

Fig. S1. Western blot analysis of transformed *S. cerevisiae* cells. Protein extracts of (A) Nvit_D12a ($M_w = 46610$ Da) and (B) Nvit_D12b ($M_w = 465247$ Da) and empty vector controls were separated on 13% SDS-PAGE gels and blotted on PVDF membranes. (FLAG)₃-tagged proteins were visualised by chemiluminescence.

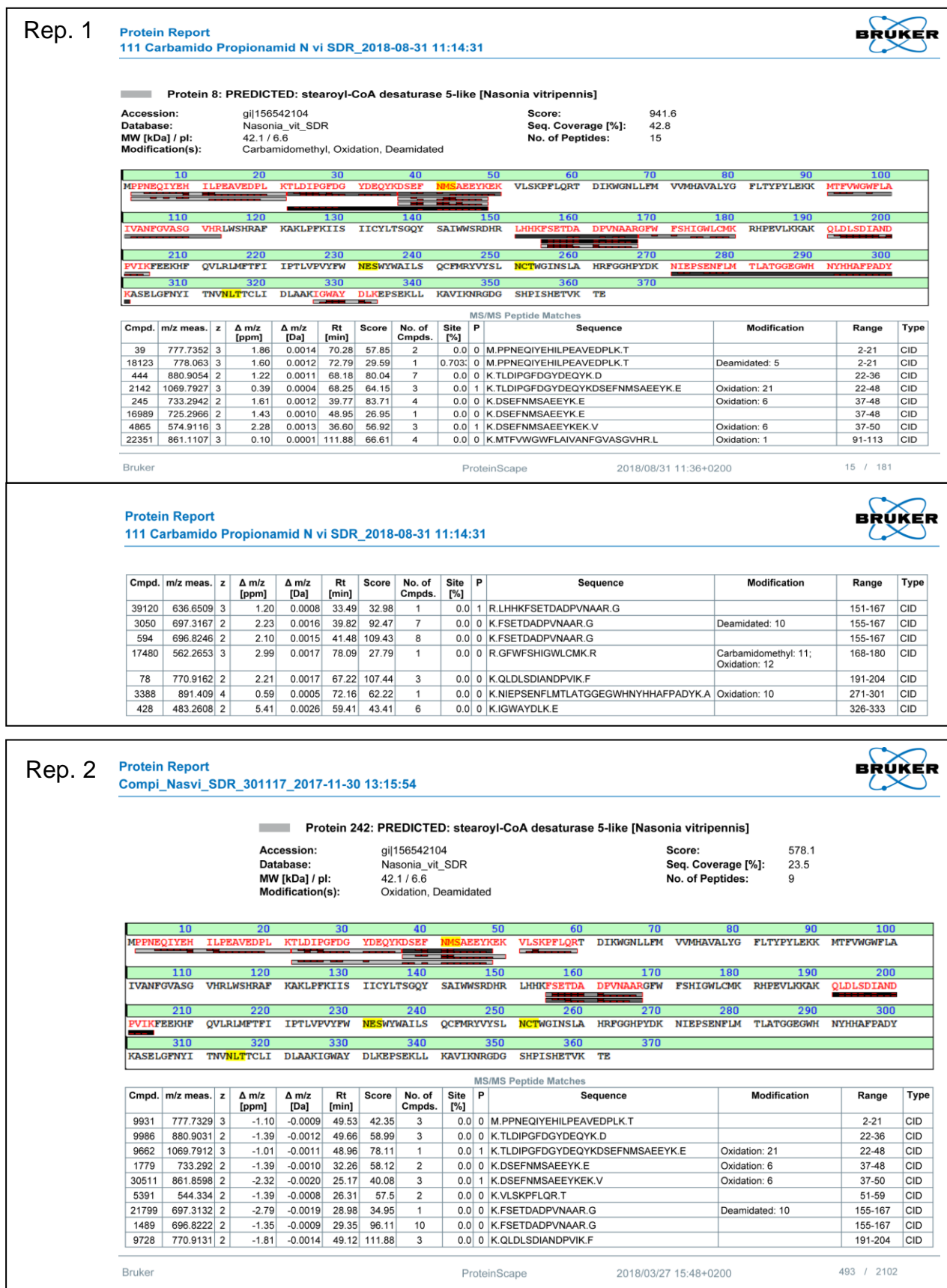


Fig. S2. Results the two replicates (Rep. 1 and Rep. 2) of the mass spectrometric analysis of the Δ12-desaturase Nvit_D12a in the rectal vesicles of male *N. vitripennis*. Ten rectal vesicles of 1–2 day old wasps were pooled per replicate and subjected to SDS-PAGE. *In-gel* trypsin digested proteins were analysed by LC/MS/MS. Protein database searching of the resulting mass spectra was performed using the Mascot search engine.

