# Functional characterisation of two Δ12-desaturases demonstrates targeted production of linoleic acid as pheromone precursor in *Nasonia*

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**Summary statement**: Two Δ12-desaturases from the parasitoid wasp *Nasonia vitripennis* synthesise linoleic acid. One of them is expressed in the male rectal vesicle where they produce a sex pheromone from linoleic acid.

## Abstract

Insect pheromones are often derived from fatty acid metabolism. Fatty acid desaturases, enzymes introducing double bonds into fatty acids, are crucial for the biosynthesis of these chemical signals. $\Delta$12-desaturases catalyse the biosynthesis of linoleic acid by introducing a second double bond into oleic acid, but have been identified in only few animal species. Here we report the functional characterisation of two $\Delta$12- desaturases, Nvit_D12a and Nvit_D12b, from the parasitic wasp *Nasonia vitripennis* (Walker)*.* We demonstrate that Nvit_D12a is expressed in the rectal vesicle of males where they produce a linoleic acid-derived sex pheromone to attract virgin females. $^{13}$C-labelling experiments with *Urolepis rufipes* (Ashmead), a closely related species belonging to the so-called "*Nasonia*-group", revealed that females but not males are able to synthesise linoleic acid. *U. rufipes* males produce an isoprenoid sex pheromone in the same gland and do not depend on linoleic acid for pheromone production. This suggests that $\Delta$12-desaturases, while common in the "*Nasonia* group", acquired a specialised function in chemical communication of those species that use linoleic acid as a pheromone precursor. Phylogenetic analysis suggests that insect $\Delta$12-desaturases have evolved repeatedly from $\Delta$9-desaturases in different insect taxa. Hence, insects have developed a way to produce linoleic acid independent of the "Omega"-desaturase subfamily which harbours the eukaryotic $\Delta$12-desaturases known so far.

## Introduction

Communication by sex pheromones is crucial for mate finding in many insects (Wyatt, 2014). To fulfil their function reliably, sex pheromones need to encode their information in a species-specific manner. Insect pheromones are often biosynthesised as variations on conserved pathways of the primary and secondary metabolism whereby the fatty acid metabolism is of particular importance (Jurenka et al., 2017; Morgan, 2004; Tillman et al., 1999). For instance, more than 90% of the lepidopteran sex pheromones known to date are fatty acid derivatives (Ando et al., 2004) and the cuticular hydrocarbons used by numerous insects as contact sex pheromones are likewise derived from the fatty acid metabolism (Blomquist, 2010). The number, position and configuration of double bonds are important structural

features of fatty acid-derived sex pheromones and contribute to the specificity of the information they convey. Thus, fatty acid desaturases (FADs) introducing double bonds into fatty acid chains are key enzymes for the species specific communication and reproductive success of many insects (Jurenka et al., 2017; Tillman et al., 1999; Tupec et al., 2017). Animals have long been assumed to be missing a crucial type of FAD, which are the Δ12-FADs that can produce linoleic acid ((9$Z$,12$Z$)-octadeca-9,12-dienoic acid, C18:2$^{\Delta9,12}$) by introducing a second double bond into oleic acid ((9$Z$)-octadec-9-enoic acid, C18:1$^{\Delta9}$) at position 12. Hence, C18:2$^{\Delta9,12}$ was considered an essential nutrient for animals (Cripps et al., 1986; Malcicka et al., 2018). However, studies on insects (Blaul et al., 2014; Blomquist et al., 1982; Buckner and Hagen, 2003; Cripps et al., 1986; Cripps et al., 1990; Haritos et al., 2012; Malcicka et al., 2018; Zhou et al., 2008), springtails (Malcicka et al., 2017), nematodes (Peyou-Ndi et al., 2000), gastropods (Weinert et al., 1993), mites (Aboshi et al., 2013; Shimizu et al., 2014) and a variety of aquatic invertebrates (Kabeya et al., 2018) have shown that not all animals depend on dietary uptake of C18:2$^{\Delta9,12}$ but are able to synthesise it *de novo*. Evidence for the presence of Δ12-FADs in insects is mostly based on stable and radioactive isotope labelling experiments (Blaul et al., 2014; Blomquist et al., 1982; Buckner and Hagen, 2003; Cripps et al., 1986; Malcicka et al., 2017). This approach, however, does not completely rule out that C18:2$^{\Delta9,12}$ is produced by associated microorganisms rather than by the studied animal itself. Unequivocal evidence for C18:2$^{\Delta9,12}$ biosynthetic capacity requires isolation and functional characterisation of insect-derived Δ12-FAD genes, which has been achieved so far only for the house cricket *Acheta domesticus* (AdD12Des, here referred to as Adom_D12) (Zhou et al., 2008), the soldier beetle *Chauliognathus lugubris* (CL10, here referred to as Clug_D12) (Haritos et al., 2012) and the red flour beetle *Tribolium castaneum* (TcD12Des, here referred to as Tcas_D12) (Haritos et al., 2014; Zhou et al., 2008). It is unknown, however, whether the Δ12-FADs in these species are involved in primary fatty acid metabolism or pheromone biosynthesis.

Males of the parasitic wasp *Nasonia vitripennis* (Walker), a pupal parasitoid of different fly species, produce in their rectal vesicle a sex pheromone composed of (4$R$,5$R$)- and (4$R$,5$S$)-5-hydroxy-4-decanolide and the minor component 4-methylquinazoline. After emergence, males deposit the pheromone to the host and other substrates to attract virgin females (Mair and Ruther, 2018; Ruther et al., 2007; Ruther et al., 2008). Stable isotope labelling experiments using fully ^{13}C-labelled fatty

acid precursors revealed that both stereoisomers of 5-hydroxy-4-decanolide are synthesised from C18:2$^{\Delta 9,12}$ or C18:1$^{\Delta 9}$ (Blaul and Ruther, 2011; Blaul et al., 2014) (**Figure 1**). Furthermore, *N. vitripennis* males are capable of converting C18:1$^{\Delta 9}$ into C18:2$^{\Delta 9,12}$, suggesting that they possess a ∆12-FAD that is involved in sex pheromone biosynthesis (Blaul et al., 2014). Sixteen predicted FAD genes have been identified and mapped in the *N. vitripennis* genome (Niehuis et al., 2011), 13 of which have been studied with respect to their sex-biased expression status (Wang et al., 2015). Such gene expression analyses revealed a male-biased expression of five predicted FADs, which are thus candidates for being involved in the biosynthesis of the sex pheromone. This is particularly true for one predicted FAD (GenBank acc. no. XP_001599836.1), which has been found to be 800-fold higher expressed in males than in females. In contrast, a highly similar paralogue showing >90% protein sequence identity (GenBank acc. no. XP_001599873.1) was found to be expressed unbiasedly (Wang et al., 2015).

The use of (4*R*,5*S*)-5-hydroxy-4-decanolide as a male sex pheromone component is also found in the three other species of the genus *Nasonia*, *N. giraulti, N. longicornis,* and *N. oneida* (Darling and Werren, 1990; Raychoudhury et al., 2010), as well as in the closely related species *Trichomalopsis sarcophagae* (Niehuis et al., 2013). This suggests that these species, like *N. vitripennis*, rely on the biosynthesis of C18:2$^{\Delta 9,12}$. The genus *Urolepis* is closely related to *Nasonia* and *Trichomalopsis* and has been suggested to form a monophyletic taxon within the Pteromalinae, the so-called "*Nasonia* group" (Burks, 2009). Unlike the *Nasonia* species, males of *Urolepis rufipes* (Ashmead)*,* the only *Urolepis* species studied so far, release from their rectal vesicle the sex pheromone 2,6-dimethyl-7-octene-1,6-diol, which is synthesised via the mevalonate pathway while C18:2$^{\Delta 9,12}$ is not involved (Ruther et al., 2019). Hence, the study of C18:2$^{\Delta 9,12}$ biosynthesis in *U. rufipes* will allow conclusions on whether ∆12-FADs are common in the *Nasonia* group or have evolved in the context of pheromone biosynthesis.

In the present study, we test the catalytic capacities of the two *N. vitripennis* FADs by functional expression in yeast and investigate whether they are expressed in the male sex pheromone gland (rectal vesicle) by a proteomics approach. Finally, we investigate the ability of *U. rufipes* to synthesise C18:2$^{\Delta 9,12}$ from C18:1$^{\Delta 9}$ by $^{13}$C-labelling experiments. We demonstrate that both FADs have ∆12-activity and that the

male-biased FAD reported by Wang et al. (2015) is expressed in the pheromone gland. A phylogenetic analysis of *N. vitripennis* FAD sequences and those of other insect $\Delta$12-FADs suggests that they have evolved independently. We furthermore show that females but not males of *U. rufipes* are capable of synthesising C18:2$^{\Delta9,12}$ suggesting that $\Delta$12-FADs have a single evolutionary origin in the *Nasonia* group but have been adapted for pheromone biosynthesis in males of the *Nasonia* species and *T. sarcophagae.*

## Materials and methods

### Insects

We used *N. vitripennis* from the inbred strain Phero_01, which originated from northern Germany and was also used in previous pheromone studies (Abdel-Latief et al., 2008; Niehuis et al., 2013; Ruther et al., 2016; Ruther et al., 2007; Ruther et al., 2008). The *U. rufipes* strain was kindly provided by K. Floate and originated from cattle feedlots in southern Alberta, Canada (Floate, 2002). Both species were reared on freeze-killed pupae of the green bottle fly *Lucilia caesar* as reported previously (Ruther et al., 2019; Steiner et al., 2006). All studies were carried out in compliance with relevant provincial and national guidelines.

### Cloning and mutagenesis

The genes of XP_001599836.1 (*Nvit_D12a*) and XP_001599873.1 (*Nvit_D12b*) (for nucleotide and amino acid sequences see Supplementary **Table S1**) were optimised for expression in *S. cerevisiae*, synthesised (Life Technologies GmbH, Darmstadt, Germany) and cloned into a modified YCplac111-pGAL-FLAG plasmid (gift from Dr. Philipp Milkereit, University of Regensburg, Germany) via unique *Bsa*I restriction sites as described previously (Rohweder et al., 2018). The modified YCplac111-pGAL-3FLAG plasmid was constructed such that three N-terminal FLAG-tags for protein detection and a cleavage site for TEV-protease for tag removal were located upstream the *Bsa*I cloning sites.

**Yeast transformation and enzyme activity assays**

Plasmids YCplac111-pGAL-3FLAG_XP_001599836.1 (*Nvit_D12a*) and YCplac111-pGAL-3FLAG_XP_001599873.1 (*Nvit_D12b*) were transformed into *S. cerevisiae* BY4741 (Euroscarf/Scientific Research and Development GmbH, Oberursel, Germany) and grown on SDC-Leu agar plates at 30°C. Single colonies were transferred to SGC-Leu agar plates, grown at 30°C for two days and tested for target protein expression by western blot analysis.

