

Supplementary Information

Supplementary Table 1. Primers utilized for qRT-PCR analyses

GOI	Accession number	Forward primer 5'-3'	Reverse Primer 5'-3'	Product (bp)
Hexamerin-like	XM_011157206	GGGTGTTACGGTTCGAATCTG	TGAGCCTCCTTGTGACTGTG	108
Astakine-like	XM_011167741	ACGGATTATCCTGCGATTTC	CCGACTAGGTTCCACATAATCC	104
Noncoding RNA	XR_850725	CGATGATTTCTGCTCACGTC	ATCACCTTCGCCAAACATTC	103
Noncoding RNA	XR_850909	TGCAACCCAAACCACACTAA	TGATTGCCATGATTGTCAAGA	92
Elf1-Beta	XM_011169118	TCGCCAAGTCAAGTATCGTG	CCAAACAAGACCGTCCATTT	107
RPL18	XM_011165253	TACACCGACCACCGATTCA	GATCACGGCGACGCAATT	80
Hexamerin 2	XM_011173560	CGTAGGATATGGACCGTTG	GCCTTGATGTTGGATTGCT	97
Arylphorin-Alpha	XM_011157183	GCCCTTTCCGTAGAGAGCTT	TGTTGACGTTGAAGCGGTAG	103
Arylphorin-Beta	XM_011157182	GCCGACTACAACACCATCAA	CGAAGGATTCACCAGAGAGG	108

Supplementary Table S2. Libraries statistics: number of reads mapping to the *Solenopsis invicta* genome for each replicate (Rep.).

	Alate queen brain		Mated queen brain	
	input	Mapped (% of input)	input	Mapped (% of input)
Rep1	13,665,083	12,464,405 (91.2)	17,305,195	16,128,864 (93.2)
Rep2	13,486,050	12,336,327 (91.5)	16,015,749	14,846,343 (92.7)
Rep3	14,717,332	13,368,742 (90.8)	18,335,231	17,143,926 (93.5)
Rep4	15,669,271	14,294,871 (91.2)	16,561,818	15,459,348 (93.3)

Supplementary Table 3: GPCRs expressed in virgin alate queen brain or mated dealate queen brain transcriptomes. GPCRs are annotated as currently identified in the *Solenopsis invicta* transcriptome and organized from highest to lowest average FPKM.

GeneInfo Identifier	Protein Accession	Transcript Accession	Locus	Current annotation	Alate FPKM	Mated FPKM
gi 751222462	XP_011164467.1	XM_011166165	LOC105199190	rhodopsin	122.76	322.869
gi 751202946	XP_011174320.1	XM_011176018	LOC105206526	dopamine receptor 1	77.9886	133.694
gi 751222464	XP_011164468.1	XM_011166166	LOC105199191	rhodopsin-like	91.2739	77.2333
gi 751236330	XP_011171996.1	XM_011173694	LOC105204568	PDF receptor	25.2926	51.0937
gi 751222675	XP_011164585.1	XM_011166283	LOC105199276	probable G-protein coupled receptor AH9.1	28.8151	35.6691