XP_001599873.1 (unbiased)
XP_001599836.1 (male biased)

```
MPPNEQIHEHVLPEAVEDPLKTLDI PGFNDYDEEHKDFNMSAEYNEKVINKPFLQRT
MPPNEQIYEHILPEAVEDPLKTLDI PGFDGYDEQYKDSEFNMSAEYKEKVL SKPFLQRT
*****: ** :*****:*****:*****:*****:*****
DIKWGNLYFMVVLHAIALYGF LTYPYFEKKMTFVWGWF LAIVANFGVAGGVHRLW SHRAF
DIKWGNLLFMVVMHAVALYGF LTYPYLEK KMTFVWGWF LAIVANFGVASGVHRL WSHRAF
***** *****: ** :*****:*****:*****:*****:*****
KAKVPLKII F ILSYLTSGQYS AIWWARDHRIH H K F S E T D A D P V N A A R G F W F S H V G W L C M K
KAKLPFKIISIICYLTSGQYS AIWWSRDH RLH H K F S E T D A D P V N A A R G F W F S H I G W L C M K
***: * :*** * : *****:*****:*****:*****:*****
RHPEVLK KAKQLDLS DI LNDPVIK FEEKHFQVLR LI FAFI IPTLVPVYFWNESWY WAILS
RHPEVLK KAKQLDLS DI ANDPVIK FEEKHFQVLR LMFTFI IPTLVPVYFWNESWY WAILS
***** *****: * :*****:*****:*****:*****
QCFMRYAYSLNCTWGIN SFAHMF GNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPADY
QCFMRYVYSLNCTWGIN SLAHRFGGHPYD KNI EPSENFLMTLATGGEGWHNYHHAFPADY
***** . *****: ** ** . *****:***:***:*****:***** . *****
KASELGFNYITNVNLT TCLIDLAAK IGWAYDLKE PSEKLLKAVIKNRGDGSHPISHEPVK
KASELGFNYITNVNLT TCLIDLAAK IGWAYDLKEPSEKLLKAVIKNRGDGSHPISHETVK
***** *****: **
TK
TE
*:
```

Fig. S3. Sequence alignment of the two $\Delta 12$ -desaturases Nvit_D12a (XP_001599836.1) and Nvit_D12b (XP_001599873.1) from *N. vitripennis*. Sequences detected in the male pheromone gland (rectal vesicle) by LC/MS/MS are indicated in colour (yellow: ranges covered by unique peptides of the male-biased desaturase Nvit_D12a; green: peptides shared by both $\Delta 12$ -desaturases).



Fig. S4. Multiple sequence alignment of functionally characterised insect $\Delta 12$ -desaturases. The three histidine boxes characteristic for “First” type desaturases (Hashimoto et al., 2008) are shown in yellow frames. Conserved amino acids are indicated by asterisks at the bottom of each column. Sequences identities and similarities are given in Table S3. Adom_D12 = *Acheta domesticus* (Zhou et al., 2008), Clug_D12 = *Chauliognathus lugubris* (Haritos et al., 2012), Tcas_D12 = *Tribolium castaneum* (Zhou et al., 2008), Nvit_D12a/b = *Nasonia vitripennis* (this study).

Table S1. Nucleotide and amino acid sequences of Nvit_D12a (XP_001599836.1) and Nvit_D12b (XP_001599873.1). The nucleotide sequences were optimised for expression in *S. cerevisiae*. Recognition sites for *Bsal* are underlined and restriction sites are bold.

Nucleotide sequences

Nvit_D12a (XP_001599836.1)

