

Supporting Information

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SI Text

Preparation of (\pm)-[¹³C₁₈]-Erythro-12,13-Epoxy-9Z-Octadecenoic acid

((\pm)-[¹³C₁₈]-Vernolic Acid). Ten milliliters of methanol and 1 ml of acetyl chloride were stirred under dry nitrogen at 0°C for 10 min. One hundred milligrams of (0.34 mmol) [¹³C₁₈]-linoleic acid (Campro Scientific) was added and the solution was stirred for 1 h at room temperature. Subsequently, the mixture was diluted with 5 ml of a 5% NaHCO₃ solution and extracted twice with 50 ml of hexane. The hexane phase was washed with brine, dried with Na₂SO₄, and evaporated under reduced pressure. The crude ester was dissolved in 8 ml of CH₂Cl₂ and 58.6 mg (0.34 mmol) of *m*-chloroperbenzoic acid was added and stirred overnight. Sixty milligrams (1.5 mmol) of CaO was added to the solution and subsequently stirred for 20 min, filtrated, and evaporated.

The mixture of reaction products was subjected to flash chromatography with 30 g of silica gel (Merck; 0.015–0.04 mm) with gradient elution using a mixture of petroleum benzene and ethyl acetate (200 ml of 95:5, 100 ml of 92.5:7.5, and 200 ml of 90:10). The elution sequence and amount of products was: (i) [¹³C₁₈]-methyl linoleate 30 mg, (ii) (\pm)-[¹³C₁₈]-erythro-12,13-epoxy-9Z-octadecenoic acid methyl ester ((\pm)-[¹³C₁₈]-vernolic

acid methyl ester) 7 mg, (iii) (\pm)-[¹³C₁₈]-erythro-9,10-epoxy-12Z-octadecenoic acid methyl ester ((\pm)-[¹³C₁₈]-coronarinic acid methyl ester) 7 mg, 4) 9,10,12,13-diepoxystearic acid methyl ester 35 mg. 7 mg (23 μ mol) of (\pm)-[¹³C₁₈]-vernolic acid methyl ester was dissolved in 100 μ l of acetonitrile, 0.55 mg (46 μ mol) of LiOH was added and stirred for 48 h at room temperature. The solution was neutralized with 1 N HCl, stirred for 1 h, diluted with 10 ml, water, acidified to pH 3 with 1 N HCl, and finally extracted with diethyl ether (3 \times 30 ml). The combined ether phase was washed with brine, dried over Na₂SO₄, and evaporated. The crude acid was applied to a LC column containing 5 g of silica gel (Merck, 0.063–0.2 mm) and eluted with 200 ml of petroleum benzene:ethyl acetate:acetic acid 94:5:1 to yield 5 mg of pure (\pm)-[¹³C₁₈]-erythro-12,13-epoxy-9Z-octadecenoic acid. GC-MS-analysis of the product was done with the methyl ester by derivatization with trimethylsulfonium hydroxide (Macherey & Nagel). The ¹³C-labeled compound coeluted with an unlabeled reference chemical (vernolic acid methylester, purity 99%, Sigma-Aldrich). The mass spectrum of the labeled product is shown in Fig. S4.

(Nas.EH1.F4)

1 *ATGTGGAAGGGCGTGC**GTTTCC**ACTAGCCACGCTGGC**CATCGG*
 1 M W K G A A V F L L A T L A I G W H L R
 61 TATCAAGGACCCGTGGAGGTACCTGACTTGCCGAATCAATACTGGGGTCCTGGAAAGCCT
 21 Y Q G P V E V P D L P N Q Y W G P G K P
 121 GTGCCAGATCCCAGGATATCAAACCGTTCAAAATGACGTTCCAAAAGAGGTAATCGAT
 41 V P D P K D I K P F K I D V P K E V I D
 181 GATCTAAACAAACGCCCTGATAGTACGAGATCATTGTGGAACCGCTGGAAGGTTCTGCC
 61 D L N K R L D S T R S F V E P L E G S A
 241 TGGACTTACGGTATCTCGTGACCTACTTGAAGACCCTGGAGGAAGAAA
 81 W T Y G I S S T Y L K T V L N H W R K K

(Nas.EH1.F3)

301 TATA*ACTGGAGCCAACCGCAGGCT**CTGCTCAACAAATACCGCAGTT**CAAGACCAAGATT*
 101 Y N W S Q R Q A L L N K Y P Q F K T K I
 361 CAAGGTCTGGACATCCACTTCTACCATGTAAAGCCCCAGGTACCTAAAGACCGCAAGGTC
 121 Q G L D I H F Y H V K P Q V P K D R K V
 421 CGTGTCTGCCACTTTGATGCTCCACGGCTGGCCAGGATCCATCGTCGAGTTCCAGAAG
 141 R V L P L L M L H G W P G S I V E F Q K
 481 ATTATCCCCATGCTCACGACAGCCAAGCCGATGAAAACCTCGTCTCGAGCTGATCATC
 161 I I P M L T T A K P D E N F V F E L I I
 541 CCATCTCTGCCGGATATGGATTCTCCAAGCTGCCCTAGACCCGGCTTGGACCTGCT
 181 P S L P G Y G F S Q A A A R P G L G P A

(Nas.EH1.dF1 and F1)

601 CAGATGGCTGTTGCTTCAAAA*ACCTGATGCAAAGACTCGGTTTCGAGCAGTTCTACACT*
 201 Q M A V V F K N L M Q R L G F E Q F Y T
 661 CAGGGTGGTGACTGGGGCAGTCTCATCACTGCCAACATGGCTGTGCTATCCAAAAAG
 221 Q G G D W G S L I T A N M A V L Y P K K

(Nas.EH1.F2)

721 *GTCATAGGAACTCACCTGA**ATATGTGCTTCATCGAATCGCATAAGGCTC**ACTTCTTATCG*
 241 V I G T H L N M C F I E S H K A H F L S
 781 CTCGTGGCGCGTACATCCCACATCTGGTCGACAGCGAGCATTACTCCAAGATGTAT
 261 L V G A Y I P S L V V D S E H Y S K M Y

(Nas.EH1.R2)

841 CCGTTGCCTACCACCTCGGCCGTTGATCGAAGAGACC*GGTTACTTGCACATCCAGGCT*
 281 P L S Y H F G R L I E E T G Y L H I Q A
 901 ACGAAACCTGAAACAGTTGGCGCCGCACTTACGGATTGCGCCGGCTGGTCTGCCCGTAC
 301 T K P E T V G A A L T D S P A G L A A Y

(Nas.EH1.dR1 and R1)