gi 751209716	XP_011157518.1	XM_011159216	LOC105194339	probable G-protein coupled receptor 52	25.6449	34.8058
gi 751223066	XP_011164802.1	XM_011166501	LOC105199400	muscarinic acetylcholine receptor DM1 isoform X1	23.8323	28.1483
gi 751205599	XP_011176389.1	XM_011178087	LOC105208274	tachykinin-like peptides receptor 99D, partial	17.3657	32.4827
gi 751223401	XP_011164985.1	XM_011166683	LOC105199537	opsin, ultraviolet-sensitive-like	37.043	12.2558
gi 751238153	XP_011173009.1	XM_011174707	LOC105205353	dopamine receptor 2-like	22.8932	21.386
gi 751212977	XP_011159291.1	XM_011160989	LOC105195542	PREDICTED: G-protein coupled receptor moody isoform X2	17.3692	20.2122
gi 751212975	XP_011159290.1	XM_011160988	LOC105195542	PREDICTED: G-protein coupled receptor moody isoform X1	20.1014	14.7491
gi 751232987	XP_011170144.1	XM_011171842	LOC105203085	tachykinin-like peptides receptor 99D	12.7003	18.8504
gi 751223068	XP_011164803.1	XM_011166500	LOC105199400	muscarinic acetylcholine receptor DM1 isoform X2	14.0267	11.4862
gi 751233286	XP_011170315.1	XM_011172013	LOC105203239	allatostatin-A receptor-like isoform X1	13.6399	11.547
gi 751219449	XP_011162809.1	XM_011164507	LOC105197902	calcitonin receptor-like	12.5495	12.6063
gi 751210001	XP_011157676.1	XM_011159374	LOC105194460	diuretic hormone receptor-like	13.131	11.574
gi 751222422	XP_011164446.1	XM_011166144	LOC105199174	5-hydroxytryptamine receptor 2A-like isoform X1	14.5434	10.0597
gi 751207421	XP_011156273.1	XM_011157971	LOC105193512	tyramine receptor 1	9.84056	12.2824

gi 751223477	XP_011165026.1	XM_011166724	LOC105199572	5-hydroxytryptamine receptor 2B-like	6.77122	14.5504
gi 751231536	XP_011169333.1	XM_011171031	LOC105202476	octopamine receptor beta-1R-like	12.9282	6.29347
gi 751205401	XP_011176282.1	XM_011177980	LOC105208194	uncharacterized protein LOC105208194	9.47505	9.49876
gi 751211218	XP_011158340.1	XM_011160038	LOC105194900	5-hydroxytryptamine receptor 2A-like	7.82188	10.8045
gi 751206701	XP_011155882.1	XM_011157580	LOC105193213	latrophilin Cirl-like	8.47064	8.25634
gi 751237973	XP_011172906.1	XM_011174604	LOC105205262	probable muscarinic acetylcholine receptor gar-1	6.71836	9.72939
gi 751230013	XP_011168496.1	XM_011170194	LOC105201914	alpha-1A adrenergic receptor-like	9.25873	6.82297
gi 751201048	XP_011165260.1	XM_011166958	LOC105199739	probable G-protein coupled receptor No9	6.04333	8.33983
gi 751208599	XP_011156916.1	XM_011158614	LOC105193922	tachykinin-like peptides receptor 86C, partial	6.81062	7.03414
gi 751218298	XP_011162177.1	XM_011163875	LOC105197486	gastrin/cholecystokinin type B receptor-like isoform X2	6.27368	6.64
gi 751199635	XP_011157701.1	XM_011159399	LOC105194469	tyramine receptor 1-like	6.55474	6.09929
gi 751212247	XP_011158889.1	XM_011160587	LOC105195234	neuropeptides capa receptor-like	6.25855	6.23513
gi 751207849	XP_011156508.1	XM_011158206	LOC105193663	histamine H2 receptor isoform X2	3.49217	8.65461