5'AAAAAAGGTCTC**CATG**CCACCAAACGAACAAATCCATGAACATGTTTTGCCAGAAGCTGTT
GAGGATCCATTGAAAACTTTGGATATTCCAGGTTTCAACGACTACGATGAAGAACATAAGGAC
TCCGAATTC AACATGTCTGCTGAAGAGTACAACGAAAAGGTTATCAACAAGCCATTCTTGCAA
AGAACC GATATTAAGTGGGGTAACTTGTACTTCATGGTTGTCTTGCATGCTATTGCCTTGTAC
GGTTTTT GACTTACCATACTTCGAGAAGAAGATGACTTTTGTGGGGTTGGTTTTTGGCTA
TCGTTGCTAATTTTGGTGTGCTGGTGGTGTTCATAGATTGTGGTCACATAGAGCTTTTAAGG
CTAAGTTCCACTGAAGATCATTTTCATCTTGTCTACTTGACCTCCGGTCAATATTCTGCTAT
TTGGTGGGCTAGAGATCATAGAATCCATCACAAGTTCTCTGAAACTGATGCTGATCCAGTTAA
TGCTGCTAGAGGTTTTTGGTTTTCTCATGTTGGTTGGTTGTGCATGAAGAGACATCCAGAAGT
TTTGAAGAAGGCCAAGCAATTGGATTTGTCCGATATTTGAATGACCCCGTCATCAAGTTTGA
AGAAAAGCACTTCAAGTCCTGAGATTGATTTTCGCCTTCATCATTCCAAC TTTGGTTCCAGTT
TACTTCTGGAACGAATCTTGGTATTGGGCCATTTTGTCTCAATGCTTTATGAGATACGCCTACT
CTTTGAACTGTACCTGGGGTATTA ACTCTTTGCTCATATGTTGGTAACCATCCATACGATAA
GCACATTGAACCAGCCGAAAAC TTCATTATGACTTTGGCTACTGGTGGTGAAGTTGGCATAA
TTACCATCATGTTTTTCCAGCTGATTACAAGGCTTCTGAATTGGGTTTCAACTACATCACCAAC
GTTAACTTGACCACCTGTTTGATTGATTTGGCTGCTAAAATTGGTTGGGCCTACGATTTGAAA
GAACCATCTGAAAAGTTGTTGAAGGCCGTGATTAAGAATAGAGGTGATGGTTCTCATCCAATC
TCTCATGAACCAGTTAAGACCAAGT**AAGAGAGACCA**AAAAA3'

Nvit_D12b (XP_001599873.1)

5'AAAAAAGGTCTC**CATG**CCACCAAACGAACAAATCTACGAACATATTTT GCCAGAAGCCGTT
GAAGATCCATTGAAAAC TTTGGATATTCCAGGTTTCGATGGTTACGATGAACAGTACAAAGAC
TCCGAATTC AACATGTCTGCTGAAGAGTACAAGAGAAGGTTTTGTCTAAGCCATTCTTGCAA
AGAACC GATATTAAGTGGGGTAACTTGTGTTTCATGGTTGTTATGCATGCTGTTGCCTTGTAC
GGTTTTT GACTTATCCATACTTGGAGAAGAAGATGACTTTTGTGGGGTTGGTTTTTGGCTA
TCGTTGCTAATTTTGGTGTGCTCTGGTGTTCATAGATTGTGGTCACATAGAGCTTTTAAGG
CTAAGTTGCCATTCAAGATCATCTCCATTATTTGCTACTTGACCTCCGGTCAATATTCTGCTAT
TTGGTGGTCTAGAGATCACAGATTGCATCACAATTCTCTGAAACTGATGCCGATCCAGTTAA
TGCTGCTAGAGGTTTTTGGTTCTCTCATATTGGTTGGTTGTGCATGAAGAGACATCCAGAAGT
TTTGAAGAAGGCCAAGCAATTGGATTTGTCCGATATTGCTAATGACCCAGTCATCAAGTTTGA
AGAAAAGCACTTCCAAGTCCTGAGATTGATGTTACCTTCATTATCCCAACTTTGGTTCCAGTT
TACTTCTGGAATGAATCTTGGTACTGGGCCATTTTGTCTCAATGTTTCATGAGATACGTCTACT
CATTGAACTGTACCTGGGGTATTA ACTCTTTGGCACATAGATTCCGGTGGTTCATCCATACGATA
AGAACATTGAACCATCCGAGAATTTCTTGATGACATTGGCTACTGGTGGTGAAGTTGGCATA
ATTATCATCATGCTTTTCCAGCTGACTACAAGGCTTCTGAATTGGGTTTAACTACATCACC
CGTTAACTTGACCACCTGTTTGATTGATTTGGCTGCTAAAATTGGTTGGGCCTACGATTTGAA
AGAACCCTCTGAAAAGTTGTTGAAGGCCGTTATTAAGAACAGAGGTGATGGTTCTCATCCAAT
TTCTCACGAAACTGTTAAGACCGAGT**AAGAGAGACCA**AAAAA 3'

Amino acid sequences

Nvit_D12a (XP_001599836.1)

MPPNEQIHEHVLPEAVEDPLKTLDIPGFDYDEEHKDSEFNMSAEEYNEKVINKPFLQRTDIKWG
NLYFMVVLHAIALYGFLTYPYFEKKMTFWGWFLAIVANFGVAGGVHRLWSHRAFKAKVPLKIIFIL
SYLTSGQYSAIWWARDHRIHHKFSETDADPVNAARGFWFSHVGWLCMKRHPEVLKAKQLDLS
DILNDPVIKFEEKHFQVLRLLIFAFIIPTLVPVYFWNESWYWAILSQCFMRYAYSLNCTWGINSAHM
FGNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPADYKASELGFNYITNVNLTCLIDLAAKIGW
AYDLKEPSEKLLKAVIKNRGDGSHPISEPVKTK*