961 *ATCCTTGAGAAGTTCAGCACCTGGACGAATCCAGACTATAGGTTCCCGACGATGGTGA*
 321 I L E K F S T W T N P D Y R F R D D G G
 1021 CTTCTGGAGAAGTTCACCATGGACGAGCTTTGGACAACCTCATGGTCACTGGGTGACC
 341 L L E K F T M D E L L D N L M V Y W V T
 1081 AACTCCATCACCAACCAGTCAGCGACTCACGCCGAATGCTTCAGCAAGGCCAACAGGGAG
 361 N S I T T S Q R L Y A E C F S K A N R E

(Nas.EH1.R3)

1141 CTCGGAGTTGACAAA*ATGCCGATCTCGTGC**CAACCGCCTGTG**CCAACCTCCCGCACGAG*
 381 L G V D K M P I F V P T A C A N F P H E
 1201 TTGGCCTACAGGTGGAAACC*CATCCTCAAGGAACGTT**CACGAACCTGGTCCAGTTCA*
 401 L A Y R S E T I L K E R F T N L V Q F T
 1261 CATCCTCCACGAGGTGGTCACTCGCCGTTTGAGGAGCCGA*ACTTCTGGCCAACGAC*
 421 H P P R G G H F A A F E E P E L L A N D
 1321 GTCTGGAGCTTGTCACAAGCTCGAGCGACTCAGTGATGAGAAACGACAGAAGCAG
 441 V W S F V H K L E Q R L S D E K R Q K Q

(Nas.EH1.R4)

1381 *GAGGCCAAGAAAGAAAAGCGAAGAAGGCGTGA*
 461 E A K K E K A K K A *

Fig. S1. Nucleotide and deduced amino acid sequences of the putative *Nasonia vitripennis* epoxide hydrolase gene *Nasvi-EH1*. The sequences are numbered at the left. Stop codon indicated by asterisk. The *Nasvi-EH1* N terminus (EHN) is coded in italics, the abhydrolase_1 region is encoded in bold. Nucleotide sequences (5'-3') of primers that were used in the gene amplification are underlined and written in italics. The primer names are given above the respective sequences.

Athro-JHEH	MTILKYSLIGVGLIVGISYLNFNQGGNQAKPKLGDQWWGPGKE-QKIVKDVPFKVNFSKG	59
Nasvi-EH1*	MGVILLWGFLGIVLIGGATIFISPP-VQQPPKLTVDVYWGPGEPEKKTDTSRPFKITFQKE	59
Apime-EH	- - - - - YLF SVN - ELNVPTLPETNWGSKKN-EKESIEIRPFKIDVSKS	47
	* * *** * *** *	*
Athro-JHEH	DIEDLKTRLKNTRNLTPALENAGWTYGVDGKFVPKIVDYLWNKYDFKKREQYLNQYDQFV	119
Nasvi-EH1*	MLDDLRNRLKNTRKMQPPLENVGWTYGLSGDFVVPVIVDHWLWNKYDFKKREAHLNQYPQFI	119
Apime-EH	VLDLLKYRLAHRRTFKKPLENVGWTYGISSTYLNTVLDYWRDKYNWTERQALLNKYPQFM	107
	** ** * *** **** * * * *** * *** *	*** * *** *
Athro-JHEH	TNIQGLNIHFLHVR - - - PKNSGGKRVLPPLLQHQHGWPGSVEFYKII PMLTTPRDDYDFVF	176
Nasvi-EH1*	TNVQGLDIHFIFHVKPQLPKNSK-IQVLPLYLQHQHGWPGSIVEFQKII PMLTTPRSDRNFVF	178
Apime-EH	TTIQGLDIHFYHVKPNLNNK-LKVLPLMLHGWPGSVVEFQKII PMLTKPWPQNQFVF	166
	* *** *** * * * * *** * *** * *** * *** * *** * *** * ***	***
Athro-JHEH	EVIAPSLPGFGFPSGAVRPGLGAAQIAVVLKNLMLRLGFNKFYTQGGDWGAIITAHMAVL	236
Nasvi-EH1*	EVVAPSLPGFGYSQAASKAGLGAVQMAHLMKNLMLRLGFDFKFYTQGGDWGAVIGANMASM	238
Apime-EH	EVIVPSLPGYGFSEGAVRPGMANAQIAVIFKNLMQRLGFEKFYVQGGDWGSVIASDMAVL	226
	*** ***** * * * * * * * * *** * *** * *** * *** * *** * ***	**
Athro-JHEH	FPEHVLGIHSNMCAVLQPQTFTTYLYSYWPSLLVPDEDYHLMYPLSKKWSRTIEETGYF	296
Nasvi-EH1*	YPQHVLGMHSNMCLVVRPWTWLKIAAYSIMPSLLPEDE-RQLMFPLSTKLALGIEETGYL	297
Apime-EH	FPEKIIGLHNMMCTSILNSNLFWLFVGTYFPSLIGANEHYSKFFFVSEILSFLIEESGYF	286
	* * * *** * *** * * * * *** * *** * *** * *** * *** * ***	*** **
Athro-JHEH	HIQATKPDTLGAALADSPAGLAWISEILEKFSTGTNPELRFKEDGGLFDIHSPDELLNVML	356
Nasvi-EH1*	HLQATKPDТИGIGVSDSPAGLAAYILEKMACTKPDNKFTDDGNLLEKFTMDELIDNLMM	357
Apime-EH	HIQATKPDTİGAALTASPDALAAYILEKFVSWTNKTYKKQDDGITEKFVLDELLDNIMI	346
	* ***** * * * * *** * * * * *** * *** * *** * *** * *** * ***	*** * ***
Athro-JHEH	YWMPNSMTTAIRIYAETFSAANRALRMDYVPIEVPSACAQFPHEISYQPPSLLSARYKKL	416
Nasvi-EH1*	YWAPNKASSSFRIYAESFNKQTFGYKMDNVTVPSACAFPYEIIFQSANFLDRDFVNL	417
Apime-EH	YWITNSITTSVRLYAENYTSSYRSLKIDQLPIKVFTACAVFPNEILVLPESLLKQKYPNI	406
	*** * * * *** * * * * *** * *** * *** * *** * *** * ***	*
Athro-JHEH	IRARKMPKGHFAAFEQOPOLLADEVWTSIGIMEADKKORE-DKLKKSA-	463
Nasvi-EH1*	LRVTKMPRGGHFAVLEEPQLLADDIWASVQAFRDYYAAQEQRLEKQKS	466
Apime-EH	IQYNIISRGHHFAAFEEPRLLADDIFSFKVKKIENLTSKSS-----	446
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Fig. S2. Amino acid sequence alignment of *Nasvi-EH1* from *Nasonia vitripennis* with epoxide hydrolase genes from *Apis mellifera* (*Apime-EH*) and *Athalia rosae* (*Athro-JHEH*), using CLUSTAL W analysis. Name of gene sequences are given to the left and amino acids are numbered to the right. Identical amino acid positions are indicated by asterisks.