For enzyme activity assays, overnight cultures (10mL SGC-Leu media) of protein expressing clones were used to inoculate 25 mL SGC-Leu media supplemented with 10% NP40 (Sigma Aldrich/Merck KGaA, Darmstadt, Germany) to an $OD_{600}$ of 0.1. Cells were grown at 30°C to an $OD_{600}$ of 0.6 and then $C18:1^{\Delta 9}$ (25 mM, dissolved in ethanol) was added to a final concentration of 0.5 mM. Growth was continued for 3 days at 30°C and cells were harvested by centrifugation (2700g, 4°C), washed first with 25 mL 1% NP40, then with 25 mL 0.5% NP40 and finally twice with 25 mL water. Yeast cell pellets were dried 2.5 h in an Eppendorf Concentrator 5301 (Eppendorf AG, Hamburg, Germany) at 30°C.

**Western blot analysis**

Detection of $(FLAG)_3$-tagged proteins was performed with the monoclonal Anti-FLAG antibody (catalogue number: 200474, lot: 0006262954, dilution: 1:1000, Agilent Technologies, Santa Clara, USA) and the HRP-coupled anti-rat secondary antibody (code number: 112035068, lot: 73749, dilution: 1:5000, Dianova, Hamburg, Germany). Protein signals were visualised using the BM Chemiluminescence Western blotting kit (Roche Diagnostics GmbH, Mannheim, Germany) in a Fluorescence Image Reader LAS3000 (Fujifilm Europe GmbH, Düsseldorf, Germany).

**Fatty acid extraction, transesterification and GC/MS-analysis**

Fatty acid composition was analysed by coupled gas chromatography/mass spectrometry (GC/MS). Yeast cell pellets were cut to small pieces and extracted for 30 min in 400 µl dichloromethane in an ultrasonic bath. The extracts were transferred to a new vial and dried under a gentle nitrogen stream. Then, 300 µl methanol and 30

µl acetyl chloride (10% dissolved in methanol) were added to the residue and transesterification was carried out for 4 h at 70°C. Subsequently, 400 ml of sodium hydrogen carbonate (5%, dissolved in sterile water) was added and the fatty acid methyl esters (FAMEs) were extracted with 200 µl hexane. For analysis, a Shimadzu QP2010 Plus GC/MS system (Shimadzu Corporation, Kyoto, Japan) equipped with a polar BP-20 capillary column (30 m x 0.25 mm inner diameter, 0.25 mm film thickness, SGE Analytical Science Europe, Milton Keynes, UK) was used. Helium served as carrier gas at a linear velocity of 40 cm s$^{-1}$. The MS was operated in the electron impact ionisation (EI) mode at 70 eV, the mass range was m/z 35-500. Samples were injected splitless at 240°C by using an AOC 20i auto sampler. The initial oven temperature was 50°C and then increased at 3°C min$^{-1}$ to 240°C, which was held for 30 min. FAME analysis of *U. rufipes* wasps was done on a non-polar BPX5 column (60 m x 0.25 mm inner diameter, film thickness 0.25 µm, SGE Analytical Science Europe, Milton Keynes, UK) with the same GC/MS parameters as described above but with 280°C as maximum oven and injector temperatures. Identification of FAMEs was done by comparison of retention times and mass spectra with those of authentic reference chemicals (reference mixture of 37 FAMES, Sigma-Aldrich, Deisenhofen, Germany). Positions of double bonds of unsaturated compounds were additionally confirmed by derivatisation with dimethyl disulfide (Sigma-Aldrich, Deisenhofen, Germany) as described by Blaul et al. (2014).

**Protein analysis by LC/MS/MS**

To test which of the two FADs is expressed in the pheromone glands of *N. vitripennis* males, we extracted proteins from 10 male rectal vesicles (two replicates) and analysed the in-gel digested proteins by coupled liquid chromatography/tandem mass spectrometry (LC/MS/MS) as described previously (Ruther et al., 2016).

**Sequence comparison and phylogenetic analysis**

The amino acid sequences were aligned using the MAFFT alignment web server (https://mafft.cbrc.jp/alignment/server/) with standard parameters. Positions containing more than 50% gaps were removed by using Gblocks (Castresana, 2000) followed by the construction of a phylogenetic tree with MrBayes (version 3.2.6, Ronquist et al., 2012). Based on invariate gamma rates and the gtr model, four runs, each with six chains were executed for 1,000,000 generations. The first 25% of the

generations were removed as burn-in and the option contype = allcompat was used to determine the consensus tree and the posterior probabilities. The accession numbers for FADs used in alignments and the phylogenetic tree are: Ades_D12, *A. domesticus* ABY26957.1; Ades_D9, *A. domesticus* AAK25797.1; Tcas_D12, *T. castaneum* NP_001137206.1; Tcas_D9, *T. castaneum* NP_001182164.1; Tcas_D9A, *T. castaneum* NP_001180578.1; Tcas_D9B, *T. castaneum* AHH30808.1; Tcas_D9C, *T. castaneum* AHH30811.1; Tcas_D9D, *T. castaneum* AHH30812.1; Tcas_D5, *T. castaneum* AHH30809.1; Tcas_D5A, *T. castaneum* AHH30810.1; Clug_D12, *C. lugubris* AFJ66832.1; Msex_D11, *Manduca sexta* AM076339.2; Nvit_D12a, *N. vitripennis* XP_001599836.1 ; Nvit_D12b, *N. vitripennis* XP_001599873.1; as well as annotated but uncharacterised sequences from *N. vitripennis*: XP_00159579.1, XP_003425691.1, XP_001599665.2, XP_001607533.2, XP_001602540.1, XP_001599877.2, XP_001600683.2, XP_001599899.2, XP_001607893.1, XP_008217358.1, XP_001602565.1 was reannotated to XP_001602565.1_reannot as reported previously (Blaul et al., 2014).

## $^{13}$C-labelling experiment with *U. rufipes*

To investigate whether *U. rufipes* wasps are capable of synthesising C18:2$^{\Delta 9,12}$ from C18:1$^{\Delta 9}$, we performed $^{13}$C-labelling experiments as described previously (Blaul et al., 2014). Briefly, single randomly chosen 2-d-old parasitoids were cold-sedated on an ice bath and 0.2 µl of a solution containing 100 µg/µl $^{13}$C18:1$^{\Delta 9}$ (Campro Scientific, Berlin, Germany, molecular mass 314) dissolved in acetone was applied to the abdominal tip (n = 12 for each sex) using a 5-µl microsyringe designed for GC on-column injection (Hamilton, Bonaduz, Switzerland). The pure solvent was applied to randomly chosen control wasps of either sex (n = 12). After 20 h, wasps were freeze-killed and lipids of homogenised wasps (3 wasps per sample resulting in 4 samples per treatment) were extracted with 200 µl dichloromethane and transesterified as described for the yeast lipids. FAME extracts were analysed by GC/MS as described above. The incorporation of $^{13}$C in C18:2$^{\Delta 9,12}$ was concluded from the occurrence of the diagnostic molecular ion m/z 312 at the expected retention time of C18:2$^{\Delta 9,12}$ methyl ester (Blaul et al., 2014). The percentage of $^{13}$C-labelled precursor that was converted into C18:2$^{\Delta 9,12}$ 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$^{\Delta9,12}$ methyl ester plus C18:1$^{\Delta9}$ methyl ester (m/z 314).

## Results

### Western blot analysis

The western blot analysis of the transformed yeast revealed protein bands at the expected molecular masses of Nvit_D12A and Nvit_D12B, which were absent in the respective controls expressing only the empty vector (**Figure S1)**.

### Chemical analysis of transesterified yeast lipids

FAME analysis of lipid extracts from yeast clones expressing both Nvit_D12a and Nvit_D12b showed an additional peak of C18:2$^{\Delta9,12}$ methyl ester, which was absent in the empty vector controls (**Figure 2**). This confirms that both enzymes are $\Delta$12-FADs converting C18:1$^{\Delta9}$ into C18:2$^{\Delta9,12}$. In contrast, the methyl ester of (9*Z*,12*Z*)-hexadeca-9,12-dienoic acid (C16:2$^{\Delta9,12}$) was not detected suggesting that (9*Z*)-hexadec-9-enoic acid (palmitoleic acid, C16:1$^{\Delta9}$) is not accepted as a substrate by the two FADs.

### Protein analysis by LC/MS/MS

The protein analysis by LC/MS/MS demonstrated that the highly male-biased FAD Nvit_D12a (XP_001599836.1) reported by Wang et al. (2015) is expressed in the male pheromone glands. Eleven of the thirteen different peptides detected in the two replicates of the LC/MS/MS analysis were unique for Nvit_D12a, while the sequences of two detected peptides were shared by Nvit_D12a and its highly homologous but unbiased paralogue Nvit_D12b (XP_001599873.1) (**Table 1**, Supplementary **Figures S2 and S3)**. Unique peptides of Nvit_D12b were not detected indicating that exclusively Nvit_D12a is expressed in the male pheromone gland.

### $^{13}$C-labelling experiment with *U. rufipes*

Our $^{13}$C-labelling experiment revealed that *U. rufipes* females are able to convert externally supplied C18:1$^{\Delta9}$ into C18:2$^{\Delta9,12}$ (**Figure 3**). As calculated by the molecular ion peak areas of the $^{13}$C-labelled FAMEs, 11.1$\pm$4.6% (mean$\pm$SEM) of the labelled C18:1$^{\Delta9}$ was converted into C18:2$^{\Delta9,12}$. In contrast, no $^{13}$C-labelled C18:2$^{\Delta9,12}$ was

detected in lipid extracts from male wasps (Supplementary **Table S2**). Neither diagnostic ions indicative of ^{13}C-labelled C18:1$^{\Delta 9}$ nor C18:2$^{\Delta 9,12}$ were detected in lipid extracts from wasps of either sex treated with the pure solvent.

