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Molecular characterization and expression analysis of putative odorant carrier proteins in *Adelphocoris lineolatus*

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ABSTRACT

The alfalfa plant bug *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), a serious pest of several agricultural crops, is extremely attracted by flowering plants to perform season host switching. Generally, chemoreception of *A. lineolatus* plays a crucial role in detecting food sources, locating mates and oviposition sites. In addition, odorant carrier proteins are believed to participate in the initial chemical communication and perception in insects. In this work, we identified 31 putative odorant carrier protein genes from the antennal transcriptomes of *A. lineolatus*, including 17 new odorant binding proteins (OBPs), 10 new chemosensory proteins (CSPs) and four Niemann-Pick type C2 proteins (NPC2s). Phylogenetic analysis demonstrated that the OBP and CSP genes from four mirid bug species (*A. lineolatus*, *Adelphocoris suturalis*, *Apolygus lucorum* and *Lygus lineolaris*) had a relatively close evolutionary relationship. Regular patterns and key conserved motifs of OBPs and CSPs in Hemiptera insects are also identified by using Multiple EM for Motif Elicitation (MEME) tool. Tissue expression profiles analysis showed that nine of the 17 OBPs, 10 CSPs and two of the four NPC2s were primarily or uniquely expressed in the adult antennae suggesting their putative roles in chemoreception. Our study provides the first insight into the roles of odorant carrier proteins in chemoreception of *A. lineolatus*, which will help to facilitate biological functions of odorant carrier proteins and develop novel insect behavioral regulation strategy in future.

Introduction

Chemoreception is an indispensable biological process which allows insects to recognize the important chemical signals to locate food sources, search mates, interact amongst conspecific colony and determine oviposition site. Generally, the initial chemical communication and perception in insects are mediated by several soluble carrier proteins including odorant binding proteins (OBPs), chemosensory proteins (CSPs) and Niemann-Pick type C2 proteins (NPC2s); which are highly concentrated in olfactory and gustatory organs. These odorant carrier proteins can bind and carry semiochemicals to activate specific chemoreceptors (Pelosi et al., 2014).

Insects' OBPs (water-soluble proteins, −150 amino acids) are made of six α-helical domains and three disulfide bridges that paired by six cysteines in an interlocked fashion (1–3, 2–5 and 4–6), which assembled in a compact and stable structure (Tegoni et al., 2004). OBPs are suggested to be involved in the initial biochemical recognition steps of olfactory signal transduction by acting as molecular carriers that solubilize and transport fat-soluble odorants to the odorant receptors (ORs) (Pelosi et al., 2014). Insect OBPs can be divided into five groups based on the number of Cys residues: 'classical' OBPs (six Cys residues) (Zhou, 2010), 'dimer' OBPs (two classical Cys signature motifs) (Zhou et al., 2004), 'Plus-C' OBPs (a Pro residue and two additional Cys residues) (Zhou et al., 2004), 'Minus-C' OBPs (lacking two of the six conserved Cys) and 'Atypical' OBPs (9–10 Cys residues) (Xu et al., 2003).

Insect OBPs were first identified in the silkmoth *Antheraea polyphemus*, which could bind to sex pheromones (Vogt et al., 1991). Most of the OBPs are highly expressed in antennae of Hemiptera insects, which play roles in the perception of chemical signals, for instance, alarm pheromones, sex pheromones and host volatiles. However, some identified OBPs with high expression in non-olfactory organs instead of

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Table 1
Odorant binding proteins (OBPs) identified in *A. lineolatus* antennae.

