



# Expressed sequenced tags from *Lygus lineolaris* (Hemiptera: Miridae), the tarnished plant bug

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**ABSTRACT.** Expressed sequenced tags (ESTs) were prepared to establish a baseline for molecular genetic studies of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). The largest class of identifiable ESTs (15.2%) was from genes involved in cellular metabolic functions, including physiological processes. Twenty-seven ESTs (9.8%) were from genes associated with transcription and translation, including ribosomal genes. One hundred and forty-two of the 276 unique ESTs were from genes not previously identified from any organism. Twelve sequences appear to be associated with feeding and digestion and may be targets for pest control studies.

**Key words:** Expressed sequence tags, Tarnished plant bug, Feeding, *Lygus lineolaris*, Digestion

## INTRODUCTION

*Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) is a pest of many agricultural crops, often damaging young fruit and growing stems by injecting salivary enzymes into plant tissue (Wheeler, 2001). In the cotton-growing regions of North America, insect damage to crops has changed dramatically over the past 10 years. While damage from lepidopterous pests has decreased due to the adoption of insect-resistant transgenic varieties of corn, soybean, and cotton, *L. lineolaris* has emerged as a leading pest insect in cotton, based on loss estimates (Williams, 2005).

An understanding of the genetics associated with feeding and digestion processes in *L. lineolaris* is vital to development of genetic strategies to curtail losses caused by this insect. Because the genome of *L. lineolaris* is not currently planned for sequencing, cDNA expressed sequenced tag (EST) sequences from this insect present the most basic functional genetic information available in the short term. A small expression library was constructed, sequenced and analyzed to obtain a basic list of gene sequences from *L. lineolaris*. Expressed sequences that correspond to insect structural genes, digestive enzymes, and common housekeeping gene sequences were segregated and compared to other publicly available arthropod sequences.

## MATERIAL AND METHODS

*Lygus lineolaris* colony was established from field collections made near Starkville, MS, USA, and originally maintained at the Biological Control & Mass Rearing Research Unit, Starkville, MS. Insects used for library construction were reared at the USDA National Biological Control Laboratory in Stoneville, MS. They were maintained on a 16-h light/8-h dark cycle, at a temperature of 24° and 20°C, respectively, and at 70% relative humidity. Insects were fed a standard production diet (Cohen, 2000). Last-instar male rather than female nymphs were used for library construction with the expectation of maximizing identification of feeding and digestive sequences and minimizing identification of egg development and maturation sequences.

Male nymphs were collected fresh and total RNA was extracted using the Multi-enzymatic liquefaction of tissue (MELT™) total nucleic acid isolation system (Ambion) kit, following instructions provided by the manufacturer. RNA was further processed using the Poly(A) Purist™ MAG magnetic poly(A) RNA purification kit (Ambion), according to instructions provided. A cDNA library was constructed using the SuperScript™ plasmid system with Gateway® technology for cDNA synthesis and cloning (Invitrogen), according to instructions provided. Fractionated cDNA was ligated into the pSPORT1 vector and then introduced into chemically competent cells. Colonies were manually picked and transferred to 96-well plates for sequencing. Sequencing was performed by the USDA ARS MidSouth Genomics Core Facility. Sequences were analyzed by comparison with the tBLASTx algorithm (Altschul et al., 1997).

## RESULTS AND DISCUSSION

We found many previously unknown genes for an increasingly important pest. Two hundred and seventy-two unique ESTs were generated, 48% of which could be identified as highly similar (expected value less than  $1 \times 10^{-10}$ ). The average sequence length was 768 bases, with only 11 ESTs having a length of less than 300 bases. Sequences less than 200 bases were

not deposited as ESTs. Twenty-four of the sequences represented contigs, with the remainder as singlets. Accession numbers assigned to ESTs were DY470827 through DY470922, DY473180 through DY473230, and DY524480 through DY524596. Twelve sequences that appeared to represent complete or nearly complete genes, including actins, tubulins, cathepsins, pectinases, and a hexamerin, were submitted as standard GenBank submissions (accession numbers DQ386914, DQ450899, DQ471300, DQ471301, DQ399525-DQ399527, and DQ474245-DQ474249).

Sequences that could be identified to an expected value of less than  $1 \times 10^{-25}$  were categorized into six groups (Table 1).