## Sequence comparison and phylogenetic analysis

Multiple sequence alignment of the *N. vitripennis* Δ12-FADs with functionally characterised insect Δ12-FADs from house cricket *A. domesticus* (Adom_D12) (Zhou et al., 2008), red flour beetle, *T. castaneum* (Tcas_D12) (Zhou et al., 2008), and soldier beetle, *C. lugubris* (Clug_D12) (Haritos et al., 2012), revealed moderate sequence identities of 32-42% and sequence similarities of 46-58% (Supplementary **Figure S4**, Supplementary **Table S3**). All insect Δ12-FADs exhibit the typical sequence motifs of the "First" desaturase subfamily *sensu* Hashimoto et al. (Hashimoto et al., 2008). Members of this subfamily are supposed to introduce the first double bond in the saturated acyl chain of fatty acids and are characterised by three histidine boxes, the first of which has four amino acids between the histidines (Hashimoto et al., 2008). The phylogenetic analysis of Nvit_D12a and Nvit_D12b revealed that the hitherto characterised insect Δ12-FADs do not cluster together in one clade (**Figure 4)**. Rather, *N. vitripennis* Δ12-FADs cluster with other predicted, hitherto uncharacterised, FADs from the *Nasonia* genome. Likewise, Tcas_12 occurs in a clade with Δ9- and Δ5-FADs of the same species and Adom_D12 is closer related to Adom_D9 than to the other Δ12-FADs from insects. Finally, the two coleopteran Δ12-FADs Clug_D12 and Tcas_D12 are located in different clades with Clug_D12 clustering with two Δ9-FADs from *T. castaneum.*

## Discussion

Here we report the functional characterisation of two Δ12-FADs from *N. vitripennis.* Nvit_D12a and Nvit_D12b are the fourth and fifth functionally assigned Δ12-FADs in insects and yet the first in the Hymenoptera. The two Δ12-FADs may furnish the demand of C18:2$^{\Delta 9,12}$ in *N. vitripennis* for the numerous functions of C18:2$^{\Delta 9,12}$ including growth, reproduction, and brain development (Wathes et al., 2007). As an abundant constituent of phospholipids C18:2$^{\Delta 9,12}$ ensures the fluidity of cell membranes and the mobility of imbedded proteins (Cripps et al., 1986; Malcicka et al., 2018; Opekarova and Tanner, 2003). Furthermore, C18:2$^{\Delta 9,12}$ is involved in the

biosynthesis of prostaglandins and other eicosanoids, which have various hormone functions in insects (Stanley, 2006). Our data and previous work (Blaul et al., 2014; Wang et al., 2015) strongly suggest that, apart from these functions, Nvit_D12a is involved in the biosynthesis of the male sex pheromone. While Nvit_D12b is expressed in both males and females, Nvit_D12a is expressed in an extremely male-biased manner (Wang et al., 2015) and our mass spectrometric analyses revealed the presence of Nvit_D12a in the male pheromone gland. These results demonstrate that Nvit_D12a enables *N. vitripennis* males to convert $C18:1^{\Delta 9}$ to $C18:2^{\Delta 9,12}$ exactly where this crucial pheromone precursor is needed. This makes pheromone production in *N. vitripennis* males widely independent of $C18:2^{\Delta 9,12}$ derived from dietary or endosymbiotic sources even though a diet rich in $C18:2^{\Delta 9,12}$ may further increase male pheromone titres (Brandstetter and Ruther, 2016).

An important question arising from the present study is whether the ability to synthesise $C18:2^{\Delta 9,12}$ is restricted to *N. vitripennis* or is shared by related species or parasitic wasps in general. All *Nasonia* species, as well as *T. sarcophagae* share $C18:2^{\Delta 9,12}$-derived (4*R*,5*S*)-5-hydroxy-4-decanolide as the major pheromone component (Niehuis et al., 2013). Furthermore, the homologue of Nvit_D12a in *N. giraulti* is, like in *N. vitripennis*, expressed in a strongly male-biased manner (Wang et al., 2015). These findings suggest that homologues of Nvit_D12a have the same function in pheromone biosynthesis in these species. Our ^{13}C-labelling experiments revealed that females but not males of *U. rufipes* are capable of synthesising $C18:2^{\Delta 9,12}$. The abdominal sex pheromone of *U. rufipes* males is used in a similar manner as in *Nasonia* but is synthesised independently of $C18:2^{\Delta 9,12}$ via the mevalonate pathway (Ruther et al., 2019). Hence, Δ12-FADs appear to be common in species of the *Nasonia* group, and have acquired an additional function in the context of sexual communication in species using fatty acid derived male sex pheromones. This adaptation might have been achieved by male-biased gene expression and/or sequence-related modifications.

Eukaryotic FADs show the characteristic features of membrane-bound, oxygen-dependent di-iron-containing enzymes sharing three conserved histidine-rich motifs (histidine boxes) coordinating two iron ions in the active site (Shanklin et al., 1994; Tupec et al., 2017). A phylogenetic and motif analysis of 275 FAD sequences from 56 eukaryotic genomes revealed that these formed four functionally distinct

subfamilies, which can be distinguished by the sequences of the histidine boxes (Hashimoto et al., 2008). Enzymes of the "First" subfamily are supposed to introduce the first double bond, typically at position 9, into a saturated acyl chain, while members of the "Omega" subfamily introduce a double bond between an existing double bond and the acyl end. Hence, $\Delta$12-FADs can be expected to show the structural motifs of the "Omega" subfamily. While predicted $\Delta$12-FADs from the "Omega" subfamily have indeed been found in the genomes of whitefly and locust (Kabeya et al., 2018), Nvit_D12a and Nvit_12b as well as all other functionally characterised $\Delta$12-FADs from insects match the structural motifs of "First" desaturases. This suggests that the $\Delta$12-FADs from *N. vitripennis, T. castaneum, C. lugubris,* and *A. domesticus* have evolved independently from ancestral $\Delta$9-FADs of the respective genomes. This hypothesis is supported by our phylogenetic analysis showing that the insect $\Delta$12-FADs do not cluster together but are closer related to $\Delta$9-FADs and other "First"-type desaturases. Hence, apart from using classical "Omega"-type $\Delta$12-FADs, insects have developed an alternative route to synthesise $C18:2^{\Delta9,12}$ by modification of "First"-type desaturases.

The present study sheds light on the poorly understood selection pressures driving the evolution of $\Delta$12-activity in desaturases. Pheromone quantity is an important factor for the sexual attractiveness of *N. vitripennis* males. Virgin females prefer higher pheromone doses over lower ones and discriminate against pheromone markings of multiply mated, pheromone-depleted males (Ruther et al., 2009). Thus, the production of the pheromone precursor $C18:2^{\Delta9,12}$ was likely a key innovation for their reproductive success.

**Data availability**

Additional data and raw data not given in the main manuscript are accessible as supplementary information.

**Author contributions**

JE initiated the study. FS, NK, OM, RS, and JR designed the experiments. FS, NK, and MM performed the functional expression of the desaturases. JR and BB performed the fatty acid analyses and the $^{13}$C-labelling experiment with *U. rufipes*. AB performed the proteomics analyses. KS, RM and FS performed the phylogenetic analyses. JR and FS wrote the manuscript. All authors discussed the results and reviewed the manuscript.

**Supplementary information**

Supplementary information available online at …

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**Figures**
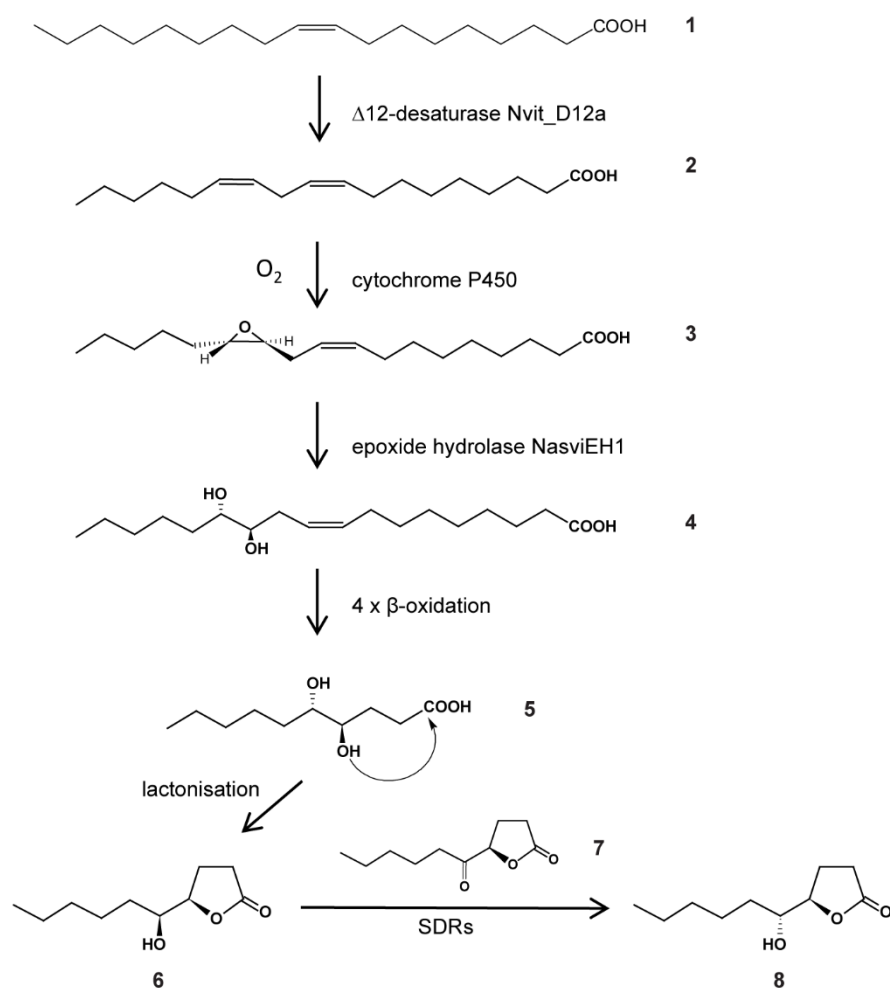