gi 751201367	XP_011167017.1	XM_011168715	LOC105200910	opsin, blue-sensitive	5.64833	6.20141
gi 751211271	XP_011158366.1	XM_011160064	LOC105194927	dopamine D2-like receptor	4.67986	6.46566
gi 751230111	XP_011168550.1	XM_011170248	LOC105201947	adenosine receptor A2b-like isoform X1	5.87984	4.76843
gi 751204439	XP_011175773.1	XM_011167349	LOC105200002	neuromedin-U receptor 2-like	5.06395	5.44275
gi 751224601	XP_011165651.1	XM_011167349	LOC105200002	neuromedin-U receptor 2-like	5.06395	5.44275
gi 751214247	XP_011159970.1	XM_011161668	LOC105195979	FMRFamide receptor isoform X2	3.61381	6.12039
gi 751236000	XP_011171810.1	XM_011173508	LOC105204438	uncharacterized protein LOC105204438	3.49725	6.16527
gi 751233288	XP_011170316.1	XM_011172014	LOC105203239	allatostatin-A receptor-like isoform X1	5.06126	4.10991
gi 751218178	XP_011162112.1	XM_011163810	LOC105197452	orexin receptor type 1-like	3.81174	5.29119
gi 751238033	XP_011172940.1	XM_011174638	LOC105205294	tachykinin-like peptides receptor 86C, partial	4.35839	3.83249
gi 751206733	XP_011155898.1	XM_011157596	LOC105193226	trace amine-associated receptor 9	4.70743	3.4473
gi 751224717	XP_011165717.1	XM_011167415	LOC105200046	gastrin-releasing peptide receptor-like	3.32243	4.03507
gi 751205799	XP_011155394.1	XM_011157092	LOC105192838	cholecystokinin receptor-like	3.71336	3.47172
gi 751225754	XP_011166274.1	XM_011167972	LOC105200419	octopamine receptor beta-2R isoform X1	2.99628	3.91515
gi 751217754	XP_011161886.1	XM_011163584	LOC105197287	octopamine receptor beta-3R-like	2.60488	3.37885

gi 751202675	XP_011173067.1	XM_011174765	LOC105205389	gonadotropin-releasing hormone II receptor isoform X2	1.65061	4.11409
gi 751207336	XP_011156229.1	XM_011157927	LOC105193474	octopamine receptor beta-3R-like	2.15744	3.39361
gi 751220611	XP_011163444.1	XM_011165142	LOC105198435	FMRFamide receptor-like	3.1209	2.29737
gi 751232028	XP_011169609.1	XM_011171307	LOC105202687	neuropeptide FF receptor 2	1.93127	2.68557
gi 751206248	XP_011155636.1	XM_011157334	LOC105193015	protocadherin-like wing polarity protein stan	1.99164	2.43573
gi 751224894	XP_011165811.1	XM_011167509	LOC105200112	growth hormone secretagogue receptor type 1	2.2874	1.8629
gi 751208123	XP_011156656.1	XM_011158354	LOC105193776	lutropin-choriogonadotropic hormone receptor	2.1355	1.84212
gi 751212866	XP_011159228.1	XM_011160926	LOC105195500	prolactin-releasing peptide receptor-like	1.74498	2.1542
gi 751214243	XP_011159967.1	XM_011161665	LOC105195979	FMRFamide receptor isoform X1	2.22963	1.61088
gi 751225756	XP_011166275.1	XM_011167973	LOC105200419	octopamine receptor beta-2R isoform X2	2.15962	1.58662
gi 751224703	XP_011165709.1	XM_011167407	LOC105200038	LOW QUALITY PROTEIN: neuromedin-B receptor-like	2.19548	1.50736
gi 751236288	XP_011171973.1	XM_011173671	LOC105204554	secretin receptor-like isoform X2	1.80764	1.47041
gi 751205399	XP_011176281.1	XM_011177978	LOC105208194	uncharacterized protein LOC105208194	1.74897	1.40877

