

Supplementary Materials

# Short Neuropeptide F and Its Receptor Regulate Feeding Behavior in Pea Aphid (*Acyrtosiphon Pisum*)

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**Table S1.** The specific primers used in qRT-PCR and dsRNA synthesis.

Usage	Primer Name	Primer Sequences
qRT-PCR	sNPF-F	GCAGCCGTTGTGTGTACTCT
qRT-PCR	sNPF-R	CGGAGATCTCTGGTTTTTGC
qRT-PCR	sNPFR-F	GTGGCTGAACGAGAACTTCC
qRT-PCR	sNPFR-R	AAGCCGGTGCAGTCTGTAGT
qRT-PCR	RPL7-F	GCGCGCCGAGGCTTAT
qRT-PCR	RPL7-R	CCGGATTTCTTTGCATTTCCTG
dsRNA synthesis	sNPF-F	<b>TAATACGACTCACTATAGGGT</b> TGGACTACGAGAATGCCAAAG
dsRNA synthesis	sNPF-R	<b>TAATACGACTCACTATAGGGC</b> TTAACCTCAGCGACGGA
dsRNA synthesis	sNPFR-F	<b>TAATACGACTCACTATAGGG</b> AAACGCTGTTGCCCACG
dsRNA synthesis	sNPFR-R	<b>TAATACGACTCACTATAGGG</b> CACTTCGACTGCATCCTGATT
dsRNA synthesis	GFP-F	<b>TAATACGACTCACTATAGGG</b> TGACCACCCTGACCTAC
dsRNA synthesis	GFP-R	<b>TAATACGACTCACTATAGGG</b> TTGATGCCGTTCTTCTGC

The bold capital letter shows the T7 RNA polymerase promoter sequence.

**Table S2.** Accession numbers of the sequences used for the gene structure analysis.

Species name	mRNA	Protein	Genome
<i>Acyrtosiphon pisum</i>	XM_003247202.3	XP_003247250.1	NC_042494.1
<i>Myzus persicae</i>	XM_022314068.1	XP_022169760.1	NW_019102905.1
<i>Nilaparvata lugens</i>	XM_022328849.1	XP_022184541.1	NW_019105702
<i>Drosophila melanogaster</i>	NM_165316.2	NP_724239.1	NT_033779.5
<i>Aedes albopictus</i>	XM_029872461.1	XP_029728321.1	NW_021838233.1
<i>Bombyx mori</i>	NM_001134257.1	NP_001127729.1	NW_004581726.1
<i>Spodoptera frugiperda</i>	XM_035582761.1	XP_035438654.1	NC_049720.1
<i>Tribolium castaneum</i>	XM_008200483.2	XP_008198705.1	NC_007425.3
<i>Aethina tumida</i>	XM_020019114.1	XP_019874673.1	NW_017853357.1
<i>Apis mellifera</i>	XM_003250107.4	XP_003250155.1	NC_037641.1
<i>Camponotus floridanus</i>	XM_011260741.2	XP_011259043.1	NW_020229547.1