Nvit_D12b (XP_001599873.1)

MPPNEQIYEHILPEAVEDPLKTLDIPGFDGYDEQYKDSEFNMSAEEYKEKVLKSKPFLQRTDIKWGN
LLFMVVMHAVALYGFLTYPYLEKKMTFWGWFLAIVANFGVASGVHRLWSHRAFKAKLPFKIISIIC
YLTSGQYSAIWWSRDHRLHHKFSETDADPVNAARGFWFSHIGWLCMKRHPEVLKAKQLDLSDI
ANDPVIKFEEKHFQVLRMLMFTFIIPTLVPVYFWNESWYWAILSQCFMRYVYSLNCTWGINSLAHRF
GGHPYDKNIEPSENFMLTLATGGEGWHNYHHAFFADYKASELGFNYITNVNLTCLIDLAAKIGW
AYDLKEPSEKLLKAVIKNRGDGSHPISETVKTE*

Table S2. Conversion of externally supplied fully labelled $^{13}\text{C}18:1^{\Delta 9}$ into $^{13}\text{C}18:2^{\Delta 9,12}$ by *Urolepis rufipes*. Single 2-d-old wasps (n = 12 for each sex) were treated with an acetone solution of $^{13}\text{C}18:1^{\Delta 9}$. After 20 h, lipids of 3 homogenised wasps each (resulting in 4 replicates for males and females, respectively) were extracted, transesterified into fatty acid methyl esters, and analysed by GC/MS. The conversion of $^{13}\text{C}18:1^{\Delta 9}$ to $^{13}\text{C}18:2^{\Delta 9,12}$ by the wasps was concluded from the occurrence of the diagnostic molecular ion m/z 312 at the retention time of C18:2 $^{\Delta 9,12}$ methyl ester. The conversion rate was calculated by relating the peak area of the ^{13}C -labelled molecular ion of C18:2 $^{\Delta 9,12}$ methyl ester (m/z 312) to the added peak areas of the ^{13}C -labelled molecular ions of C18:2 $^{\Delta 9,12}$ methyl ester plus C18:1 $^{\Delta 9}$ methyl ester (m/z 314).

	Female 01	Female 02	Female 03	Female 04	Male 01	Male 02	Male 03	Male 04
Area m/z 312	4059	14031	17465	8985	0	0	0	0
Area m/z 314	34633	115906	273316	43249	33488	46539	23350	28169
Conversion rate (%)	10.49	10.80	6.01	17.20	0	0	0	0

Table S3. Sequence identities and similarities between insect-derived $\Delta 12$ -desaturases. Number and percentage of identical amino acid positions (sequence identities) are given in the upper right triangle, number and percentage of identical plus similar amino acid positions (sequence similarity) are given in the lower left triangle.

	Adom_D12	Clug_D12	Tcas_D12	Nvit_D12a	Nvit_D12b
Adom_D12		186/357 (52%)	163/377 (43%)	159/388 (41%)	159/383 (42%)
Clug_D12	244/357 (68%)		157/371 (42%)	149/381 (39%)	141/381 (37%)
Tcas_D12	221/377 (59%)	225/371 (60%)		131/408 (32%)	129/398 (32%)
Nvit_D12a	223/388 (58%)	212/381 (55%)	189/408 (46%)		327/362 (90%)
Nvit_D12b	220/383 (57%)	210/381 (55%)	191/398 (47%)	346/362 (96%)	

References

Haritos, V. S., Horne, I., Damcevski, K., Glover, K., Gibb, N., Okada, S. and Hamberg, M. (2012). The convergent evolution of defensive polyacetylenic fatty acid biosynthesis genes in soldier beetles. *Nat. Commun.* **3**.

Hashimoto, K., Yoshizawa, A. C., Okuda, S., Kuma, K., Goto, S. and Kanehisa, M. (2008). The repertoire of desaturases and elongases reveals fatty acid variations in 56 eukaryotic genomes. *J. Lipid. Res.* **49**, 183-191.

Zhou, X. R., Horne, I., Damcevski, K., Haritos, V., Green, A. and Singh, S. (2008). Isolation and functional characterization of two independently-evolved fatty acid delta 12-desaturase genes from insects. *Ins. Mol. Biol.* **17**, 667-676.