**Figure 1. Biosynthetic pathway of the *N. vitripennis* sex pheromone components.** Biosynthesis starts from oleic acid (**1**, C18:1$^{\Delta 9}$) (Blaul et al., 2011) which is converted by the Δ-12-desaturase Nvit_D12a to linoleic acid (**2**, C18:2$^{\Delta 9,12}$) (this study and Blaul et al., 2014). C18:2$^{\Delta 9,12}$ is epoxidised by a hitherto uncharacterised cytochrome P450 enzyme. The resulting 12,13-epoxy-octadec-9*Z*-enoic acid (**3**), is hydrolysed by the epoxide hydrolase NasviEH1 (Abdel-latief et al., 2008) to (12*R*,13*S*)-12,13-dihydroxy-octadec-9*Z*-enoic acid (**4**); chain shortening by four steps of β-oxidation leads to (4*R*,5*S*)-4,5-dihydroxydecanoic acid (**5**) which lactonises to (4*R*,5*S*)-5-hydroxy-4-decanolide (**6**). Epimerisation of (**6**) by the short chain dehydrogenases/reductases (SDRs) NV10127, NV10128 and NV10129 with (4*R*)-5-oxodecanolide (**7**) occurring as an intermediate, finally leads to (4*R*,5*R*)-5-hydroxy-4-decanolide (**8**) (Niehuis et al., 2013, Ruther et al., 2016).
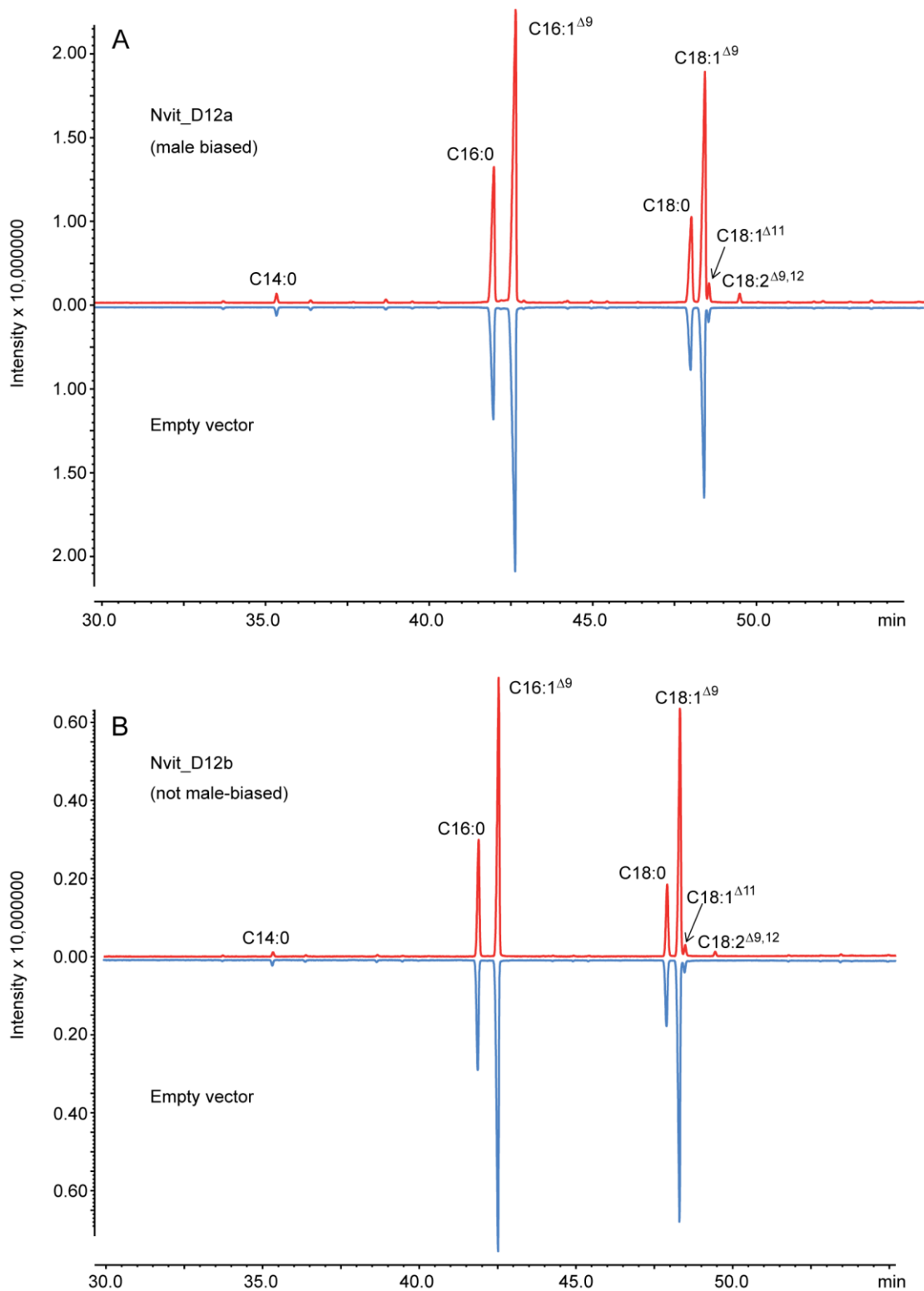
**Figure 2. Transformed yeast cells expressing Nvit_D12a or Nvit_12b synthesise C18:2$^{\Delta 9,12}$.** GC/MS analysis on a polar BP-20 column of FAMEs extracted from transformed *S. cerevisiae* cells supplemented with C18:1$^{\Delta 9}$. Cells were transformed with (A) Nvit_D12a and (B) Nvit_D12b. Cells carrying the empty vector were used as controls. The experiment was replicated three times.
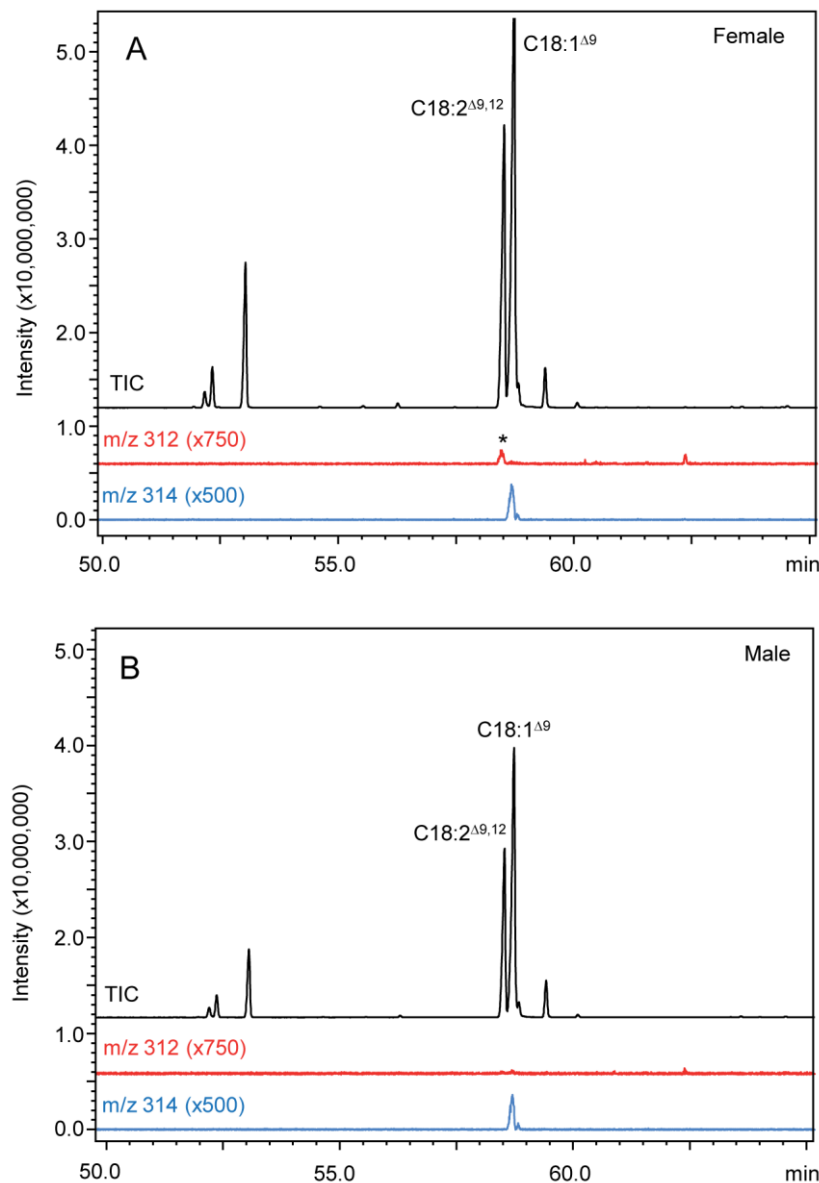
**Figure 3. Females but not males of *U. rufipes* are able to synthesise C18:2$^{\Delta 9,12}$.** GC/MS analysis on a non-polar BPX5 column of transesterified lipid extracts from three (A) female and (B) male *U. rufipes* wasps to which fully labelled $^{13}$C18:1$^{\Delta 9}$ had been applied. Shown are the total ion chromatograms (TIC) and extracted ion chromatograms (magnification factors given in brackets) of the molecular ions of fully labelled $^{13}$C18:1$^{\Delta 9}$ methyl ester (m/z 314) and $^{13}$C18:2$^{\Delta 9,12}$ methyl ester (m/z 312). Asterisk indicates peak of labelled $^{13}$C18:2$^{\Delta 9,12}$ methyl ester which is missing in males showing that females but not males are capable of synthesising C18:2$^{\Delta 9,12}$. The experiment was replicated four times for each sex.
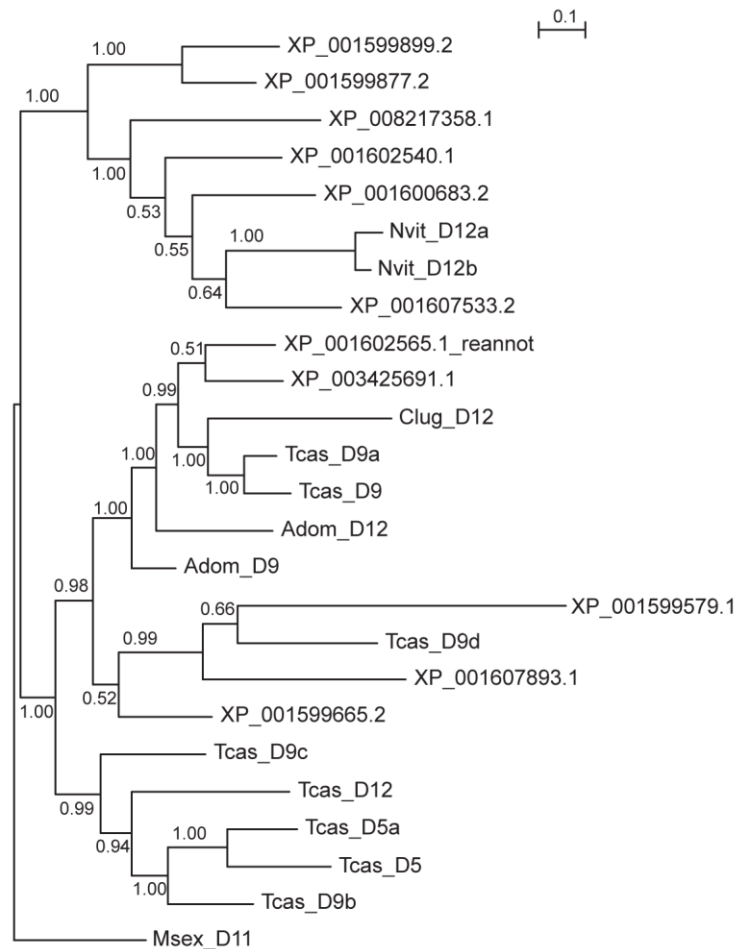
**Figure 4. Phylogenetic analysis of *N. vitripennis* Δ12-FADs.** The tree shows the relationship of Nvit_D12a and Nvit_D12b to other FADs. These are predicted FADs from the *N. vitripennis* genome (indicated by accession numbers starting with "XP_") (Blaul et al., 2014; Niehuis et al., 2011; Wang et al., 2015), insect Δ12-FADs from red flour beetle *T. castaneum* (Tcas_D12, Zhou et al., 2008), house cricket *A. domesticus* (Adom_D12, Zhou et al., 2008) and soldier beetle *C. lugubris* (Clug_D12, Haritos et al., 2012), as well as functionally characterised FADs from the same species (all accession numbers given in the Materials and Methods). Numbers following "D" indicate position of the introduced double bond. Δ11-FAD from tobacco hornworm *M. sexta* (Msex_D11) functioned as outgroup. Posterior probabilities are given at the branching points. The length of the bar corresponds to 0.1 mutations per site.