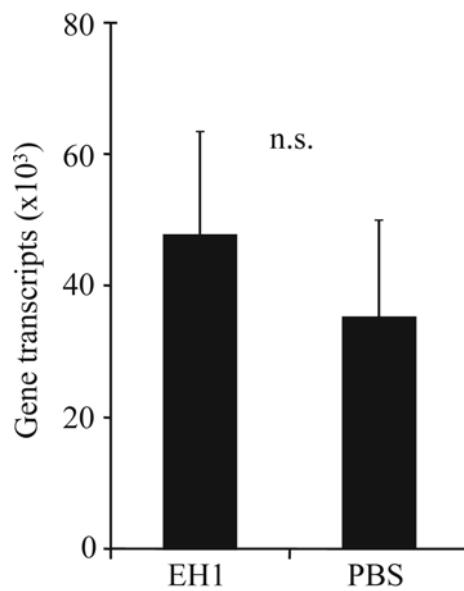


Fig. S3. Effect of the injection of dsRNA corresponding to *Nasvi-EH1* (EH1) on the quantity of gene transcripts of the nontarget gene *Nasvi-EH2* in male rectal vesicles as estimated by quantitative real time RT-PCR analysis. Control males were injected with 1× DEPC-PBS buffer. No significant differences were found (*t* test for independent samples).

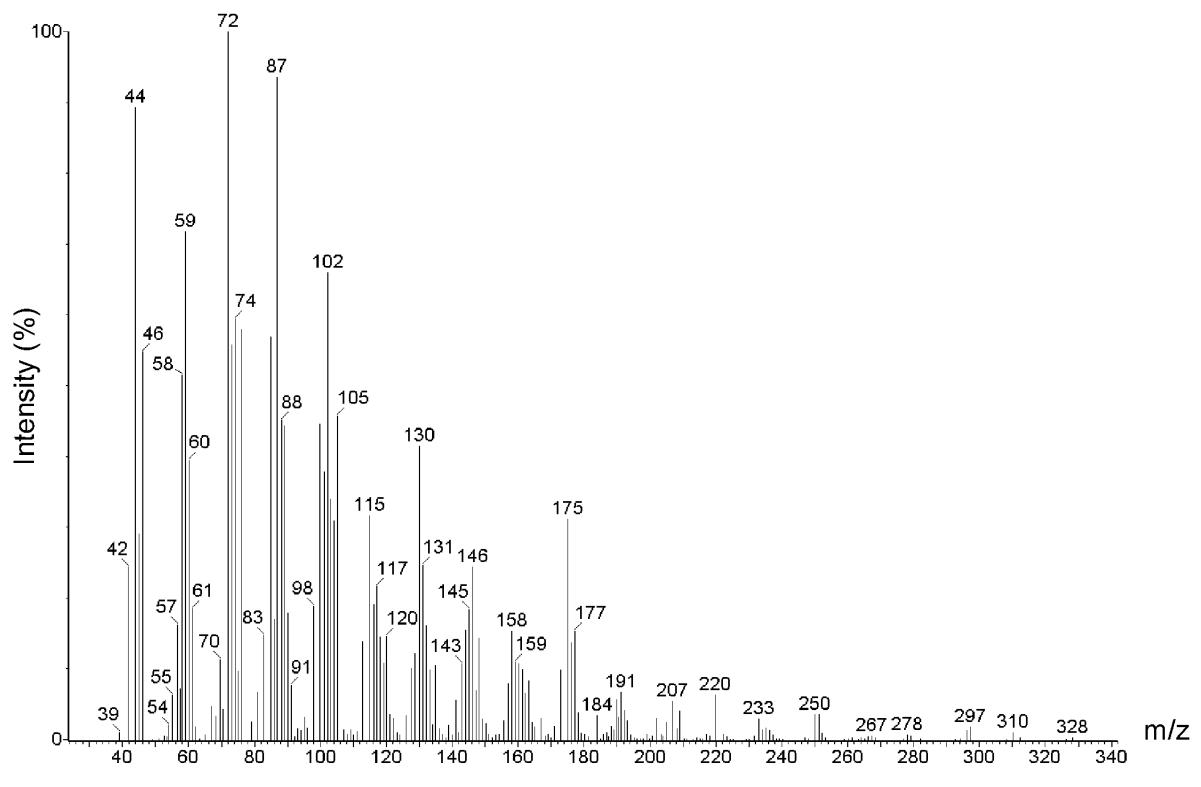


Fig. S4. Mass spectrum (EI, 70 eV) of synthetic (\pm)-[$^{13}\text{C}_{18}$]-vernolic acid methylester.