Gene name	Acc. number	Unigene	Length(bp)	ORF(aa)	Signal peptide	RPKM value		Best Blastx Match			
						Female	Male	name	Acc. number	E value	Identify
OBP1	ACZ58027.1	comp129978_c0	1142	146	1—10	9724.07	15,753.12	odorant-binding protein 1 [*Adelphocoris lineolatus*]	ACZ58027.1	1.00E-97	100%
OBP2	ACZ58028.1	comp124408_c1	570	–	–	21,488.92	19,692.24	odorant-binding protein 2 [Adelphocoris lineolatus]	ACZ58028.1	3.00E-86	99%
OBP3	ACZ58029.1	comp120864_c0	2073	153	1—19	225.47	186.43	odorant-binding protein 3 [Adelphocoris lineolatus]	ACZ58029.1	2.00E-97	97%
OBP4	ACZ58030.1	comp121995_c0	834	133	1—23	11,552.89	17,447.49	odorant-binding protein 4 [Adelphocoris lineolatus]	ACZ58030.1	4.00E-89	100%
OBP5	ACZ58031.1	comp118375_c0	616	146	1—21	7898.82	14,443.52	odorant-binding protein 5 [Adelphocoris lineolatus]	ACZ58031.1	2.00E-78	98%
OBP6	ACZ58032.1	comp138380_c0	662	153	1—20	2083.51	1519.22	odorant-binding protein 6 [Adelphocoris lineolatus]	ACZ58032.1	7.00E-102	98%
OBP7	ACZ58085.1	comp121998_c0	853	202	1—24	415.59	231.55	odorant binding protein 7 [Adelphocoris lineolatus]	ACZ58085.1	3.00E-131	99%
OBP8	ACZ58079.1	comp126406_c0	2317	153	1—24	389.99	368.11	odorant binding protein 8 [Adelphocoris lineolatus]	ACZ58079.1	1.00E-98	99%
OBP9	ACZ58080.1	comp125618_c0	922	160	1—18	33.12	45.26	odorant binding protein 9 [Adelphocoris lineolatus]	ACZ58080.1	1.00E-105	100%
OBP10	ACZ58081.1	comp126236_c0	2644	162	1—16	71.46	64.61	odorant binding protein 10 [Adelphocoris lineolatus]	ACZ58081.1	2.00E-105	100%
OBP11	ACZ58082.1	comp112938_c0	564	151	1—17	4888.4	2991.25	odorant binding protein 11 [Adelphocoris lineolatus]	ACZ58082.1	7.00E-105	99%
OBP12	ACZ58083.1	comp126512_c0	1018	156	1—20	3977.24	3184.09	odorant binding protein 12 [Adelphocoris lineolatus]	ACZ58083.1	1.00E-106	99%
OBP13	ACZ58084.1	comp120776_c0	869	143	1—24	1281.71	1458.01	odorant binding protein 13 [Adelphocoris lineolatus]	ACZ58084.1	4.00E-93	98%
OBP14	ACZ58086.1	comp110436_c0	704	205	1—18	4.34	1.57	odorant binding protein 14 [Adelphocoris lineolatus]	ACZ58086.1	5.00E-82	99%
OBP15	AMD02855.1	comp120604_c0	1091	214	1—22	309.2	151	odorant binding protein 15 [Adelphocoris lineolatus]	AMD02855.1	4.00E-56	100%
OBP16	AMD02856.1	comp123505_c0	749	148	1—25	954.72	571.38	odorant binding protein 16 [Adelphocoris lineolatus]	AMD02856.1	7.00E-45	100%
OBP17	AMD02857.1	comp112975_c0	684	144	1—16	2007.83	1235.86	odorant binding protein 17 [Adelphocoris lineolatus]	AMD02857.1	2.00E-80	100%
OBP18	MG191320	comp119790_c0	1018	275	1—18	46.34	23.24	odorant-binding protein 18a [*Lygus lineolaris*]	AHF71046.1	3.00E-89	85%
OBP19	MG191321	comp553989_c0	606	194	1—24	1.34	0.23	odorant-binding protein 20 [Apolygus lucorum]	AMQ76473.1	1.00E-89	72%
OBP20	MG191322	comp127812_c0	863	146	1—19	1668.44	963.38	odorant-binding protein 26 [Apolygus lucorum]	AMQ76479.1	9.00E-62	66%
OBP21	MG191323	comp150315_c0	653	143	1—22	14.56	14.31	odorant-binding protein 3 [Adelphocoris suturalis]	ANA10229.1	1.00E-99	99%
OBP22	MG191324	comp109524_c0	698	176	1—16	43.21	1.96	odorant-binding protein 30 [Lygus lineolaris]	AHF71061.1	2.00E-91	85%
OBP23	MG191325	comp126012_c0	787	203	1—24	10.64	6.77	odorant-binding protein 31 [Adelphocoris suturalis]	ANA10241.1	1.00E-92	88%
OBP24	MG191326	comp121234_c0	820	193	1—21	117.74	169.13	odorant-binding protein 32 [Adelphocoris suturalis]	ANA10242.1	2.00E-127	91%
OBP25	MG191327	comp119197_c0	626	153	1—49	3.6	1.37	odorant-binding protein 32 [Adelphocoris suturalis]	ANA10242.1	9.00E-47	44%
OBP26	MG191328	comp47947_c0	686	155	1—15	26.67	12.62	odorant-binding protein 33 [Apolygus lucorum]	AMQ76486.1	7.00E-87	85%
OBP27	MG191329	comp114303_c0	556	134	1—33	50.11	112.76	odorant-binding protein 5 [Adelphocoris suturalis]	ANA10231.1	9.00E-77	96%
OBP28	MG191330	comp120428_c0	724	167	1—20	104.23	162.09	odorant-binding protein 8 [Lygus lineolaris]	AHF71035.1	4.00E-09	31%
OBP29	MG191340	comp120169_c0	821	–	–	200.81	351.97	odorant binding protein 11 [Apolygus lucorum]	AFJ54052.1	2.00E-93	70%
OBP30	MG191343	comp24826_c0	543	–	–	1.32	0	odorant-binding protein 32 [Adelphocoris suturalis]	ANA10242.1	3.00E-42	43%
OBP31	MG191344	comp46007_c0	276	–	–	0	2.27	odorant binding protein 4 [Apolygus lucorum]	AEP95761.1	5.00E-45	76%
OBP32	MG191341	comp86942_c0	275	–	–	1.49	0	odorant binding protein 6 [Apolygus lucorum]	AEA07664.1	3.00E-40	76%
OBP33	MG191345	comp86942_c1	328	–	–	1.39	0	odorant binding protein 6 [Apolygus lucorum]	AEA07664.1	2.00E-41	69%
OBP34	MG191342	comp2676196_c0	241	–	–	0.87	0	odorant-binding protein [Helicoverpa assulta]	AEX07273.1	2.00E-44	99%

Note: Signal peptides were predicted using SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/).