gi 751204377	XP_011175739.1	XM_011177437	LOC105207791	neuropeptides capa receptor-like	1.38895	1.70664
gi 751199398	XP_011156359.1	XM_011158057	LOC105193552	prostaglandin E2 receptor EP3 subtype	1.73066	1.25177
gi 751202673	XP_011173059.1	XM_011174757	LOC105205389	gonadotropin-releasing hormone II receptor isoform X1	1.0036	1.87529
gi 751210368	XP_011157876.1	XM_011159574	LOC105194591	allatostatin-A receptor-like	1.52128	1.32521
gi 751217576	XP_011161788.1	XM_011163486	LOC105197213	neuropeptides capa receptor-like isoform X2	1.63435	1.16905
gi 751202512	XP_011172409.1	XM_011174107	LOC105204861	allatostatin-A receptor	1.59364	1.14899
gi 751205345	XP_011176251.1	XM_011177949	LOC105208166	protein trapped in endoderm-1	1.51694	1.10737
gi 751222424	XP_011164447.1	XM_011166145	LOC105199174	5-hydroxytryptamine receptor 2A-like isoform X2	0.000951	2.46655
gi 751234791	XP_011171145.1	XM_011172843	LOC105203919	uncharacterized protein LOC105203919	1.27474	1.18695
gi 751212979	XP_011159292.1	XM_011160990	LOC105195543	G-protein coupled receptor moody	1.24731	1.17934
gi 751212250	XP_011158890.1	XM_011160588	LOC105195236	neuropeptides capa receptor-like	1.88112	0.468675
gi 751212870	XP_011159230.1	XM_011160929	LOC105195500	prolactin-releasing peptide receptor-like	0.676592	1.60495
gi 751236286	XP_011171972.1	XM_011173670	LOC105204554	secretin receptor-like isoform X1	0.805082	1.36281
gi 751199777	XP_011158500.1	XM_011160198	LOC105195005	relaxin receptor 1	1.34767	0.788181

gi 751221554	XP_011163962.1	XM_011165660	LOC105198818	alpha-2A adrenergic receptor	0.988972	1.14645
		XR_850821	LOC105197486	gastrin/cholecystokinin type B receptor-like (LOC105197486), transcript variant X2, misc_RNA	0.130718	1.98553
gi 751236292	XP_011171975.1	XM_011173673	LOC105204554	secretin receptor-like isoform X4	0.991241	1.06065
gi 751236290	XP_011171974.1	XM_011173672	LOC105204554	secretin receptor-like isoform X3	1.58782	0.348021
gi 751204965	XP_011176051.1	XM_011177749	LOC105208023	green-sensitive opsin-like	0.330279	1.35461
gi 751212868	XP_011159229.1	XM_011160928	LOC105195500	prolactin-releasing peptide receptor-like	1.67119	0.00067
gi 751203152	XP_011175066.1	XM_011176764	LOC105207336	cardioacceleratory peptide receptor-like	0.592684	0.971376
gi 751236282	XP_011171969.1	XM_011173667	LOC105204554	secretin receptor-like isoform X1	0.597832	0.886539
gi 751212872	XP_011159231.1	XM_011160927	LOC105195500	prolactin-releasing peptide receptor-like	0.000375	1.4159
gi 751214494	XP_011160104.1	XM_011161802	LOC105196073	LOW QUALITY PROTEIN: probable G-protein coupled receptor B0563.6	0.529527	0.875558
gi 751209558	XP_011157431.1	XM_011159129	LOC105194275	gonadotropin-releasing hormone receptor-like	0.330771	0.972118
gi 751224902	XP_011165816.1	XM_011167514	LOC105200117	thyrotropin-releasing hormone receptor-like	0.349814	0.849813