Trini-JHEH	-----MARLLFI-LPVLAIVFLPVYFLFLQSPPVPNVDMNDWWG-----PESAKEKQDTS 50
Trini-mEH	-----MGRLLFL-VPVLAIVLLPVYYFLQGPPPLPDLDYNEWWG-----PESGKQKQDTS 50
Bommo-JHEH	-----MSRLLFIALPLLVLASIPLYLLVLKSPPPMPKLDLEEWWG-----PPELKQKQDTS 51
Spoex-JHEH	-----MG-FIVKAVLVAALGVAAWYYYFIGCCPKTIPKLDNNNEWWG-----PKELVGKQDNA 50
Manse-JHEH	-----MYKILSSFVAGVAIGSGLVITYVLYNVPEPPELDLQRWWG-----IGTRPTEEDKS 51
Nasvi-EH2	-----MWKGAAVFLLATLAIGWHLRYQGPVEVPDLPNQYWG-----PGKP-VPDPKD 46
Apime-EH	-----MYYFHFIYLFS-----VNELNVPTLPETNWG-----SKKN-EKESIE 36
Athro-JHEH	-----MTILKYSILIGVGLIVG-----ISYLFNQGGNQKAPKLGDQWWG-----PGKE-QKIVKD 48
Nasvi-EH1*	-----MGVLLWGFLGIVLIGG--ATIFISPP-VQQPPKLTVDVYWG-----PGDEPKKTDT 48
Trica-EH	-----MGCGCVIVTAIAVIFILKFVHKIKRFFKVPTVPLYETTWG-----PRDK-TEEDDS 51
Trica-mEH	-----MNALIKFIVLLIALAIGNVIYKIN---TKESVKVPPETWWG-----PGDP-SKEDTR 48
Ctefe-JHEH2	-----MSNCRILWIAIVIGLGVLYYEITKEFPKPNIPLDTTWG-----TGKS-QKIDTS 49
Ctefe-JHEH1	-----MGKCCRMLIIFAAIAGIAVLYYQITKELPKPNIPLDTTWG-----PGKP-QNVDIS 49
Drome-mEH	-----MANIWPRILVGALTILVAVGYKNYRDLsapgkRPDLDNNAYWG-----PTLKEPYRENK 54
Drome-JHEH2	-----MANIWPRILVGALTILVAVGYKNYRDLsapgkRPDLDNNAYWG-----PTLKEPYRENK 54
Drome-JHEH1	-----MGVTVKILVLILAIAGGLV---YRNVTQLWADLPAPKLDQEWGDEAQPKDYEAYLKNN 57
Drome-JHEH3	-----MKCLIVFGLIVALFGAFVGYGYVVFTELTKPLPKPEFKDDTYWG-----PGDAKDFVPDE 55
Aedae-EH1	-----MGFCARVLVFVGATLLLAVSYKQYKDATGPLPVPSLDPNEYWG-----PGDVRQYKEDV 53
Anoga-EH	-----MGFMLRVLVFVGISLATAVVFKQYRNLTAPMPVPDFLNMKQYWG-----PGDVVKQYREDT 53
Aedae-EH2	-----MGFAGRFLVFTLLVGVLFKVQFDLSAPAAIPAIDYQEYWG-----PGDVKNKENV 53
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Trini-JHEH	-----IRPFKISFGNNNVKDLKDRLRPLTPPLEGVGFYDFYGFNTNEIDSWLKYWAKDYN-- 106
Trini-mEH	-----VRPFKINFGENLVKDLKDRLRPLTPPLEGVGFYDFYGFNTNEINSWLKYWAEGYN-- 106
Bommo-JHEH	-----IKPFEITFSETMVKELKERIKKKRPFAPPLEGVGFKYGFNSKQLDSWLKYWAEEYP-- 107
Spoex-JHEH	-----IRPKVKFDEAMIKDLKLRLKNHRAFRPPLLEGVGFEYGFNTAQIDSWINYWADKYN-- 106
Manse-JHEH	-----IRPFSIDFNDTVIDDLKERLKNRRPFTKPLEGINSEYGMNTELETVLEYWLNEYN-- 107
Nasvi-EH2	-----IKPKFIDVPKEVIDDLNKRLDSTRSFVEPLEGSWATYGISSTYLTKLVLNHWRKKYN-- 102
Apime-EH	-----IRPKFIDVSKVSLDDLKYLRLAHRTFKKPLENVGWTYGISSTYLNTVLDYWRDKYN-- 92
Athro-JHEH	-----VVPFKVNFSKGDIEDLKTRLKNTRNLTPALENAGWTYGVDGKFVPKIVDYWLKYD-- 104
Nasvi-EH1*	-----VRPFKITFQKEMDDLNRNLKRNRMQPPLENVGWTYGLSGDFVPVIVDHWLKYD-- 104
Trica-EH	-----IQPFTVKVPDEVITDLQQRLQNARPLTPPLEGVQHQYGINNTNLKIVDFWRNEYN-- 107
Trica-mEH	-----IVPKIQLVPNQILEDLQRQLKNARKFAPPLEGVHGHYGINNTNLKEIVNYWLTKYD-- 104
Ctefe-JHEH2	-----MRPFKIAINDEVNLTLKVKLSDVS-FTPPLLEGIDFYGFNTNTLKKLVDFWRTQYN-- 104
Ctefe-JHEH1	-----IRPKKININNKVIENLKLKLNDVQ-YTLPLEGEGINSEYGFNTDSLKKIVDFWRTQYN-- 104
Drome-mEH	-----AILPFDISVKPVEVIEDLIGQLSRPLKAQAPLEGVGQYGFNANEELAKVVKYWRDTYLPK 113
Drome-JHEH2	-----AILPFDISVKPVEVIEDLIGQLSRPLKAQAPLEGVGQYGFNANEELAKVVKYWRDTYLPK 113
Drome-JHEH1	-----SEVIGNRLSYDPKTIADLKERLNRTLRLTPPLEGVAFEYGFNTNLYKEVVEYWRDDYLPK 117
Drome-JHEH3	-----KIYEFKLQVPQSEIDDLRKELNRTLRLTEPLDGIACEYGFNTYALEQFVVDYWRDNLYLT 114
Aedae-EH1	-----SIKPKVAYSPEVIEKLRSKLNDVPTLVKPLEGAAFEYGFNSNRLQDILKYWKTSYLNK 112

Fig. S5. CLUSTAL W amino acid sequences alignment of the epoxide hydrolase genes of *N. vitripennis* (Nasvi-EH1*), (Nasvi-EH2 = GenBank accession no. XP_001602953), *Apis mellifera* (Apime-EH), *Athalia rosae* (Athro-JHEH), *Manduca sexta* (Manse-JHEH), *Bombyx mori* (Bommo-JHEH), *Spodoptera exigua* (Spoex-JHEH), *Trichoplusia ni* (Trini-mEH, and EH), *Drosophila melanogaster* (Drome-JHEH1, 2, and 3 and mEH), *Aedes aegypti* (Aedae-JHEH1, and 2), *Ctenocephalides felis* (Canfe-EH1, and 2), and *Tribolium castaneum* (Trica-EH, and mEH). Gene names are given to the left, Identical amino acid positions are indicated by asterisks.