**Table 1.** Results of the LC/MS/MS analysis of Nvit_D12a in the pooled rectal vesicles from 10 *N. vitripennis* males after SDS-PAGE and *in-gel* trypsin digestion; protein database searching of the resulting mass spectra was performed using the Mascot search engine.

| GenBank accession no. | Mascot score | | Sequence coverage (%) | | Number of peptides | | emPAI value[a] | |
|---|---|---|---|---|---|---|---|---|
| | I[b] | II | I | II | I | II | I | II |
| XP_001599836.1 | 941.6 | 578.1 | 42.8 | 23.5 | 15 | 9 | 2.6 | 1.04 |

[a]exponentially modified protein abundance index; [b]roman numbers indicate the two individual replicates.

## List of Symbols and Abbreviations used

| | |
|---|---|
| $C18:1^{\Delta 9}$ | oleic acid |
| $C18:2^{\Delta 9,12}$ | linoleic acid |
| FAD | fatty acid desaturase |
| FAME | fatty acid methyl ester |
| GC/MS | gas chromatography/mass spectrometry |
| HRP | horseradish peroxidase |
| Leu | leucine |
| LC/MS/MS | liquid chromatography/tandem mass spectrometry |
| SDC | synthetic dextrose complete |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| SGC | synthetic galactose complete |
| TEV | Tobacco Etch Virus |

# Supplementary information

## Functional characterisation of two Δ12-desaturases demonstrates targeted production of linoleic acid as pheromone precursor in *Nasonia*

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**Figure 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.

Rep. 1

**Protein Report**
111 Carbamido Propionamid N vi SDR_2018-08-31 11:14:31

BRUKER

Protein 8: PREDICTED: stearoyl-CoA desaturase 5-like [Nasonia vitripennis]

| | | | |
|---|---|---|---|
| Accession: | gi\|156542104 | Score: | 941.6 |
| Database: | Nasonia_vit_SDR | Seq. Coverage [%]: | 42.8 |
| MW [kDa] / pI: | 42.1 / 6.6 | No. of Peptides: | 15 |
| Modification(s): | Carbamidomethyl, Oxidation, Deamidated | | |

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      10         20         30         40         50         60         70         80         90        100
MPPNEQIYEH ILPEAVEDPL KTLDIPGFDG YDEQYKDSEF NMSAEEYKEK VLSKPFLQRT DIKWGNLLFM VVMHAVALYG FLTYPYLEKK MTFVWGWFLA
     110        120        130        140        150        160        170        180        190        200
IVANFGVASG VHRLWSHRAF KAKLPFKIIS IICYLTSGQY SAIWWSRDHR LHHKFSETDA DPVNAARGFW FSHIGWLCMK RHPEVLKKAK QLDLSDIAND
     210        220        230        240        250        260        270        280        290        300
PVIKFEEKHF QVLRLMFTFI IPTLVPVYFW NESWYWAILS QCFMRYVYSL NCTWGINSLA HRFGGHPYDK NIEPSENFLM TLATGGEGWH NYHHAFPADY
     310        320        330        340        350        360        370
KASELGFNYI TNVNLTTCLI DLAAKIGWAY DLKEPSEKLL KAVIKNRGDG SHPISHETVK TE
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**MS/MS Peptide Matches**

| Cmpd. | m/z meas. | z | Δ m/z [ppm] | Δ m/z [Da] | Rt [min] | Score | No. of Cmpds. | Site [%] | P | Sequence | Modification | Range | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 39 | 777.7352 | 3 | 1.86 | 0.0014 | 70.28 | 57.85 | 2 | 0.0 | 0 | M.PPNEQIYEHILPEAVEDPLK.T | | 2-21 | CID |
| 18123 | 778.063 | 3 | 1.60 | 0.0012 | 72.79 | 29.59 | 1 | 0.703 | 0 | M.PPNEQIYEHILPEAVEDPLK.T | Deamidated: 5 | 2-21 | CID |
| 444 | 880.9054 | 2 | 1.22 | 0.0011 | 68.18 | 80.04 | 7 | 0.0 | 0 | K.TLDIPGFDGYDEQYK.D | | 22-36 | CID |
| 2142 | 1069.7927 | 3 | 0.39 | 0.0004 | 68.25 | 64.15 | 3 | 0.0 | 1 | K.TLDIPGFDGYDEQYKDSEFNMSAEEYK.E | Oxidation: 21 | 22-48 | CID |
| 245 | 733.2942 | 2 | 1.61 | 0.0012 | 39.77 | 83.71 | 4 | 0.0 | 0 | K.DSEFNMSAEEYK.E | Oxidation: 6 | 37-48 | CID |
| 16989 | 725.2966 | 2 | 1.43 | 0.0010 | 48.95 | 26.95 | 1 | 0.0 | 0 | K.DSEFNMSAEEYK.E | | 37-48 | CID |
| 4865 | 574.9116 | 3 | 2.28 | 0.0013 | 36.60 | 56.92 | 3 | 0.0 | 1 | K.DSEFNMSAEEYKEK.V | Oxidation: 6 | 37-50 | CID |
| 22351 | 861.1107 | 3 | 0.10 | 0.0001 | 111.88 | 66.61 | 4 | 0.0 | 0 | K.MTFVWGWFLAIVANFGVASGVHR.L | Oxidation: 1 | 91-113 | CID |

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**Protein Report**
111 Carbamido Propionamid N vi SDR_2018-08-31 11:14:31

BRUKER

| Cmpd. | m/z meas. | z | Δ m/z [ppm] | Δ m/z [Da] | Rt [min] | Score | No. of Cmpds. | Site [%] | P | Sequence | Modification | Range | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 39120 | 636.6509 | 3 | 1.20 | 0.0008 | 33.49 | 32.98 | 1 | 0.0 | 1 | R.LHHKFSETDADPVNAAR.G | | 151-167 | CID |
| 3050 | 697.3167 | 2 | 2.23 | 0.0016 | 39.82 | 92.47 | 7 | 0.0 | 0 | K.FSETDADPVNAAR.G | Deamidated: 10 | 155-167 | CID |
| 594 | 696.8246 | 2 | 2.10 | 0.0015 | 41.48 | 109.43 | 8 | 0.0 | 0 | K.FSETDADPVNAAR.G | | 155-167 | CID |
| 17480 | 562.2653 | 3 | 2.99 | 0.0017 | 78.09 | 27.79 | 1 | 0.0 | 0 | R.GFWFSHIGWLCMK.R | Carbamidomethyl: 11; Oxidation: 12 | 168-180 | CID |
| 78 | 770.9162 | 2 | 2.21 | 0.0017 | 67.22 | 107.44 | 3 | 0.0 | 0 | K.QLDLSDIANDPVIK.F | | 191-204 | CID |
| 3388 | 891.409 | 4 | 0.59 | 0.0005 | 72.16 | 62.22 | 1 | 0.0 | 0 | K.NIEPSENFLMTLATGGEGWHNYHHAFPADYK.A | Oxidation: 10 | 271-301 | CID |
| 428 | 483.2608 | 2 | 5.41 | 0.0026 | 59.41 | 43.41 | 6 | 0.0 | 0 | K.IGWAYDLK.E | | 326-333 | CID |

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Rep. 2

**Protein Report**
Compi_Nasvi_SDR_301117_2017-11-30 13:15:54

BRUKER

Protein 242: PREDICTED: stearoyl-CoA desaturase 5-like [Nasonia vitripennis]

| | | | |
|---|---|---|---|
| Accession: | gi\|156542104 | Score: | 578.1 |
| Database: | Nasonia_vit_SDR | Seq. Coverage [%]: | 23.5 |
| MW [kDa] / pI: | 42.1 / 6.6 | No. of Peptides: | 9 |
| Modification(s): | Oxidation, Deamidated | | |