- 1) Transcription and translation (10% of provisionally identified sequences) including 10 ribosomal proteins.
- 2) Cellular metabolism and physiological process sequences (15%).
- 3) Structural and cytoskeletal sequences (7%).
- 4) Sequences associated with feeding and digestion (4%).
- 5) Mitochondrial sequences (4%).
- 6) Sequences homologous to genes for which a function was not identified (8%).

Sequences that corresponded to *L. lineolaris* genes that were already identified and deposited in GenBank were found, including 16S ribosomal sequence and a digestive enzyme trypsin (Zhu et al., 2003).

We identified several gene sequences that may serve as endogenous controls for expression studies. ESTs from the transcription and translation group include ribosomal and mitochondrial sequences that have been useful for phylogenetic and population studies of arthropods (Murrell et al., 2001). Sequences that may be associated with feeding and digestion are of particular interest, especially the pectin-degrading enzymes identified as the primary cause of plant damage by this insect (Strong and Kruitwagen, 1968; Shackel et al., 2005). Additional putative digestive enzymes were identified by cross referencing the GenBank homologue with protein databases, as described in a study of another phygophagous insect (Pedra et al., 2003). Gene sequences previously identified from *L. lineolaris* include ribosomal sequences (Whiting et al., 1997), cytochrome oxidase (Tilmon et al., 2003), and cytochrome P450 monooxygenase sequences (Zhu and Snodgrass, 2003), odorant binding sequences (Vogt et al., 1999), esterases (Zhu et al., 2004), and trypsins (Zeng et al., 2002; Zhu et al., 2003). These previously submitted sequences were not duplicated in our submissions. Our purpose was to provide a broader window into the genetic composition of this important pest insect.

This is the first EST library publicly deposited for this pest, and it includes genes associated with the damage-producing activities of the insect, namely feeding. There were 272 different ESTs generated, of which 260 were deposited in the NCBI dbEST repository. Twelve genes were deposited into GenBank as standard Core Nucleotide submissions. The mitochondrial EST sequences identified may be used to construct a whole mitochondrial genome sequence for this insect. Many sequences will be used in future research as endogenous controls, and to characterize functional genes for this pest and related species.

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**Table 1.** Expressed sequenced tags from the tarnished plant bug, *Lygus lineolaris*, and the most homologous tBLASTx match. Species names are only provided for insect matches.