Anoga-EH	-SIKPKVSYDAAVIEKLRGKLTDVPKLTPLDGVAPEYGFNTNRLQDILKYWRTTYLDK	112
Aedae-EH2	-EVKSFELNYGEDVIGKLRNRLLDDVPKFAEPLGTAPEYGFNSKKLGEILKYWRSDYLER	112
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Trini-JHEH	FKERETFLNQFPQFKTNIQGLDIHFIRVTPKVPQG--VEVVPLLLHGWPGBREFYEA	163
Trini-mEH	FKERETFLNQFPQFKTNIQGLDIHFIFKVTPKVPAG--VQVVPMLLHGWPGBREFYES	163
Bommo-JHEH	FAERQKFLNQYPHKFTNIQGLNIHFMRITPKVPKD--VEIVPLLLHGWPGBREFYEA	164
Spoex-JHEH	FSEREAFLNKFPHFKTNIQGLDIHFIRVKPEVPKN--VEVLPLLMIHGPGBREFYEA	163
Manse-JHEH	FKKRAELLNKPFHYKTRIQGLDLHFIRVKPEIKEG--VQVLPLMMHGWPSSKEFDKV	164
Nasvi-EH2	WSQRQALLNKYPQFKTKIQGLDIHFYHVKPQVPKDRK-VRVLPLMLMHGPGBSIVEFQKI	161
Apime-EH	WTERQALLNKYPQFMTTIQGLDIHFYHVKPQVNNKN-LKVLPLMLMHGPGBSIVEFQKI	151
Athro-JHEH	FKKREQYLNQYDQFVNTIQGLNIHFHLVRR--PKNSGGKRVLPPLLQHGWPGBSVEFYKI	161
Nasvi-EH1*	FKKREAHLNQYPQFITNVQGLDIHFIFHVKPQLPKNSK-IQVLPLYLQHGWPGBSIVEFQKI	163
Trica-EH	WKERETFLNKLKPQFTVSQVQGLRLHYIHVKP--EKT-DGLKVLPLMLMHGPGBREFYEI	164
Trica-mEH	WRERENFLNQYPQFKTNIQGLDVHFIHVKP--KNVPSGVKTQPLLLVHGWPGBREFYEI	162
Ctefe-JHEH2	WREREALLNKYPHKFTNIQGLDIHYVHIKPQVSKN--IHVLPMIMVHGWPGBSVEFYKI	161
Ctefe-JHEH1	WREREALLNKYPHKFTNIQGLDIHYVHIKPQVSKN--IEVLPVLMIHGPGBSVEFYKI	161
Drome-mEH	WSEREEYLKKLDHYQTEIQLKLKHAKPSQVKQGPCKVLPPLLMHGWPGBTREFYDF	173
Drome-JHEH2	WSEREEYLKKLDHYQTEIQLKLKHAKPSQVKQGPCKVLPPLLMHGWPGBTREFYDF	173
Drome-JHEH1	WREREVFVLWQFNHFTTDIQLRTHFLHLMVDDNKVG-KKHPVPLLLHGWPGBSVEFYDF	176
Drome-JHEH3	WDERQELFNSFKQYKTEIQLNIHYIHEKVSEEAKEK-KHVYPLLLHGWPGBSREFPDF	173
Aedae-EH1	WTEREAFLNKPFHKTQIQGLNIHFVHKPVPAGTK--VLPPLLLHGWPGBSREFYDV	169
Anoga-EH	WSEREKFLNKFPHFQTQIQGLNIHYIHVKPVPAGTK--VLPPLLLHGWPGBSREFYDI	169
Aedae-EH2	WDERQKYLNRFPQFKTQIQGLDIHFRLRKPEVQNPKR--IVPLLMLHGWPGBSQQKFYEI	169
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Trini-JHEH	IPLLTAV--SKDRDFAFEVIVPSLPGYGFSDPAVRPGLGAPQIGVVMKNLMSRLGYKQFY	221
Trini-mEH	IPLLTAV--SKDRDFALEVIVPSLPGYGFSDGAVRPGMAGHIGIIMRNLMNRGLGYKRYF	221
Bommo-JHEH	IPLHTAV--SRDRNFALEIIAPSLPGYGFSDAAVRPGLAAAEEVAVIFKNLMLARLGKQYY	222
Spoex-JHEH	IPLLTRO--TAGYNFVFELIIPSPICPGYGFSDPAVRPGLGMPQVAVIFRNLMNRGLHKYYY	221
Manse-JHEH	IPILTTP--KHEYNIVFEVVAVDLPGYGFSEGTNPKGLNPVQIGVMMRNLMLRLGFEKFY	222
Nasvi-EH2	IPLMLTTA--KPDENFVFELIIPSLPGYGFQAAARPGLGPAQMAVFKNLMLQRLGFEQFY	219
Apime-EH	IPLMLTP--WPNQNFVFEVIVPSLPGYGFSEGAVRPGMANAQIAVIFKNLMLQRLGFEKFY	209
Athro-JHEH	IPLMLTP--RDDYDFVFEVIAPSLPGFGFPGSAVRPGLGAAQIAVVLKNLMLRLGFKFY	219
Nasvi-EH1*	IPLMLTP--RSDRNFVFEVVAPSLPGFGYSQAASKAGLGAQVMAHLMKNLMLRLGFDKFY	221
Trica-EH	IPLLTTP--QPGRDVFVFEVIAPSLPGYGFSEAVRPGLGAQMAVLFKNFMNRGLGFKYY	222
Trica-mEH	IPLLLTV--QDKKKFVFEVIIPSLPGYGFQAAVRPGLGAHQTAIFKNLMKRLGFDRYY	220
Ctefe-JHEH2	IPLMLTP--RTDYNFVFELILPSIPGYGFSQAAAKPGLGATQIAVIMHNLMRIGFKKKY	219
Ctefe-JHEH1	IPLMLTP--RAGYNFVFELILPSIPGYGFSQAAAKPGLGATQIAVIMHNLMRIGFKKKY	219
Drome-mEH	IPLLLTP--SDKSDYVFEVIAPSLPGYGWSQGSSKTGFGVAQVAVVMRNLMLRVGFDKFL	231
Drome-JHEH2	IPLLLTP--SDKSDYVFEVIAPSLPGYGWSQGSSKTGFGVAQVAVVMRNLMLRVGFDKFL	231

Fig. S5. (Continued)