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MPPNEQIYEH ILPEAVEDPL KTLDIPGFDG YDEQYKDSEF NMSAEEYKEK VLSKPFLQRT DIKWGNLLFM VVMHAVALYG FLTYPYLEKK MTFVWGWFLA
     110        120        130        140        150        160        170        180        190        200
IVANFGVASG VHRLWSHRAF KAKLPFKIIS IICYLTSGQY SAIWWSRDHR LHHKFSETDA DPVNAARGFW FSHIGWLCMK RHPEVLKKAK QLDLSDIAND
     210        220        230        240        250        260        270        280        290        300
PVIKFEEKHF QVLRLMFTFI IPTLVPVYFW NESWYWAILS QCFMRYVYSL NCTWGINSLA HRFGGHPYDK NIEPSENFLM TLATGGEGWH NYHHAFPADY
     310        320        330        340        350        360        370
KASELGFNYI TNVNLTTCLI DLAAKIGWAY DLKEPSEKLL KAVIKNRGDG SHPISHETVK TE
```

**MS/MS Peptide Matches**

| Cmpd. | m/z meas. | z | Δ m/z [ppm] | Δ m/z [Da] | Rt [min] | Score | No. of Cmpds. | Site [%] | P | Sequence | Modification | Range | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9931 | 777.7329 | 3 | -1.10 | -0.0009 | 49.53 | 42.35 | 3 | 0.0 | 0 | M.PPNEQIYEHILPEAVEDPLK.T | | 2-21 | CID |
| 9986 | 880.9031 | 2 | -1.39 | -0.0012 | 49.66 | 58.99 | 3 | 0.0 | 0 | K.TLDIPGFDGYDEQYK.D | | 22-36 | CID |
| 9662 | 1069.7912 | 3 | -1.01 | -0.0011 | 48.96 | 78.11 | 1 | 0.0 | 1 | K.TLDIPGFDGYDEQYKDSEFNMSAEEYK.E | Oxidation: 21 | 22-48 | CID |
| 1779 | 733.292 | 2 | -1.39 | -0.0010 | 32.26 | 58.12 | 2 | 0.0 | 0 | K.DSEFNMSAEEYK.E | Oxidation: 6 | 37-48 | CID |
| 30511 | 861.8598 | 2 | -2.32 | -0.0020 | 25.17 | 40.08 | 3 | 0.0 | 1 | K.DSEFNMSAEEYKEK.V | Oxidation: 6 | 37-50 | CID |
| 5391 | 544.334 | 2 | -1.39 | -0.0008 | 26.31 | 57.5 | 2 | 0.0 | 0 | K.VLSKPFLQR.T | | 51-59 | CID |
| 21799 | 697.3132 | 2 | -2.79 | -0.0019 | 28.98 | 34.95 | 1 | 0.0 | 0 | K.FSETDADPVNAAR.G | Deamidated: 10 | 155-167 | CID |
| 1489 | 696.8222 | 2 | -1.35 | -0.0009 | 29.35 | 96.11 | 10 | 0.0 | 0 | K.FSETDADPVNAAR.G | | 155-167 | CID |
| 9728 | 770.9131 | 2 | -1.81 | -0.0014 | 49.12 | 111.88 | 3 | 0.0 | 0 | K.QLDLSDIANDPVIK.F | | 191-204 | CID |

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**Figure 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)


MPPNEQIHEHVLPEAVEDPLKTLDIPGFNDYDEEHKDSEFNMSAEEYNEKVINKPFLQRT
MPPNEQIYEHILPEAVEDPLKTLDIPGFDGYDEQYKDSEFNMSAEEYKEKVLSKPFLQRT
******:**:****************:.***::*************:***:.*******

DIKWGNLYFMVVLHAIALYGFLTYPYFEKKMTFVWGWFLAIVANFGVAGGVHRLWSHRAF
DIKWGNLLFMVVMHAVALYGFLTYPYLEKKMTFVWGWFLAIVANFGVASGVHRLWSHRAF
*******  ****:**:*********:.****:*********************.**********

KAKVPLKIIFILSYLTSGQYSAIWWARDHRIHHKFSETDADPVNAARGFWFSHVGWLCMK
KAKLPFKIISIICYLTSGQYSAIWWSRDHRLHHKFSETDADPVNAARGFWFSHIGWLCMK
***:*:*** *:.************:.****:.***********************.******

RHPEVLKKAKQLDLSDILNDPVIKFEEKHFQVLRLIFAFIIPTLVPVYFWNESWYWAILS
RHPEVLKKAKQLDLSDIANDPVIKFEEKHFQVLRLMFTFIIPTLVPVYFWNESWYWAILS
****************** *****************.*.:***************

QCFMRYAYSLNCTWGINSFAHMFGNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPADY
QCFMRYVYSLNCTWGINSLAHRFGGHPYDKNIEPSENFLMTLATGGEGWHNYHHAFPADY
******.************:** **.*****:***:***:*************.*****

KASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHPISHEPVK
KASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHPISHETVK
**************************************************************.**

TK
TE
*:
```

**Figure S3. Sequence alignment of the two Δ12-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 Δ12-desaturases).

```
                                                                    60
                                                                    |
Adom_D12    MDLNEE-----------------SAPSGVLFEEDVAEQEAKMANGG-PKKGKKLEEPYRL
Clug_D12    MAPNAR-----------------DS-TGVLSEED-----AEITPEQ-IEEVHKTSKNRHL
Tcas_D12    MSAQT-------------------------------ITTTE-TTQNAQKPQQYHW
Nvit_D12a   MPPNEQIHEHVLPEAVEDPLKTLDIPGFNDYDEEHKDSEFNMSAEEYNEKVINKPFLQRT
Nvit_D12b   MPPNEQIYEHILPEAVEDPLKTLDIPGFDGYDEQYKDSEFNMSAEEYKEKVLSKPFLQRT
                *

                                                                    120
                                                                    |
Adom_D12    EIVWFNVLWFVLLHAGALYGVYLIFASAKIYTTLYGFLLCELSLLSITAGVHRLWAHRAY
Clug_D12    PLVWRDVIKFAVLHALGFYGFYLMFTSARIWTSVLVFINYHLGILGITAGAHRLWAHKAY
Tcas_D12    RMVWRNIILYIIMHLTGFYGLYLAMFYAQWKTVFYSWFLLVIALQGVTAGSHRLWAHKAY
Nvit_D12a   DIKWGNLYFMVVLHAIALYGFLTYPYFEKKMTFVWGWFLAIVANFGVAGGVHRLWSHRAF
Nvit_D12b   DIKWGNLLFMVVMHAVALYGFLTYPYLEKKMTFVWGWFLAIVANFGVASGVHRLWSHRAF
                *         *     **         *              * **** * * *

                                                                    180
                                                                    |
Adom_D12    KAKWPLRLTLMVLNLLAYQNSIYEWARDHRVHHKFSETNADPVNAKRGFFFSHVGWLLCR
Clug_D12    KARWPLKLFLAYIQTLAFQFDIIFWAKYHRVHHKYTETDADPHNAKRGFFYSHMGWTMVK
Tcas_D12    KARLPLRMLLCIFQTLSLQNHIYDWATYHRVHHKFVDTNADPHNSRRGFFFSHMGWLFIE
Nvit_D12a   KAKVPLKIIFILSYLTSGQYSAIWWARDHRIHHKFSETDADPVNAARGFWFSHVGWLCMK
Nvit_D12b   KAKLPFKIISIICYLTSGQYSAIWWSRDHRLHHKFSETDADPVNAARGFWFSHIGWLCMK
            **  *          *       *   ** ***   * *** *   *** ** **

                                                                    240
                                                                    |
Adom_D12    KHPEVRAKGGRIDLSDLERDPIVMFQKRHYYK-LVPFVSFVIPTLIPMYFWGETLSNSWY
Clug_D12    KNPEFETRCNEIDLSDLYSDPIVNFQYKYYYQ-ILFVVFLVVPTFIPMYFWNETFVNA-F
Tcas_D12    PHKDVEDKYKSIDFSDLHADSVVMIQKKYYHTFFAPVIGFYLPAAIPWYFWGENFWTAFF
Nvit_D12a   RHPEVLKKAKQLDLSDILNDPVIKFEEKHFQV-LRLIFAFIIPTLVPVYFWNESWYWAIL
Nvit_D12b   RHPEVLKKAKQLDLSDIANDPVIKFEEKHFQV-LRLMFTFIIPTLVPVYFWNESWYWAIL
                      * **     *              *   * *** *

                                                                    300
                                                                    |
Adom_D12    VSTMFRYCLSLNLTWLVNSAAHMWGNKPYDKNINPVENLAVAIGSLGEGWHNFHHVFPWD
Clug_D12    CLNLSRYLLSLHCTWLVNSAAHLYGNKPYDKSLYSSENFWVTILVNGEGWHNYHHAFPWD
Tcas_D12    VATMLRYCACTNITFLVNSWAHIYGSRPYDKNIYPTESATIAVLTGGEGWHNYHHTFPWD
Nvit_D12a   SQCFMRYAYSLNCTWGINSFAHMFGNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPAD
Nvit_D12b   SQCFMRYVYSLNCTWGINSLAHRFGGHPYDKNIEPSENFLMTLATGGEGWHNYHHAFPAD
                  **         *    ** **   *  ****       *      ****** ** ** *
                                                                    360
                                                                    |
Adom_D12    YKTSELG---NYSLNFTNAFIDLAVLLGLAYDLKTVPVSMIKTRVGRTGDGSHD------
Clug_D12    YKASELG---IYSTNMTAFFIDAMAKLGLAYDLKSVAPEVVKRRVERTGDGSHP------
Tcas_D12    YKTGEFG---KYRSNLTTGFLDFMAAIGWAYDLKTVSEEMIMKRVLRTGDGTRKFDKIDK
Nvit_D12a   YKASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHP------
Nvit_D12b   YKASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHP------
            **  * *          * *      *       * *****            ***

Adom_D12    -------------VWGWGDKDLPKELADQTMIENR--KTE
Clug_D12    -------------IWGWGDKDQSEEDKRSALISHK--DT-
Tcas_D12    ILNVDDDHHHEDMLWGWGDSDMAKEEMNYVKIHNR--KED
Nvit_D12a   -----------------------------ISHEPVKTK
Nvit_D12b   -----------------------------ISHETVKTE
                                            *
```

**Figure S4. Multiple sequence alignment of functionally characterised insect Δ12-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 *Bsa*I are underlined and restriction sites are bold.

---

## Nucleotide sequences

### *Nvit_D12a* (XP_001599836.1)

5'AAAAAA<u>GGTCTCA</u>**CATG**CCACCAAACGAACAAATCCATGAACATGTTTTGCCAGAAGCTGTT
GAGGATCCATTGAAAACTTTGGATATTCCAGGTTTCAACGACTACGATGAAGAACATAAGGAC
TCCGAATTCAACATGTCTGCTGAAGAGTACAACGAAAAGGTTATCAACAAGCCATTCTTGCAA
AGAACCGATATTAAGTGGGGTAACTTGTACTTCATGGTTGTCTTGCATGCTATTGCCTTGTAC
GGTTTTTTGACTTACCCATACTTCGAGAAGAAGATGACTTTTGTTTGGGGTTGGTTTTTGGCTA
TCGTTGCTAATTTTGGTGTTGCTGGTGGTGTTCATAGATTGTGGTCACATAGAGCTTTTAAGG
CTAAGGTTCCACTGAAGATCATTTTCATCTTGTCCTACTTGACCTCCGGTCAATATTCTGCTAT
TTGGTGGGCTAGAGATCATAGAATCCATCACAAGTTCTCTGAAACTGATGCTGATCCAGTTAA
TGCTGCTAGAGGTTTTTGGTTTTCTCATGTTGGTTGGTTGTGCATGAAGAGACATCCAGAAGT
TTTGAAGAAGGCCAAGCAATTGGATTTGTCCGATATTTTGAATGACCCCGTCATCAAGTTTGA
AGAAAAGCACTTTCAAGTCCTGAGATTGATTTTCGCCTTCATCATTCCAACTTTGGTTCCAGTT
TACTTCTGGAACGAATCTTGGTATTGGGCCATTTTGTCTCAATGCTTTATGAGATACGCCTACT
CTTTGAACTGTACCTGGGGTATTAACTCTTTCGCTCATATGTTTGGTAACCATCCATACGATAA
GCACATTGAACCAGCCGAAAACTTCATTATGACTTTGGCTACTGGTGGTGAAGGTTGGCATAA
TTACCATCATGTTTTTTCCAGCTGATTACAAGGCTTCTGAATTGGGTTTCAACTACATCACCAAC
GTTAACTTGACCACCTGTTTGATTGATTTGGCTGCTAAAATTGGTTGGGCCTACGATTTGAAA
GAACCATCTGAAAAGTTGTTGAAGGCCGTGATTAAGAATAGAGGTGATGGTTCTCATCCAATC
TCTCATGAACCAGTTAAGACCAAG**TAAG**A<u>GAGACC</u>AAAAAA3'