Clone name	Sequence length	GenBank accession No.	Closest GenBank GI No. (tBLASTx)	Closest GenBank match organism	Closest match description	Similarity (e-value)
<b>Transcription and translation</b>						
NBCL000007	924	DY470858	56199451	<i>Culicoides sonorensis</i>	Elongation factor	0
NBCL000142	878	DY473222	62484463	<i>Drosophila melanogaster</i>	Kruppel target	1e-145
NBCL000095	921	DY470917	24645296	<i>D. melanogaster</i>	Tryptophanyl-tRNA synthetase	1e-135
NBCL000172	895	DY524501	66513775	<i>Apis mellifera</i>	ATP-binding cassette	1e-134
NBCL000253	1301	DY524581	19073318	<i>Lygus lineolaris</i>	16S ribosomal subunit	1e-126
NBCL000093	810	DY470915	54609282	<i>Bombyx mori</i>	Ribosomal protein S2	1e-123
NBCL000198	781	DY524527	27462591	<i>Spodoptera frugiperda</i>	Elongation factor	1e-123
NBCL000111	892	DY473190	45361526	western clawed frog	Chaperonin	1e-114
NBCL000166	784	DY524495	66530281	<i>Ap. mellifera</i>	Ribosomal protein L7	1e-111
NBCL000123	862	DY473202	66525284	<i>Ap. mellifera</i>	Ribosomal protein L4	4e-94
NBCL000083	834	DY470905	1209408	<i>Chironomus tentans</i>	mRNA binding/transport	7e-94
NBCL000006	854	DY470857	9438111	<i>Aedes triseriatus</i>	Chaperonin	8e-92
NBCL000163	519	DY524492	15213831	<i>S. frugiperda</i>	Ribosomal protein S23	1e-91
NBCL000265	604	DY524593	24585788	<i>D. melanogaster</i>	Ribosomal protein L21	1e-80
NBCL000225	754	DY524553	37703938	<i>Forficula auricularia</i>	Elongation factor	9e-75
NBCL000137	926	DY473217	66559207	<i>Ap. mellifera</i>	Ribosomal protein L6	3e-69
NBCL000034	964	DY470881	24647906	<i>D. melanogaster</i>	Initiation factor	2e-67
NBCL000138	820	DY473218	57924402	<i>Anopheles gambiae</i>	Tyrosyl-DNA phosphodiesterase	9e-66
NBCL000130	453	DY473210	67083786	black-legged tick	Ribosomal protein L30	1e-60
NBCL000026	827	DY470873	24644482	<i>D. melanogaster</i>	mRNA splicing regulation	4e-59
NBCL000021	935	DY470868	27626145	<i>An. gambiae</i>	Small nuclear ribonucleoprotein	1e-53
NBCL000059	545	DY470835	24584115	<i>D. melanogaster</i>	Ribosomal protein L24	2e-51
NBCL000067	877	DY470843	24643987	<i>D. melanogaster</i>	Elongation factor	2e-46
NBCL000062	877	DY470838	21450322	mouse	Small nuclear ribonucleoprotein	2e-39
NBCL000035	865	DY470882	61674086	<i>Aedes aegypti</i>	Ribosomal protein L7	2e-28
NBCL000164	942	DY524493	24654755	<i>D. melanogaster</i>	Nucleic acid binding	3e-28
NBCL000099	813	DY470921	24658444	<i>D. melanogaster</i>	Engrafted-associated protein	7e-28
<b>Cellular metabolism and physiological processes</b>						
NBCL000252	1344	DY524580	71370845	ribbon worm	ATP synthase	1e-170
D9D	934	DQ474245	13430288	<i>Acheta domesticus</i>	Delta9 desaturase	1e-137
NBCL000025	747	DY470872	58394105	<i>An. gambiae</i>	6-phosphofructo-2-kinase	1e-128
NBCL000213	967	DY524541	58384144	<i>An. gambiae</i>	Malate dehydrogenase	1e-125

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Table 1. Continued.

Clone name	Sequence length	GenBank accession No.	Closest GenBank GI No. (tBLASTx)	Closest GenBank match organism	Closest match description	Similarity (e-value)
NBCL000216	977	DY524544	58387252	<i>An. gambiae</i>	CytC protein transporter	1e-122
NBCL000082	900	DY470904	28571121	<i>D. melanogaster</i>	Fumarate hydratase	1e-112
NBCL000226	868	DY524554	83307263	clam worm	Ubiquitin C-terminal hydrolase	1e-110
NBCL000227	877	DY524555	24584172	<i>D. melanogaster</i>	Isomerase/aldose epimerase	3e-99
NBCL000182	537	DY524511	58380262	<i>An. gambiae</i>	Ubiquitin-conjugating enzyme	1e-96
NBCL000231	850	DY524559	27570482	<i>An. gambiae</i>	Calmodulin binding	5e-95
NBCL000032	881	DY470879	24662536	<i>D. melanogaster</i>	Ubiquitin fusion-degradation protein	4e-93
NBCL000234	889	DY524562	42763906	<i>Ae. aegypti</i>	Golgi-associated protein carrier	4e-82
Nitr	753	DQ474249	58384354	<i>An. gambiae</i>	Nitrilase	2e-78
NBCL000241	978	DY524569	72114865	purple sea urchin	Autophagy endopeptidase	5e-75
NBCL000178	634	DY524507	45479210	tick	Ferritin	5e-71
NBCL000162	884	DY524491	66510284	<i>Ap. mellifera</i>	Trehalose phosphate synthase	1e-69
NBCL000096	826	DY470918	46561759	<i>Homalodisca coagulata</i>	Vacuolar ATPase	8e-69
NBCL000190	387	DY524519	66550693	<i>Ap. mellifera</i>	Glycogenin	6e-67
NBCL000175	797	DY524504	24654208	<i>D. melanogaster</i>	Oxidoreductase	7e-64
NBCL000050	612	DY470895	67083824	black-legged tick	Superoxide dismutase	1e-61
NBCL000161	792	DY524490	42763975	<i>Ae. aegypti</i>	Hydrogen-transporting ATPase	2e-61
NBCL000189	443	DY524518	58385846	<i>An. gambiae</i>	Phosphoenolpyruvate carboxylkinase	2e-59
NBCL000090	805	DY470912	66529836	<i>Ap. mellifera</i>	SON DNA-binding	2e-56
NBCL000151	876	DY524480	24656870	<i>D. melanogaster</i>	Glycerol-3-phosphate transporter	7e-55
NBCL000244	279	DY524572	17861429	<i>D. melanogaster</i>	Protein serine/threonine phosphatase	2e-53
Hex 1	1126	DQ471300	1580795	<i>Periplaneta americana</i>	Hexamerin	1e-51
NBCL000018	569	DY470866	53148460	<i>Plutella xylostella</i>	Proteasome subunit	3e-51
NBCL000010	801	DY470861	73543347	purple sea urchin	Calcium-transporting ATPase	3e-50
NBCL000140	895	DY473220	14331151	<i>Photinus pyralis</i>	Regucalcin; luciferin-regenerating enzyme	2e-46
NBCL000080	922	DY470902	24648434	<i>D. melanogaster</i>	Glutamyl-tRNA amidotransferase	3e-41
NBCL000239	268	DY524567	72015265	purple sea urchin	Serine/threonine protein phosphatase	4e-41
NBCL000102	830	DY473181	24645792	<i>D. melanogaster</i>	ATP binding; electron transport	1e-31
NBCL000187	810	DY524516	61675133	<i>Ae. aegypti</i>	Lipid storage; transport	2e-30
NBCL000224	777	DY524552	55733683	orangutan	Ubiquitin targeting receptor	2e-30
NBCL000157	861	DY524486	5006440	<i>D. melanogaster</i>	Signal transducing adaptor protein	3e-30
NBCL000002	928	DY470853	24652223	<i>D. melanogaster</i>	Pyruvate carboxylase	2e-29
NBCL000232	603	DY524560	66525724	<i>Ap. mellifera</i>	Thioredoxin	2e-28