Drome-JHEH1	IHLHQTNLDNNNKYIFNVVVPSLPGYGWSQGTSRKGLGPAQAVMMRNLMRLGYNKKFF 236
Drome-JHEH3	IPLMLTKH--SNITDYAFEVVAPSLVGWGSDAATRPGFNAEMATVMRNLMRLGHKKFF 231
Aedae-EH1	IPKLLTTK--SDDKDFVFEVVPSPLGYSQGASKGKGLSPSEVAIVMKNLMSRVGFQFY 227
Anoga-EH	IPLLTTT--SKDKDFVFEVIVPSPLGYSQGASKGKGLSPSEVAIVMKNLMSRVGFQFY 227
Aedae-EH2	IPLRVAR--SDDKEYVFEVIVPSPLGYSQGASKGKGLSPAKIAVIMRNLMARLGFKYY 227
* * * * *	
Trini-JHEH	LQGGDWGALIGNCIVTLFPKDILGYHTNMPIVMSAKSTLFELLGSVFPSSLIEDMSTYER 281
Trini-mEH	VQGGDWGSVIGTSLATFFPEEVLYGHANIGLVLSTKAMVWQAIGSVWPSLIMDDLSLVDR 281
Bommo-JHEH	VQGGDWGALIGSAMATSFPKEIIGFHYSYMTLSPAATFLEFGALFPSLIVEP-ELANR 281
Spoex-JHEH	VQGGDWGAGIVSTMSTLFPEDILGHHSNMLFTQHTCATVRTLVAFLPSLIIEE-HLASR 280
Manse-JHEH	IQAGDWGSQCATHMATLFPDQVLGLHTNMPLSSRPLSTVKLFIGALFPSLIVDA-KYMDR 281
Nasvi-EH2	TQGGDWGSLITANMAVLYPKVIGHLNMCFCIESHKAHFLSLVGAYIPSLVVDS-EHYSK 278
Apime-EH	VQGGDWGSVIASDMAVLFPEKIIGLHNMCFTSINLSNLFWLFGVGTYPFSLIGAN-EHYSK 268
Athro-JHEH	TQGGDWGAIITAHHMVLFPEHVLGIHSNCAVLQPQTFTTYLYSYWPSLLVPD-EDYHL 278
Nasvi-EH1*	TQGGDWGAVIGANMASMYPQHVLGMHSNMCLVVRPTWLKIAAYSLMPSSLPED-E-RQL 279
Trica-EH	IQGGDWGAVITQHMATLYPEKILGLHSNMCFTNLKSQVKLFLFSFYPTLIVKQ-EHVNK 281
Trica-mEH	VQGGDWGSAVTSAMALYYPDRVKGHIHLNCVSNSYLAKLKLLAGSVWPSLVVEE-KQKHK 279
Ctefe-JHEH2	VQGGDWGSRIVSAMSTLFPENVLGHHSNLCFLNTLSSNIKFVGSLFPEWFAGK-QNVHK 278
Ctefe-JHEH1	VQGGDWGSMIISAMSTLFPENVLGQHNSNMCFVNTPSSNIKAIIGSFFPESFAGT-GNAHK 278
Drome-mEH	VQGGDWGSIIIGSNVASLFPENVLGYHSNMCGNNSPMGQLKMVLASFFPSWFVDSE-YADF 290
Drome-JHEH2	VQGGDWGSIIIGSNVASLFPENVLGYHSNMCGNNSPMGQLKMVLASFFPSWFVDSE-YADF 290
Drome-JHEH1	IQGGDWGSIIIGSNIATLYENVLGYHSNMCNNLSPKSLAKGLVAEFWPSLFPVPSG-FEDF 295
Drome-JHEH3	IQGGDWGSIIIGSNLATLYENVIGYHSNMCVLHTPLAILKGIVGSFFPEKYLPSRFFVDH 291
Aedae-EH1	VQGGDWGSILIANLISTLYQDNVLGVHMNMCANGLQAILKSIIASFRPSMFIEEK-YVDY 286
Anoga-EH	IQGGDWGSILIGNYIATYFQSNVLGVHLMNCMSIMTPLSYPKMFLAALKPSLFIQE-YTDF 286
Aedae-EH2	VHGGDWGSVIGNIMATFFQDEVLVGHLTMCMNTAPIGTLKNILGAVAPSLLVVEQ-YKDF 286
***** * * *	
Trini-JHEH	LYPLSTRFANLLRETGYMHIQSTKPDVGVALSDSPAGLLAYILEKFATWTRPDLMSPN 341
Trini-mEH	IYPLSKTLSFQVRESGYLHIQASKPDVGVALTDSPAGLLAYIVEKFSIWTRPELTSPN 341
Bommo-JHEH	LYPLSEKYSTLLEELGYMHIQATKPDVGIGLTDSPAGLLAYILEKFSTWTNPDLRSKED 341
Spoex-JHEH	IYPLSSFFAYVLEFGYMHIQATKPDVGVLSDSPAGLLAYILEKFSTWTKKEYKFKAG 340
Manse-JHEH	IYPLKNLFSYIILRETGYFHIQATKPDTGVALTDSPAGLAGYLIEKMAICNSNRDQLDTPH 341
Nasvi-EH2	MYPLSYHFGRLLIETGYLHIQATKPDVGAAALTDSAGLAAYILEKFSTWTNPDYFRRDD 338
Apime-EH	FFPVSEILSFLIEESGYFHIQATKPDTGVALTDSPAGLAAYILEKFSTWTNPDYFRRDD 328
Athro-JHEH	MYPLSKKWSRTIEETGYFHIQATKPDTLGAALADSPAGLAAYILEKFSTGTNPELRKED 338
Nasvi-EH1*	MFPLSTKLALGIEETGYLHLQATKPDTGIGVSDSPAGLAAYILEKMACTKPDNKFTDD 339
Trica-EH	VYPLSSKFAKTLLETGYMHLQATKPDVGVALNDSPAGLAAYILEKFITWTNPAPWRDLED 341
Trica-mEH	IYPLSNYFSNALLEFGYMHQLQATKPDTGVALNDSPVGGLAAYIEKFTTWTNPPEWKNRAD 339
Ctefe-JHEH2	IYPLSEHFTLLEESGYFHIQATKPDVGVALRDSPAGLAAYILEKFSTGTNKAWSAKD 338

Fig. S5. (Continued)