gi 751236194	XP_011171921.1	XM_011173619	LOC105204531	G-protein coupled receptor Mth2-like	0.508774	0.580146
gi 751228090	XP_011167439.1	XM_011169137	LOC105201199	adenosine receptor A2b-like	0.391874	0.666697
gi 751236294	XP_011171976.1	XM_011173674	LOC105204554	secretin receptor-like isoform X5	0.545303	0.399329
gi 751228088	XP_011167438.1	XM_011169136	LOC105201199	adenosine receptor A2b-like	0.381256	0.409619
gi 751232585	XP_011169919.1	XM_011171617	LOC105202925	5-hydroxytryptamine receptor 1-like	0.222941	0.555203
gi 751218295	XP_011162176.1	XM_011163874	LOC105197486	gastrin/cholecystokinin type B receptor-like isoform X1	0.34228	0.164363
gi 751236280	XP_011171968.1	XM_011173666	LOC105204554	secretin receptor-like isoform X1	0	0.484745
gi 751234850	XP_011171180.1	XM_011172878	LOC105203942	gonadotropin-releasing hormone receptor-like, partial	0.456537	0
gi 751225429	XP_011166100.1	XM_011167798	LOC105200319	probable G-protein coupled receptor Mth-like 2	0.21404	0.034813
gi 751205397	XP_011176280.1	XM_011177979	LOC105208194	uncharacterized protein LOC105208194	0.171619	0
gi 751225427	XP_011166099.1	XM_011167797	LOC105200318	uncharacterized protein LOC105200318	0.036088	0.068215
gi 751225752	XP_011166273.1	XM_011167971	LOC105200419	octopamine receptor beta-2R isoform X1	0.002057	0.098628
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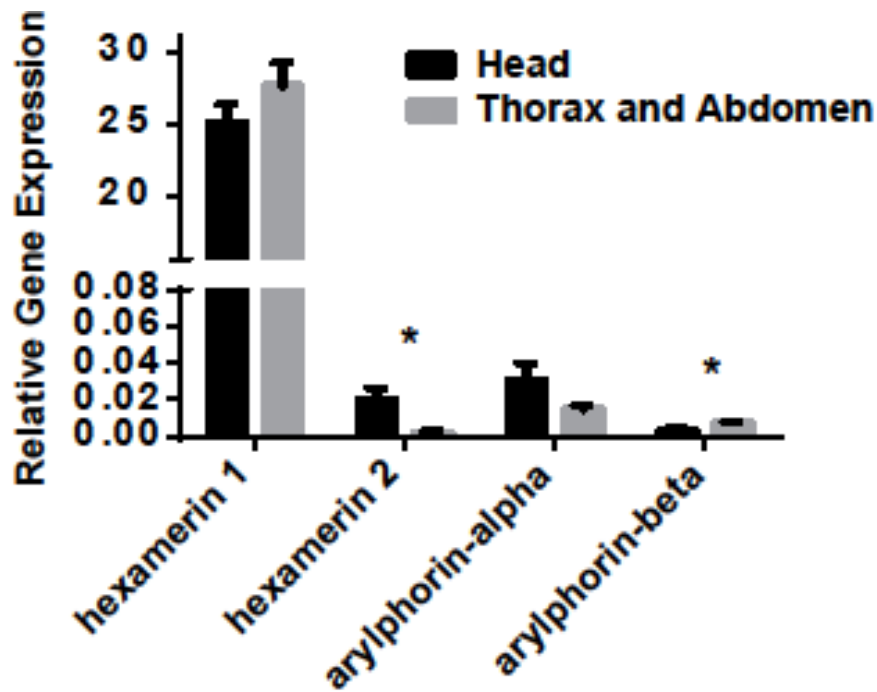
gi 751213593	XP_011159613.1	XM_011161311	LOC105195745	vasopressin V1a receptor-like isoform X2	0.075512	0
gi 751213591	XP_011159612.1	XM_011161310	LOC105195745	vasopressin V1a receptor-like isoform X1	0	0.033531
gi 751203577	XP_011175311.1	XM_011177009	LOC105207504	uncharacterized protein LOC105207504 isoform X4	0	0.027589
gi 751205347	XP_011176253.1	XM_011177951	LOC105208166	protein trapped in endoderm-1	0.002619	0.000177
gi 751207847	XP_011156507.1	XM_011158205	LOC105193663	histamine H2 receptor isoform X1	0	0.002322
gi 751199633	XP_011157692.1	XM_011159390	LOC105194469	tyramine receptor 1-like	0.0005	0.001214
gi 751230113	XP_011168551.1	XM_011170249	LOC105201947	adenosine receptor A2b-like isoform X2	0.000606	0.000314
gi 751214241	XP_011159966.1	XM_011161664	LOC105195979	FMRFamide receptor isoform X1	0.000179	0
gi 751217574	XP_011161787.1	XM_011163485	LOC105197213	neuropeptides capa receptor-like isoform X1	3.14E-05	8.00E-05
gi 751236196	XP_011171922.1	XM_011173620	LOC105204531	G-protein coupled receptor Mth2-like	7.72E-05	2.01E-05
gi 751209556	XP_011157430.1	XM_011159128	LOC105194275	gonadotropin-releasing hormone receptor-like	6.94E-06	0
gi 751203575	XP_011175310.1	XM_011177008	LOC105207504	G-protein coupled receptor Mth2-like isoform X3	0	4.18E-06
gi 751203573	XP_011175309.1	XM_011177007	LOC105207504	uncharacterized protein LOC105207504 isoform X2	0	2.33E-06