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Table 1. Continued.

Clone name	Sequence length	GenBank accession No.	Closest GenBank GI No. (tBLASTx)	Closest GenBank match organism	Closest match description	Similarity (e-value)
<b>Structural and cytoskeletal</b>						
Act 1	1237	DQ386914	91088364	<i>Tribolium castaneum</i>	Cytoplasmic actin	0
Tub 1	1099	DQ471301	158730	<i>D. melanogaster</i>	Alpha tubulin	0
NBCL000039	932	DY470886	66506578	<i>Ap. mellifera</i>	Beta tubulin	0
Act 2	1513	DQ450899	13430417	<i>Drosophila virilis</i>	Muscle actin	1e-159
NBCL000079	828	DY470901	158734	<i>D. melanogaster</i>	Alpha tubulin	1e-149
NBCL000221	804	DY524549	46561751	<i>H. coagulata</i>	Tropomyosin	1e-102
NBCL000131	856	DY473211	66530944	<i>Ap. mellifera</i>	Nonmuscle myosin-II heavy chain	1e-85
Tub 2	938	DQ474248	71991834	nematode	Alpha tubulin	1e-77
NBCL000229	786	DY524557	4928475	<i>Libellula pulchella</i>	Tropomyosin T	8e-74
NBCL000023	949	DY470870	73981495	dog	Actin capping protein	5e-72
NBCL000014	808	DY470862	56684880	<i>Gryllotalpa orientalis</i>	Myosin light chain	8e-65
NBCL000255	956	DY524583	66548658	<i>Ap. mellifera</i>	Myosin heavy chain	3e-62
NBCL000174	639	DY524503	46019971	<i>Lethocerus indicus</i>	Myofilin	3e-53
NBCL000212	349	DY524540	24583211	<i>D. melanogaster</i>	Peritrophic membrane protein	6e-51
NBCL000240	393	DY524568	66547346	<i>Ap. mellifera</i>	Alpha adaptin	6e-34
NBCL000149	691	DY473229	24655678	<i>D. melanogaster</i>	Cuticle protein	3e-28
NBCL000179	525	DY524508	58395673	<i>An. gambiae</i>	Cuticle protein	3e-28
NBCL000024	581	DY470871	59939332	<i>Drosophila buzzatii</i>	Cuticle protein	2e-27
<b>Feeding and digestive</b>						
NBCL000103	898	DY473182	24648290	<i>D. melanogaster</i>	Caseolytic proteinase	1e-145
Cath-L 1	957	DQ474246	38147394	<i>Triatoma infestans</i>	Cathepsin-L	1e-107
NBCL000076	867	DY470898	66505779	<i>Ap. mellifera</i>	Prolyl endopeptidase	4e-80
Cath-L 2	648	DQ474247	17062057	<i>Rhodnius prolixus</i>	Cathepsin-L	6e-72
PG1	993	DQ399525	10933619	fungus	Polygalacturonase	2e-70
PG3	886	DQ399527	2951782	yeast	Polygalacturonase	4e-57
NBCL000180	471	DY524509	33352212	alga	Chlapsin aspartic proteinase	1e-54
PG2	912	DQ399526	45270073	yeast	Polygalacturonase	1e-51
NBCL000064	477	DY470840	23577280	transparent sea squirt	Palmitoyl-protein thioesterase	2e-36
NBCL000236	330	DY524564	57530287	chicken	rPOP prolyl endopeptidase	5e-34
NBCL000203	612	DY524532	21750241	human	Prolyl carboxypeptidase	3e-29
<b>Mitochondrial</b>						
NBCL000261	1633	DY524589	11139100	<i>Triatoma dimidiata</i>	Cytochrome oxidase	0