### *Nvit_D12b* (XP_001599873.1)

5'AAAAAA<u>GGTCTCA</u>**CATG**CCACCAAACGAACAAATCTACGAACATATTTTGCCAGAAGCCGTT
GAAGATCCATTGAAAACTTTGGATATTCCAGGTTTCGATGGTTACGATGAACAGTACAAAGAC
TCCGAATTCAACATGTCTGCTGAAGAGTACAAAGAGAAGGTTTTGTCTAAGCCATTCTTGCAA
AGAACCGATATTAAGTGGGGTAACTTGTTGTTCATGGTTGTTATGCATGCTGTTGCCTTGTAC
GGTTTTTTGACTTATCCATACTTGGAGAAGAAGATGACTTTTGTTTGGGGTTGGTTTTTGGCTA
TCGTTGCTAATTTTGGTGTTGCCTCTGGTGTTCATAGATTGTGGTCACATAGAGCTTTTAAGG
CTAAGTTGCCATTCAAGATCATCTCCATTATTTGCTACTTGACCTCCGGTCAATATTCTGCTAT
TTGGTGGTCTAGAGATCACAGATTGCATCACAAATTCTCTGAAACTGATGCCGATCCAGTTAA
TGCTGCTAGAGGTTTTTGGTTCTCTCATATTGGTTGGTTGTGCATGAAGAGACATCCAGAAGT
TTTGAAGAAGGCCAAGCAATTGGATTTGTCCGATATTGCTAATGACCCAGTCATCAAGTTTGA
AGAAAAGCACTTCCAAGTCCTGAGATTGATGTTCACCTTCATTATCCCAACTTTGGTTCCAGTT
TACTTCTGGAATGAATCTTGGTACTGGGCCATTTTGTCTCAATGTTTCATGAGATACGTCTACT
CATTGAACTGTACCTGGGGTATTAACTCTTTGGCACATAGATTCGGTGGTCATCCATACGATA
AGAACATTGAACCATCCGAGAATTTCTTGATGACATTGGCTACTGGTGGTGAAGGTTGGCATA
ATTATCATCATGCTTTTCCAGCTGACTACAAGGCTTCTGAATTGGGTTTTAACTACATCACCAA
CGTTAACTTGACCACCTGTTTGATTGATTTGGCTGCTAAAATTGGTTGGGCCTACGATTTGAA
AGAACCCTCTGAAAAGTTGTTGAAGGCCGTTATTAAGAACAGAGGTGATGGTTCTCATCCAAT
TTCTCACGAAACTGTTAAGACCGAG**TAAG**A<u>GAGACC</u>AAAAAA 3'

## Amino acid sequences

### Nvit_D12a (XP_001599836.1)

MPPNEQIHEHVLPEAVEDPLKTLDIPGFNDYDEEHKDSEFNMSAEEYNEKVINKPFLQRTDIKWG
NLYFMVVLHAIALYGFLTYPYFEKKMTFVWGWFLAIVANFGVAGGVHRLWSHRAFKAKVPLKIIFIL
SYLTSGQYSAIWWARDHRIHHKFSETDADPVNAARGFWFSHVGWLCMKRHPEVLKKAKQLDLS
DILNDPVIKFEEKHFQVLRLIFAFIIPTLVPVYFWNESWYWAILSQCFMRYAYSLNCTWGINSFAHM
FGNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPADYKASELGFNYITNVNLTTCLIDLAAKIGW
AYDLKEPSEKLLKAVIKNRGDGSHPISHEPVKTK*

### Nvit_D12b (XP_001599873.1)

MPPNEQIYEHILPEAVEDPLKTLDIPGFDGYDEQYKDSEFNMSAEEYKEKVLSKPFLQRTDIKWGN
LLFMVVMHAVALYGFLTYPYLEKKMTFVWGWFLAIVANFGVASGVHRLWSHRAFKAKLPFKIISIIC
YLTSGQYSAIWWSRDHRLHHKFSETDADPVNAARGFWFSHIGWLCMKRHPEVLKKAKQLDLSDI
ANDPVIKFEEKHFQVLRLMFTFIIPTLVPVYFWNESWYWAILSQCFMRYVYSLNCTWGINSLAHRF
GGHPYDKNIEPSENFLMTLATGGEGWHNYHHAFPADYKASELGFNYITNVNLTTCLIDLAAKIGW
AYDLKEPSEKLLKAVIKNRGDGSHPISHETVKTE*

**Table S2. Conversion of externally supplied fully labelled $^{13}C18:1^{\Delta 9}$ into $^{13}C18:2^{\Delta 9,12}$ by *Urolepis rufipes*.** Single 2-d-old wasps (n = 12 for each sex) were treated with an acetone solution of $^{13}C18:1^{\Delta 9}$. 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 $^{13}C18:1^{\Delta 9}$ to $^{13}C18:2^{\Delta 9,12}$ 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 Δ12-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.
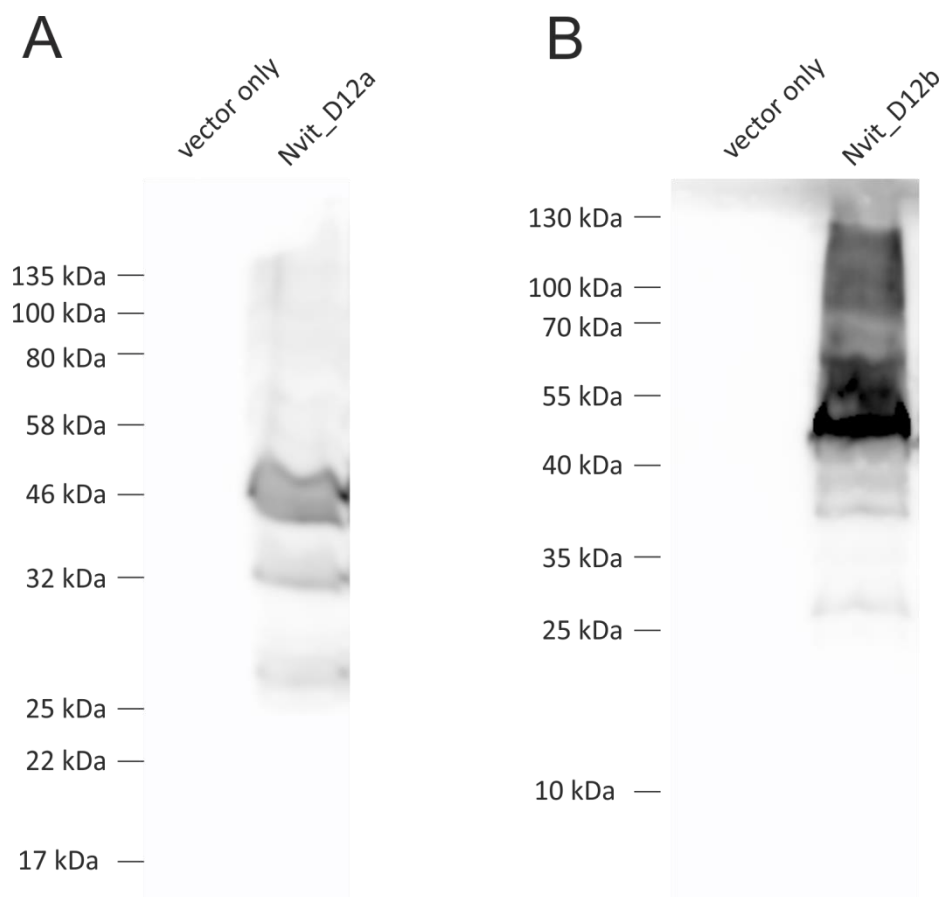
**Fig. S1. Western blot analysis of transformed *S. cerevisiae* cells.** Protein extracts of (**A**) Nvit_D12a ($M_W$ = 46610 Da) and (**B**) Nvit_D12b ($M_W$ = 465247 Da) and empty vector controls were separated on 13% SDS-PAGE gels and blotted on PVDF membranes. (FLAG)$_3$-tagged proteins were visualised by chemiluminescence.

**Rep. 1**

**Protein Report**
111 Carbamido Propionamid N vi SDR_2018-08-31 11:14:31

BRUKER

Protein 8: PREDICTED: stearoyl-CoA desaturase 5-like [Nasonia vitripennis]

| | | | |
|---|---|---|---|
| Accession: | gi\|156542104 | Score: | 941.6 |
| Database: | Nasonia_vit_SDR | Seq. Coverage [%]: | 42.8 |
| MW [kDa] / pl: | 42.1 / 6.6 | No. of Peptides: | 15 |
| Modification(s): | Carbamidomethyl, Oxidation, Deamidated | | |

```
        10            20          30           40          50         60           70          80          90          100
MPPNEQIYEH  ILPEAVEDPL  KTLDIPGFDG  YDEQYKDSEF  NMSAEEYKEK  VLSKPFLQRT  DIKWGNLLFM  VVMHAVALYG  FLTYPYLEKK  MTFVWGWFLA

       110           120         130          140         150        160          170         180         190         200
IVANFGVASG  VHRLWSHRAF  KAKLPFKIIS  IICYLTSGQY  SAIWWSRDHR  LHHKFSETDA  DPVNAARGFW  FSHIGWLCMK  RHPEVLKKAK  QLDLSDIAND

       210           220         230          240         250        260          270         280         290         300
PVIKFEEKHF  QVLRLMFTFI  IPTLVPVYFW  NESWYWAILS  QCFMRYVYSL  NCTWGINSLA  HRFGGHPYDK  NIEPSENFLM  TLATGGEGWH  NYHHAFPADY

       310           320         330          340         350        360          370
KASELGFNYI  TNVNLTTCLI  DLAAKIGWAY  DLKEPSEKLL  KAVIKNRGDG  SHPISHETVK  TE
```