**Table S3.** Accession numbers of the sequences used for phylogenetic relationship analysis.

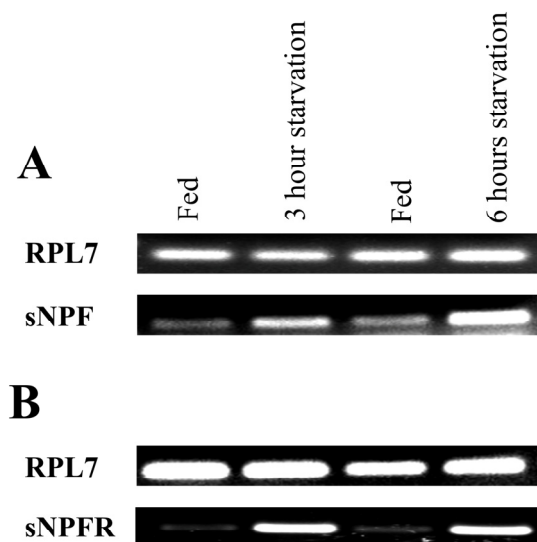
<b>Species name-sNPF</b>	<b>Accession number</b>
<i>Acyrtosiphon pisum</i>	XP_003247250.1
<i>Apis mellifera</i>	XP_003250155.1
<i>Bombus bifarius</i>	XP_033296993.1
<i>Osmia lignaria</i>	XM_034317951.1
<i>Camponotus floridanus</i>	XP_011259043.1
<i>Aphis craccivora</i>	KAF0767496.1
<i>Drosophila melanogaster</i>	NP_724239.1
<i>Bactrocera dorsalis</i>	KY652383
<i>Zeugodacus cucurbitae</i>	XM_011192244.2
<i>Eumeta japonica</i>	GBP76345.1
<i>Bombyx mori</i>	NP_001127729.1
<i>Manduca sexta</i>	XM_030172318.1
<i>Spodoptera littoralis</i>	JQ646093.1
<i>Helicoverpa armigera</i>	XM_021333218.1
<b>Species name-sNPFR</b>	<b>Accession number</b>
<i>Acyrtosiphon pisum</i>	XM_029489088.1
<i>Bombus terrestris</i>	XM_020864331.1
<i>Apis mellifera</i>	XM_006561685.3
<i>Harpegnathos saltator</i>	EFN78163.1
<i>Solenopsis invicta</i>	AAV88918
<i>Bombyx mori</i>	NM_001134235.1
<i>Tribolium castaneum</i>	XP_966794
<i>Nilaparvata lugens</i>	XM_039439868.1
<i>Drosophila melanogaster</i>	NP_524176
<i>Bactrocera dorsalis</i>	KY652384.1
<i>Mus musculus</i>	NP_963909.2
<i>Oreochromis niloticus</i>	XM_025909417.1
<i>Oncorhynchus kisutch</i>	XM_020463106.2
<i>Danio rerio</i>	AAZ04130.1
<i>Loa loa</i>	XM_020451317.1

**Table S4.** Table represents the Ct values of qRT-PCR obtained in fed and starvation experiment. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of starvation with sNPF (A) and sNPFR (B) compared with the expression of ribosomal protein L7 (RPL7).

<b>Sample</b>	<b>Gene</b>	<b>Ct value</b>			
		<b>Fed</b>	<b>3 h starvation</b>	<b>Fed</b>	<b>6 h starvation</b>
Adult	sNPF	28.18	27.75	28.56	26.47
	RPL 7	23.18	24.30	23.78	24.13
CNS	sNPF	27.95	26.94	28.47	25.31
	RPL 7	23.26	23.86	24.26	23.69
Adult	sNPFR	28.27	27.70	28.61	26.85
	RPL 7	23.92	24.61	25.44	25.77
CNS	sNPFR	28.29	27.61	28.41	26.29
	RPL 7	23.55	24.42	23.65	23.90

**Table S5.** Ct values of qRT-PCR obtained from spatio-temporal expression (A) and gene silencing experiment (B).

Ct value-A						
Gene	Antennae	Brain	integument	Embryos	Midgut	
sNPF	31.08	23.06	30.17	26.44	32.62	
RPL7	19.34	18.51	20.87	18.46	18.35	
	1st Instar	2nd Instar	3rd Instar	4th Instar	Adult	
sNPF	22.64	25.67	25.29	26.34	24.57	
RPL7	18.60	20.29	19.34	19.85	18.25	
	Antennae	Brain	integument	Embryos	Midgut	
sNPFR	26.29	23.25	26.14	28.06	26.03	
RPL7	18.77	18.11	17.29	17.50	18.46	
	1st Instar	2nd Instar	3rd Instar	4th Instar	Adult	
sNPFR	23.05	26.24	26.07	25.81	25.27	
RPL7	18.39	20.50	18.84	19.85	19.10	
Ct value-B						
Time	6 hours	12 hours	24 hours	36 hours	48 hours	72 hours
sNPF	26.88	27.92	27.18	24.83	20.42	20.93
RPL7	21.01	21.33	20.84	18.45	14.56	15.54
Time	6 hours	12 hours	24 hours	36 hours	48 hours	72 hours
GFP	19.70	19.83	20.57	20.48	21.74	21.92
RPL7	13.87	14.66	15.27	14.84	16.37	16.77
Time	6 hours	12 hours	24 hours	36 hours	48 hours	72 hours
sNPFR	23.34	27.29	27.45	27.46	27.32	27.47
RPL7	10.42	15.02	15.91	15.57	17.12	18.24
Time	6 hours	12 hours	24 hours	36 hours	48 hours	72 hours
GFP	27.60	27.31	27.29	27.34	27.27	26.83
RPL7	14.97	15.61	16.12	15.67	17.13	17.42



**Figure S1.** Reverse transcription-polymerase chain reaction (RT-PCR) analysis of starvation with sNPF (A) and sNPFR (B) compared with the expression of ribosomal protein L7 (RPL7).