gi 751203571	XP_011175308.1	XM_011177006	LOC105207504	G-protein coupled receptor Mth2-like isoform X1	0	5.29E-07
gi 751203569	XP_011175307.1	XM_011177005	LOC105207504	G-protein coupled receptor Mth2-like isoform X1	0	4.22E-07
gi 751209550	XP_011157427.1	XM_011159125	LOC105194275	gonadotropin-releasing hormone receptor-like	0	0
gi 751209552	XP_011157428.1	XM_011159126	LOC105194275	gonadotropin-releasing hormone receptor-like	0	0
gi 751209554	XP_011157429.1	XM_011159127	LOC105194275	gonadotropin-releasing hormone receptor-like	0	0
gi 751217578	XP_011161789.1	XM_011163487	LOC105197213	neuropeptides capa receptor-like isoform X3	0	0
gi 751202671	XP_011173053.1	XM_011174751	LOC105205389	gonadotropin-releasing hormone II receptor isoform X1	0	0
gi 751214245	XP_011159968.1	XM_011161666	LOC105195979	FMRFamide receptor isoform X1	0	0
gi 751236274	XP_011171965.1	XM_011173663	LOC105204554	secretin receptor-like isoform X1	0	0
gi 751236276	XP_011171966.1	XM_011173664	LOC105204554	secretin receptor-like isoform X1	0	0
gi 751236278	XP_011171967.1	XM_011173665	LOC105204554	secretin receptor-like isoform X1	0	0

gi 751236284	XP_011171971.1	XM_011173669	LOC105204554	secretin receptor-like isoform X1	0	0
gi 751206087	XP_011155552.1	XM_011157250	LOC105192960	gonadotropin-releasing hormone receptor-like	0	0
gi 751239722	XP_011173862.1	XM_011175560	LOC105206029	glucagon receptor-like, partial	0	0
gi 751227496	XP_011167228.1	XM_011168926	LOC105201068	uncharacterized protein LOC105201068, partial	0	0
gi 751219652	XP_011162921.1	XM_011164619	LOC105197996	uncharacterized protein LOC105197996	0	0
gi 751234416	XP_011170936.1	XM_011172634	LOC105203747	somatostatin receptor type 2-like		

Supplementary methods. Analyses of hexamerins expression in the head and thorax/abdomen of queens

For comparisons of gene expression of four different hexamerins between head and thorax plus abdomen (Fig. S2), alate virgin queens were decapitated with fine forceps. Ten queens were used per independent replicate immediately placing either heads or thorax/abdomens into 100 μ L Trizol on ice. Samples were stored at -80°C overnight and thawed on ice. Tissues were homogenized in microcentrifuge tubes using sterile plastic pestles (Thermo Fisher Scientific), after which RNA was extracted and cDNA was synthesized as described.



Supplemental Figure 1: Expression of hexamerins in virgin alate queen heads and thorax/ abdomens. Gene expression determined by qPCR was normalized to both Elf1-Beta and RPL18. Bars indicate mean relative gene expression (ΔCt) \pm SEM, $n = 4$. “*” indicate a significant difference ($p \leq 0.05$) for the examined corresponding hexamerins between heads and thorax/abdomen, as determined by t -tests. Hexamerin 1, $p = 0.2590$; Hexamerin 2, $p = 0.0226$; Arylphorin-alpha, $p = 0.1707$; Arylphorin-beta, $p = 0.0254$.