton, expression profiles of digestive enzymes could not be elucidated with the technique used in the study. Wright et al. (2006) characterized contribution of different classes of proteolytic enzymes in WTPB salivary gland and gut homogenates to digestion of casein. Serine proteases with high pH optima were predominant in salivary glands while aspartic proteases with acidic pH optima and serine proteases with slightly above neutral (pH 7.5) optima were predominant in WTPB midgut. Although nucleotide sequences of some proteases from gut and salivary glands of TPB are available in databases, no significant studies have been conducted on expression profiles of sequence characterized proteases in TPB.

Thermodynamic properties of polygalacturonase (PG) and α -amylase in salivary glands of both TPB and WTPB indicated ability of these insects to rapidly modify pectic polysaccharides and starches (Agblor et al., 1994). Recently, a few PGs were cloned and characterized from TPB and WTPB (Allen & Mertens, 2008; Celorio-Mancera et al., 2008). Several salivary proteins, including a few PGs and proteases, were identified by sequencing proteins in WTPB probed diet by mass spectrometry (Cooper et al., 2013). Although these studies indicated an adaptive nature of plant bug digestive system and diversity of enzymes involved in digestion, more descriptive studies are needed for precise characterization of digestion in TPB. In our laboratory, we have cloned and characterized over 75 different PG, protease, lipase, glucosidase, and xylanase-like transcripts from salivary glands and gut of TPB (e.g. GenBank accession numbers, KC702729-KC702778 and KF208689-KF208739). We have also performed RNA-Seq experiments to study expression profiles of these digestive enzymes in midgut and salivary glands of TPB. Gene ontology (GO) terms for molecular function enriched in salivary glands of TPB ($p < 0.05$ with Benjamini-Hochberg false discovery rate correction) and number and percentage of enriched transcripts are given in Table 1. Gene ontology terms for PG activity, hydrolase activity on glycosyl bonds, and protease activity are some significantly enriched terms in salivary gland with 44 (97.78%), 15 (78.95%), and 71 (19.40%) transcripts, respectively, of the transcriptome selected. Additional enriched GO terms included structural constituents of ribosomes (i.e. ribosomal proteins), translation initiation activity, structural molecule activity, and receptor/catalytic activity that indicated enhanced levels of protein synthesis in salivary glands of TPB. Detailed evaluation of gut and salivary gland gene expression in TPB exposed to different food sources is in progress.

Table 1. Molecular function gene ontology (GO) terms enriched in *Lygus lineolaris* salivary glands. Probability values, total number of transcript sequences annotated with the GO term, number and percentage of transcripts with significantly high (nominal $p < 0.05$ with Benjamini-Hochberg false discovery rate correction for multiple testing) expression levels in salivary glands of adults are given.

Gene ontology (GO) Term	P-value	Total no. transcripts	No. selected transcripts	% Selected transcripts
Polygalacturonase activity	7.940E-39	45	44	97.78
Structural constituent of ribosome	6.050E-29	117	64	54.70
Hydrolase activity	3.720E-12	459	106	23.09
protein binding	3.780E-10	1069	51	4.77
hydrolase activity on glycoside bonds	1.650E-09	19	15	78.95
binding	8.420E-06	760	40	5.26
peptidase activity	4.950E-05	366	71	19.40
Structural molecule activity	5.420E-03	16	9	56.25
Translation factor activity	1.230E-02	35	12	34.29
Receptor activity/catalytic activity	1.260E-02	4	4	100.00
Receptor activity/kinase activity	1.490E-02	4	4	100.00
Translation initiation factor activity	3.270E-02	11	6	54.55
Electron carrier activity	4.110E-02	8	5	62.50

A population genetics study of TPB in a small geographic area near Stoneville, MS was conducted using microsatellites markers. Insects collected from five locations (Table 2) in May, July and September of 2006 were genotyped with 14 microsatellite loci characterized in TPB and WTPB (Perera et al., 2007; Shrestha et al., 2007). Statistical analysis indicated that TPB populations in the study area were genetically structured. Two genetic clusters were identified by Bayesian simulations using the STRUCTURE v2.3.4 program (Pritchard et al., 2000) and factorial correspondence analysis (FCA) using the GENETIX 4.05 program (Belkhir et al., 2004). The proportions of insects classified to each cluster (arbitrarily named Cluster 1 and Cluster 2) changed temporally; in May 2006, four collections had high proportions of Cluster 1 insects and all collections in September 2006 contained high proportions of Cluster 2 insects. The fractions of insects classified to each genetic cluster from locations in May (A1-E1), July (A2-E2), and September (A3-E3) of 2006 are shown in Table 2. This temporal shift in allele frequencies at the microsatellite loci may be a result of a combination of genetic drift, gene flow, and selection by various intrinsic and extrinsic factors. However, it is difficult to evaluate the contribution by any of the above phenomena to the changes in allele frequencies at the microsatellite loci analysed.

Table 2. The distribution of genetic clusters in five collection sites near Stoneville, MS in samples collected in May, July, and September 2006. Fraction of Cluster 1 and Cluster 2 genotypes from each collection site and collection time and the standard deviation calculated from 20 independent simulations are shown. Distances between the collection sites (Km): A-B: 5.89, A-C: 7.59, A-D: 12.53, A-E: 15.82, B-C: 1.73, B-D: 6.98, B-E: 10.28, C-D: 5.34, C-E: 8.61, D-E: 3.31.

Collection site	May		July		September	
	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 1	Cluster 2
A	0.949±0.000	0.051±0.000	0.667±0.000	0.333±0.000	0.223±0.000	0.777±0.000
B	0.389±0.001	0.611±0.001	0.318±0.000	0.682±0.000	0.114±0.000	0.886±0.000
C	0.883±0.000	0.117±0.000	0.324±0.000	0.676±0.000	0.122±0.000	0.878±0.000
D	0.895±0.000	0.105±0.000	0.835±0.000	0.165±0.000	0.241±0.001	0.759±0.001
E	0.801±0.001	0.199±0.001	0.738±0.001	0.263±0.001	0.077±0.000	0.923±0.000

Future research needs

Except for a few detailed studies of limited scope, most publications currently available on the genetics of TPB focus on cloning and sequencing of specific genes or groups of genes. There is a need for more information on TPB physiology, genetics, and genomics. Broad scope studies on genetics and physiology of development, diapause, feeding, and xenobiotic metabolism would facilitate our understanding of TPB ability to adapt to climatic extremes from tropical to sub-arctic regions, to develop and reproduce on a wide array of host plants, and to rapidly evolve resistance to several classes of insecticides. TPB transcriptome and genome sequencing efforts undertaken at our laboratory are only the first step in developing resources to study TPB genetics and physiology, but they are essential starting points for developing better understand TPB genetics and associated biological processes. For example, properly annotated transcriptomes can be used as a reference nucleotide sequence set in RNA-Seq studies to evaluate effects of host plants on TPB physiology or to identify groups of genes acting in concert in overcoming the effects of intoxication with chemical insecticides. Genome and transcriptome nucleotide sequence data can also be used to develop marker-based genetic maps to study inheritance of traits and to conduct genome-wide association studies to understand complex interactions between multiple loci. Development of collaborative research programs are complex and require skills and expertise in multiple disciplines. Researchers at universities and government research facilities in the USA and worldwide are essential to broaden our knowledge-base of TPB.

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