Ctefe-JHEH1	MYPMSEHFTLLEEMGYLHLQATKPDTVGVALRDSPAGLAAYILEKFSTWTNRSWRSVKD	338
Drome-mEH	YKGLGHLFSTIMEEMGYAHIQASKPDTIGNALIDNPTGLASYILEKFSTWTNTAFRSLPD	350
Drome-JHEH2	YKGLGHLFSTIMEEMGYAHIQASKPDTIGNALIDNPTGLASYILEKFSTWTNTAFRSLPD	350
Drome-JHEH1	FFPKSNEMRYLMEESGYFHIQATKPDTIGAALTDPVGGLAAAYILEKFSTWTNPSYRSLPD	355
Drome-JHEH3	HFPVWEKWLELLEESGYFHIQATKPDTIGAALTSSPVGGLASYILEKFQTCTNPGLK-QDF	350
Aedae-EH1	YYPSGPKFQMQLLVESGYMHLQATKPDTIGTALVGNPVGLAAYIEKFSTWTNPSYRSLAD	346
Anoga-EH	YPLSAFKANIIETGYMHIQSTKPDTIGTVLQGNPVGLAAYILEKFSTWTNPAYRNLD	346
Aedae-EH2	YYPYLDRLKLIAETGYMHIQATKPDTIGAVLTGNPVGLATYILEKFSTWTNPQYRSLAD	346
* * * * *		
Trini-JHEH	GGLDYRFTRDQLIDNLMMWTNRAITPAMRLYAENFNKRTVEMKLDEIPTPVPTWGLQTK	401
Trini-mEH	GGLDFRFTKDQLIDNLMMWTSKSITTSVRLYAESFNKVLGYQLDDIPTPVPSWFIQGK	401
Bommo-JHEH	GGLSYRWTKDQLIDNLMLYWSTKSIVTSMRLYAESFSSRHFDLKLDEIQVQVPTWVLQAK	401
Spoex-JHEH	GGLSNRFTKDQLIDNLMIYWSTNSITTSMRFAENFSHKIMSLNDQIPTDVPTWGLQAK	400
Manse-JHEH	GGLEN-LNLDVDLDTVTINWINNCIVTSTRLYAEGFSWPEV-LIVHRIPSMVPTAGINFK	399
Nasvi-EH2	GGLLEKFTMDELIDNLNMVYWWVNTSITTSQRLYAECKFSKANRELGVDKMPIFVPTACANFP	398
Apime-EH	GGITEKFVLDELLNIMIYIWITNSITTSVRLYAENYTSSYRSLSKIDQLPIKVFTACAVFP	388
Athro-JHEH	GGLFDIHSPDELLDNVMLYWMPNSMTTAIRIYAETFSANRALRMDYVPIEVPSCAQFP	398
Nasvi-EH1*	GNLLEKFTMDELIDNLMMWAPNKASSSFRIYAESFNKQTFGYKMDNVPVTPVSACAQFP	399
Trica-EH	GGLTKKYSYTSLLDNVMIYWWVNTSITTSMRLYAETFNKNQTKLGVAKIPTTVPTACARFS	401
Trica-mEH	GGLLERFTYDKILDNIMIYWWVNTSITTSMRLYAESINK-ESNVFDDRAVITVPSACALFD	398
Ctefe-JHEH2	GNLQSKFTFTELLDNVMIYYVTGSITTSMRLYAESYSDHLSLNMDRVPTVPTACAKFP	398
Ctefe-JHEH1	GNLLLKYNIPELLDNVMIYYVTDSITTSMRLYAESFTKAHLALNLDVRVNHPAACAKFP	398
Drome-mEH	GGLTKRFTYDQLLDNVMIYYVTDSITTSMRLYSESVMVASQFALAVDSVPIKAKGCTRFA	410
Drome-JHEH2	GGLTKRFTYDQLLDNVMIYYVTDSITTSMRLYSESVMVASQFALAVDSVPIKAKGCTRFA	410
Drome-JHEH1	GGLTKRYKMDALLDNLMIYYLTSITTSMRLYAEQYQAQRDLHLDRVPTVPTGCARFK	415
Drome-JHEH3	GAIIVTVFGLAVIDNLNMVYVLTNSATTAARFYLENVSCKTYRDLQLDRVQSPVPMGCARFR	410
Aedae-EH1	GGLEKYFTLDSLNDNIMIYYLTDITTSQRIYYEAFSASEFALAIDRIPRVTACAKFK	406
Anoga-EH	GGLEKYFSDLALLDNVMIYYLTDITTSQRIYAEAFASDELKKEIDRIPTAVPAACAKFR	406
Aedae-EH2	GGLEKYFTLDSLNDNIMIYYLSDITTSQRLYAETFNKELSRELDRIPTHVPAAKAKFR	406
* * *		
Trini-JHEH	YELGYQPQYILKIKFPNLVGTTLQEGGHFIAFELPEVFTNDVIKAVTEFRKLQKKNVKT	461
Trini-mEH	YEIAYQPPFVLKLKYPNIVGVTVLDDGGHFFAELPEVFSKDVLKAVTAFRKLQKNNEKT	461
Bommo-JHEH	HELAYQPPCILKLKYTKLNVNASVIEDGGHFLAFELPEIFAKDVLKAIGEFRKL--NVKT	459
Spoex-JHEH	EELFYQPPAVLPSAKFKNLIGTTVLDDGGHFLAFELPQVLSADVFKAFKAFKEWHQANKKT	460
Manse-JHEH	YEVLVQPDWILRKFPNLVRSTVLDFGGHFAALHTPQALADDIFASAVQFLKFHDRKRQ	459
Nasvi-EH2	HELAYRSETILKERFTNLVQFTHPRGGHFAAFEEPELLANDVWSFVHKLEQRSLDEKRQ	458
Apime-EH	NEILVLPESLLQKYPNIIQYNIISRGGHFAAFEEPRLADDIFSFKVKKIENLTSKSS--	446
Athro-JHEH	HEISYQPPSLLSARYKKLIRARKMPKGHHFAAFEQPOLLADEVWTSIGIMEADKKQRE-D	457
Nasvi-EH1*	YEIIIPQSANFLRDRFVNLLRVTKMPRGGHFAVLEEPQLLADDIWASVQAFRDYYAAQE	459

Fig. S5. (Continued)

Trica-EH	YDLAYSPTAVLKEKYKNIVYESDYD-AGHFAAFEEPELLAKDIYKGVEKIEQFHKNNERL	460
Trica-mEH	HEIIYQPVSIFKDRFAKLVQVNEYD-GGHFAAFEVPESLAKDIWLAVSKFEPPKPNK--	455
Ctefe-JHEH2	HEIAYKTDFQLAEKYKTLLQSSTIMPRGGHFAALEEPLLLAEIDIFSAVKKFIDHHSSKKDSK	458
Ctefe-JHEH1	NELAYVTDCQLAEKYKTLLQSNDMPSGGHFAAFEEPGLLAEDIFTAVKKFKEFYSKK-AE	457
Drome-mEH	HEITHFSDSVLANKFPNLVHSTHHRDGHHFPAFELPQQLYDDFVSVQKANFS-----	463
Drome-JHEH2	HEITHFSDSVLANKFPNLVHSTHHRDGHHFPAFELPQQLYDDFVSVQKANFS-----	463
Drome-JHEH1	SDIMQFLDVQLKDKYTNLVHSTYHKKGHHFAALEVPKVLYKDFIDFVETVERKFKIKTL-	474
Drome-JHEH3	FDLASVTDWQLRDKFPNLTHSMYFQQGSHFAALEMPAMLNFNTAFVGKIGLHGEKRK--	468
Aedae-EH1	YELMHHTIDWALKDHFTNLVQSNHFDDGGHFAAMQLPDVLYKDFVEFVKVEK-----	458
Anoga-EH	HELPQQIDWVLKDHTNLVQSNHFEDGGHFAAMQLPEVLYKDFVAFIASIQK-----	458
Aedae-EH2	YELFQQTDWALRDHFRNLIQSCHKHYDDGGHFVAMQLPDVLYEDIVEFVNRLYRREDQ---	462
** * *		
Trini-JHEH	DL-----	463
Trini-mEH	DL-----	463
Bommo-JHEH	EL-----	461
Spoex-JHEH	EL-----	462
Manse-JHEH	KSS-----	462
Nasvi-EH2	KQEAKKEKAKKA	470
Apime-EH	-----	
Athro-JHEH	KLKKSA-----	463
Nasvi-EH1*	RLEKQKS-----	466
Trica-EH	S-----	461
Trica-mEH	-----	
Ctefe-JHEH2	NQENRDL-----	465
Ctefe-JHEH1	SQKKADL-----	464
Drome-mEH	-----	
Drome-JHEH2	-----	
Drome-JHEH1	-----	
Drome-JHEH3	-----	
Aedae-EH1	-----	
Anoga-EH	-----	
Aedae-EH2	-----	