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Table 1. Continued.

Clone name	Sequence length	GenBank accession No.	Closest GenBank GI No. (tBLASTx)	Closest GenBank match organism	Closest match description	Similarity (e-value)
NBCL000262	1128	DY524590	50402839	<i>Pteronarcys princeps</i>	Cytochrome b	1e-111
NBCL000267	761	DY524595	11139100	<i>T. dimidiata</i>	Unknown	1e-111
NBCL000250	1240	DY524578	11139100	<i>T. dimidiata</i>	NADH dehydrogenase	1e-89
NBCL000264	671	DY524592	62146758	<i>Hyles annei</i>	Cytochrome oxidase	5e-74
NBCL000266	986	DY524594	11139100	<i>T. dimidiata</i>	NADH dehydrogenase	7e-72
NBCL000160	694	DY524489	58384746	<i>An. gambiae</i>	Mitochondrial S11 subunit	5e-66
NBCL000233	894	DY524561	11139100	<i>T. dimidiata</i>	Cytochrome b	1e-58
NBCL000268	861	DY524596	27372193	tadpole shrimp	Unknown	8e-43
NBCL000169	979	DY524498	24653505	<i>D. melanogaster</i>	L53 subunit	7e-32
Not otherwise classified						
NBCL000217	968	DY524545	66515999	<i>Ap. mellifera</i>	Adaptor related protein	1e-139
NBCL000101	841	DY473180	58395407	<i>An. gambiae</i>	Defense response, hypoxia upregulated	1e-113
NBCL000009	822	DY470860	58378947	<i>An. gambiae</i>	Unknown function	2e-89
NBCL000046	812	DY470891	66551761	<i>Ap. mellifera</i>	Unknown function	4e-79
NBCL000063	907	DY470839	24665376	<i>D. melanogaster</i>	Multiprotein bridging factor (mbf)	4e-65
NBCL000044	779	DY470890	27639256	<i>An. gambiae</i>	Unknown function	1e-60
NBCL000068	917	DY470844	47939403	zebrafish	Unknown function	5e-58
NBCL000070	372	DY470846	68342549	bacteria	Unknown function	1e-53
NBCL000038	889	DY470885	66500186	<i>Ap. mellifera</i>	Unknown function	8e-51
NBCL000132	983	DY473212	189700	human	Growth factor	9e-51
NBCL000194	618	DY524523	66552120	<i>Ap. mellifera</i>	Unknown function	1e-39
NBCL000238	992	DY524566	58393870	<i>An. gambiae</i>	Unknown function	2e-39
NBCL000110	902	DY473189	66539172	<i>Ap. mellifera</i>	Huntingtin interacting protein	8e-29
NBCL000069	944	DY470845	24653249	<i>D. melanogaster</i>	Unknown function	3e-27

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