Fig. S5. (Continued)

(A)

<i>Nasvi-EH1</i>	MWKGAAVFLLATLAIGWHLRYQGPVEVP-DLPNQYWPGP-KPVPDPKDIKPFKIDVPKEV	58
<i>Nasvi-EH2</i>	MGVLLWGFLGIVLIGGATIFISPPVQQPKLTDVYWPGPDEPKKTDTSVRPFKITFQKEM	60
	* *	
<i>Nasvi-EH1</i>	IDDLNKRLDSTRSFVEPLEGSAWTYGISSSTYLKTVLNHWRKKNWSQRQALLNKYPQFKT	118
<i>Nasvi-EH2</i>	LDDLRNRLKNTRKMQPPLENVGWTYGLSGDFVPVIVDHWLKNYDFKKREAHLNQYPQFIT	120
	* *	
<i>Nasvi-EH1</i>	KIQGLDIHFYHVKPQVPKDRKVRVLPLLMLHGPGSIVEFQKIPMLTTAKPDENFVFEL	178
<i>Nasvi-EH2</i>	NVQGLDIHFIFIHVKPQLPKNSKIQVLPYLQHGWPGSIVEFQKIPMLTPRSDRNFVFEV	180
	* *	
<i>Nasvi-EH1</i>	IIPSLPGYGFQSQAARPGLGPAQMAMVFKNLMQRLGFEQFYTQGGDWGSLITANMAVLYP	238
<i>Nasvi-EH2</i>	VAPSLPGFGYSQAASKAGLGAQMAHLMKNLMLRGFDKFYTQGGDWGAVIGANMASMYP	240
	* *	
<i>Nasvi-EH1</i>	KKVIGTHLNMCFIESHKAHFLSLVGAYIPSLVVDEHYSKMYPLSYHFGRLIEETGYLHI	298
<i>Nasvi-EH2</i>	QHVLGMHSNMCLVVRPWTWLKIAAYSIMPSLLPEDERQ-LMFPLSTKLALGIEETGYLHL	299
	* *	
<i>Nasvi-EH1</i>	QATKPETVGAALTDSPAGLAAYILEKFSTWTNPDYFRRDDGGLLEKFTMELLDNLMVW	358
<i>Nasvi-EH2</i>	QATKPDTIGIVSDSPAGLAAYILEKMACTKPDNKFTDDGNLLEKFTMELIDNLMMYW	359
	* *	
<i>Nasvi-EH1</i>	VTNSITTSQRLYAECFSKANRELGVDKMPIFVPTACANFPHELAYERSETILKERFTNLVQ	418
<i>Nasvi-EH2</i>	APNKASSSFRIYAESFNKQTFGYKMDNVPVTVPSCAQFPYEEIFQSANFLRDRFVNLLR	419
	* *	
<i>Nasvi-EH1</i>	FTHPPRGGHFAAFEEPELLANDVWSFVHKLEQRLSDEKRQKQEAKKEKAKKA	470
<i>Nasvi-EH2</i>	VTKMPRGGHFAVLEEPQLLADDIWASVQAFRDYYAAQEQRLEKQKS----	466
	* *	

(B)

Nas.EH2.F1

1 **AAGATCGCGGCCTACAGCCTGATGCCGTCGCTTCTGCCGGAGGACGAGAGGCAGCTGATG**
 61 TTCCCGCTGAGCACGAAACTGGCTTGGCATCGAGGAGACCGGTTACCTCCACTTGCAG
 121 GCCACCAAGCCCCACACCATCGGAATCGGAGTGAGCGATTCTCCCGCTGGACTGGCGCG
 181 TACATCCTGGAAAAGATGGCTTACTGCACGAAACCCGACAACAAGTCACCGACGACGGT
 241 AACCTCCTGGAAAAGTTACCAGGACGAGCTCATCGACAATCTCATGATGTACTGGCG
 301 CCGAACAAAGGCTAGCAGCTCCTCCGAATCTACGCCAGAGAGTTCAACAAGCAAACCTC
 361 GGCTACAAGATGGACAACGTTCCAGTAACGGTACCAAGCGCTGCGCCAGTCCCCTAC
 421 GAGATAATCTTCCAATCGCCAACCTCCTCGCGACCGTTCTGTAACCTCCTCAGGGTA
 481 ACGAAGATGCCTCGAGGTGGCCATT CGCGGTCTCGAAGAACCCAGCTCTCGCCGAC

Nas.EH2.R

541 GACATCTGGCTCGGTACAGGC**GTTCCGAGACTACTACGCCGCG**

Fig. S6. (A) Amino acid sequence alignment of the putative EH genes *Nasvi-EH1* and *Nasvi-EH2* (GenBank accession no. XP_001602895) from *Nasonia vitripennis*, using CLUSTAL W analysis. Name of the gene sequences are given to the left, the amino acids are numbered to the right, and identical amino acids are indicated with asterisks. (B) Nucleotide sequence of the *Nasvi-EH2* dsRNA that was used in RNAi analysis. Primer nucleotide sequences are in bold and their names are given above.

Table S1. Exon/Intron splice sites prediction of the *Nasvi-EH1* DNA gene, using www.fruitfly.org/seq_tools/splice.html tools

Start	End	Score	Exon/Intron
53	67	0.65	atctcaggatatcaag
72	86	0.88	cgtggaggtaacctga
165	179	0.89	aaaagaggtaatcga
393	407	0.75	gccccaggtaacctaa
411	425	0.62	ccgcaagggtccgtgt