**MS/MS Peptide Matches**

| Cmpd. | m/z meas. | z | Δ m/z [ppm] | Δ m/z [Da] | Rt [min] | Score | No. of Cmpds. | Site [%] | P | Sequence | Modification | Range | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 39 | 777.7352 | 3 | 1.86 | 0.0014 | 70.28 | 57.85 | 2 | 0.0 | 0 | M.PPNEQIYEHILPEAVEDPLK.T | | 2-21 | CID |
| 18123 | 778.063 | 3 | 1.60 | 0.0012 | 72.79 | 29.59 | 1 | 0.703 | 0 | M.PPNEQIYEHILPEAVEDPLK.T | Deamidated: 5 | 2-21 | CID |
| 444 | 880.9054 | 2 | 1.22 | 0.0011 | 68.18 | 80.04 | 7 | 0.0 | 0 | K.TLDIPGFDGYDEQYK.D | | 22-36 | CID |
| 2142 | 1069.7927 | 3 | 0.39 | 0.0004 | 68.25 | 64.15 | 3 | 0.0 | 1 | K.TLDIPGFDGYDEQYKDSEFNMSAEEYK.E | Oxidation: 21 | 22-48 | CID |
| 245 | 733.2942 | 2 | 1.61 | 0.0012 | 39.77 | 83.71 | 4 | 0.0 | 0 | K.DSEFNMSAEEYK.E | Oxidation: 6 | 37-48 | CID |
| 16989 | 725.2966 | 2 | 1.43 | 0.0010 | 48.95 | 26.95 | 1 | 0.0 | 0 | K.DSEFNMSAEEYK.E | | 37-48 | CID |
| 4865 | 574.9116 | 3 | 2.28 | 0.0013 | 36.60 | 56.92 | 3 | 0.0 | 1 | K.DSEFNMSAEEYKEK.V | Oxidation: 6 | 37-50 | CID |
| 22351 | 861.1107 | 3 | 0.10 | 0.0001 | 111.88 | 66.61 | 4 | 0.0 | 0 | K.MTFVWGWFLAIVANFGVASGVHR.L | Oxidation: 1 | 91-113 | CID |

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**Protein Report**
111 Carbamido Propionamid N vi SDR_2018-08-31 11:14:31

BRUKER

| Cmpd. | m/z meas. | z | Δ m/z [ppm] | Δ m/z [Da] | Rt [min] | Score | No. of Cmpds. | Site [%] | P | Sequence | Modification | Range | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 39120 | 636.6509 | 3 | 1.20 | 0.0008 | 33.49 | 32.98 | 1 | 0.0 | 1 | R.LHHKFSETDADPVNAAR.G | | 151-167 | CID |
| 3050 | 697.3167 | 2 | 2.23 | 0.0016 | 39.82 | 92.47 | 7 | 0.0 | 0 | K.FSETDADPVNAAR.G | Deamidated: 10 | 155-167 | CID |
| 594 | 696.8246 | 2 | 2.10 | 0.0015 | 41.48 | 109.43 | 8 | 0.0 | 0 | K.FSETDADPVNAAR.G | | 155-167 | CID |
| 17480 | 562.2653 | 3 | 2.99 | 0.0017 | 78.09 | 27.79 | 1 | 0.0 | 0 | R.GFWFSHIGWLCMK.R | Carbamidomethyl: 11; Oxidation: 12 | 168-180 | CID |
| 78 | 770.9162 | 2 | 2.21 | 0.0017 | 67.22 | 107.44 | 3 | 0.0 | 0 | K.QLDLSDIANDPVIK.F | | 191-204 | CID |
| 3388 | 891.409 | 4 | 0.59 | 0.0005 | 72.16 | 62.22 | 1 | 0.0 | 0 | K.NIEPSENFLMTLATGGEGWHNYHHAFPADYK.A | Oxidation: 10 | 271-301 | CID |
| 428 | 483.2608 | 2 | 5.41 | 0.0026 | 59.41 | 43.41 | 6 | 0.0 | 0 | K.IGWAYDLK.E | | 326-333 | CID |

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**Rep. 2**

**Protein Report**
Compi_Nasvi_SDR_301117_2017-11-30 13:15:54

BRUKER

Protein 242: PREDICTED: stearoyl-CoA desaturase 5-like [Nasonia vitripennis]

| | | | |
|---|---|---|---|
| Accession: | gi\|156542104 | Score: | 578.1 |
| Database: | Nasonia_vit_SDR | Seq. Coverage [%]: | 23.5 |
| MW [kDa] / pl: | 42.1 / 6.6 | No. of Peptides: | 9 |
| Modification(s): | Oxidation, Deamidated | | |

```
        10            20          30           40          50         60           70          80          90          100
MPPNEQIYEH  ILPEAVEDPL  KTLDIPGFDG  YDEQYKDSEF  NMSAEEYKEK  VLSKPFLQRT  DIKWGNLLFM  VVMHAVALYG  FLTYPYLEKK  MTFVWGWFLA

       110           120         130          140         150        160          170         180         190         200
IVANFGVASG  VHRLWSHRAF  KAKLPFKIIS  IICYLTSGQY  SAIWWSRDHR  LHHKFSETDA  DPVNAARGFW  FSHIGWLCMK  RHPEVLKKAK  QLDLSDIAND

       210           220         230          240         250        260          270         280         290         300
PVIKFEEKHF  QVLRLMFTFI  IPTLVPVYFW  NESWYWAILS  QCFMRYVYSL  NCTWGINSLA  HRFGGHPYDK  NIEPSENFLM  TLATGGEGWH  NYHHAFPADY

       310           320         330          340         350        360          370
KASELGFNYI  TNVNLTTCLI  DLAAKIGWAY  DLKEPSEKLL  KAVIKNRGDG  SHPISHETVK  TE
```

**MS/MS Peptide Matches**

| Cmpd. | m/z meas. | z | Δ m/z [ppm] | Δ m/z [Da] | Rt [min] | Score | No. of Cmpds. | Site [%] | P | Sequence | Modification | Range | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9931 | 777.7329 | 3 | -1.10 | -0.0009 | 49.53 | 42.35 | 3 | 0.0 | 0 | M.PPNEQIYEHILPEAVEDPLK.T | | 2-21 | CID |
| 9986 | 880.9031 | 2 | -1.39 | -0.0012 | 49.66 | 58.99 | 3 | 0.0 | 0 | K.TLDIPGFDGYDEQYK.D | | 22-36 | CID |
| 9662 | 1069.7912 | 3 | -1.01 | -0.0011 | 48.96 | 78.11 | 1 | 0.0 | 1 | K.TLDIPGFDGYDEQYKDSEFNMSAEEYK.E | Oxidation: 21 | 22-48 | CID |
| 1779 | 733.292 | 2 | -1.39 | -0.0010 | 32.26 | 58.12 | 2 | 0.0 | 0 | K.DSEFNMSAEEYK.E | Oxidation: 6 | 37-48 | CID |
| 30511 | 861.8598 | 2 | -2.32 | -0.0020 | 25.17 | 40.08 | 3 | 0.0 | 1 | K.DSEFNMSAEEYKEK.V | Oxidation: 6 | 37-50 | CID |
| 5391 | 544.334 | 2 | -1.39 | -0.0008 | 26.31 | 57.5 | 2 | 0.0 | 0 | K.VLSKPFLQR.T | | 51-59 | CID |
| 21799 | 697.3132 | 2 | -2.79 | -0.0019 | 28.98 | 34.95 | 1 | 0.0 | 0 | K.FSETDADPVNAAR.G | Deamidated: 10 | 155-167 | CID |
| 1489 | 696.8222 | 2 | -1.35 | -0.0009 | 29.35 | 96.11 | 10 | 0.0 | 0 | K.FSETDADPVNAAR.G | | 155-167 | CID |
| 9728 | 770.9131 | 2 | -1.81 | -0.0014 | 49.12 | 111.88 | 3 | 0.0 | 0 | K.QLDLSDIANDPVIK.F | | 191-204 | CID |

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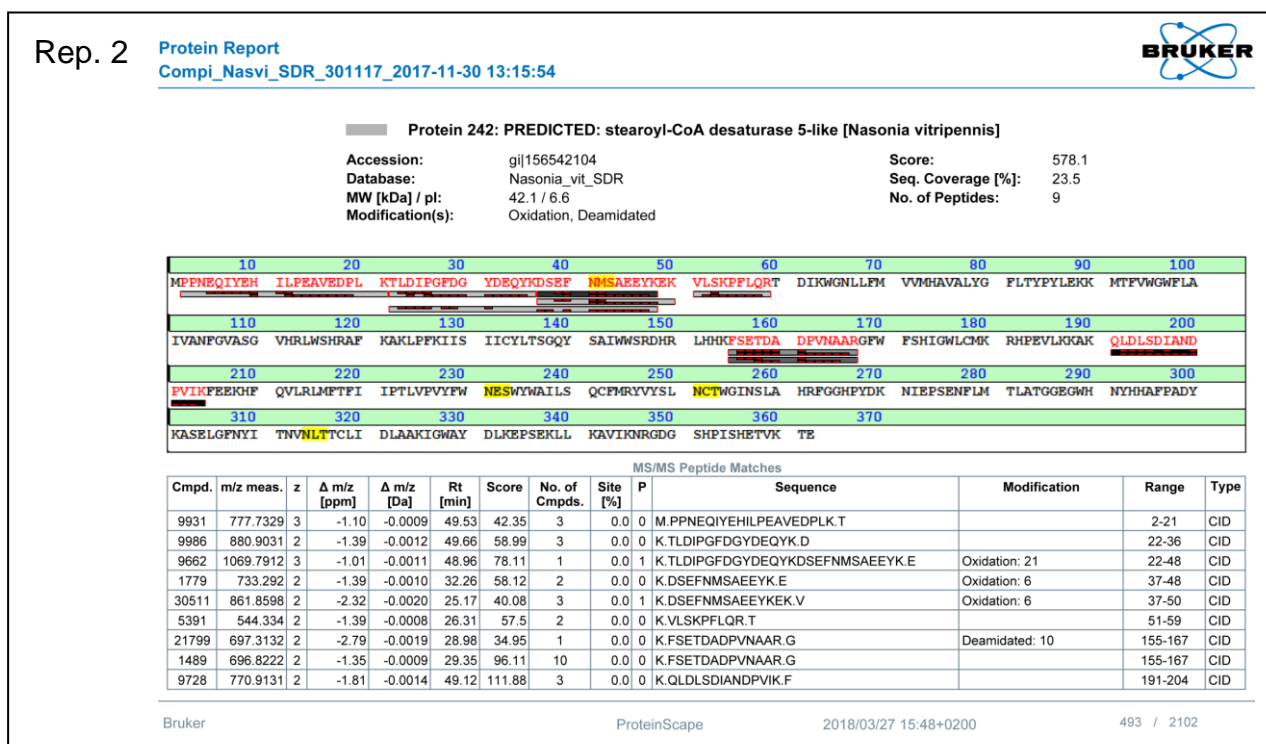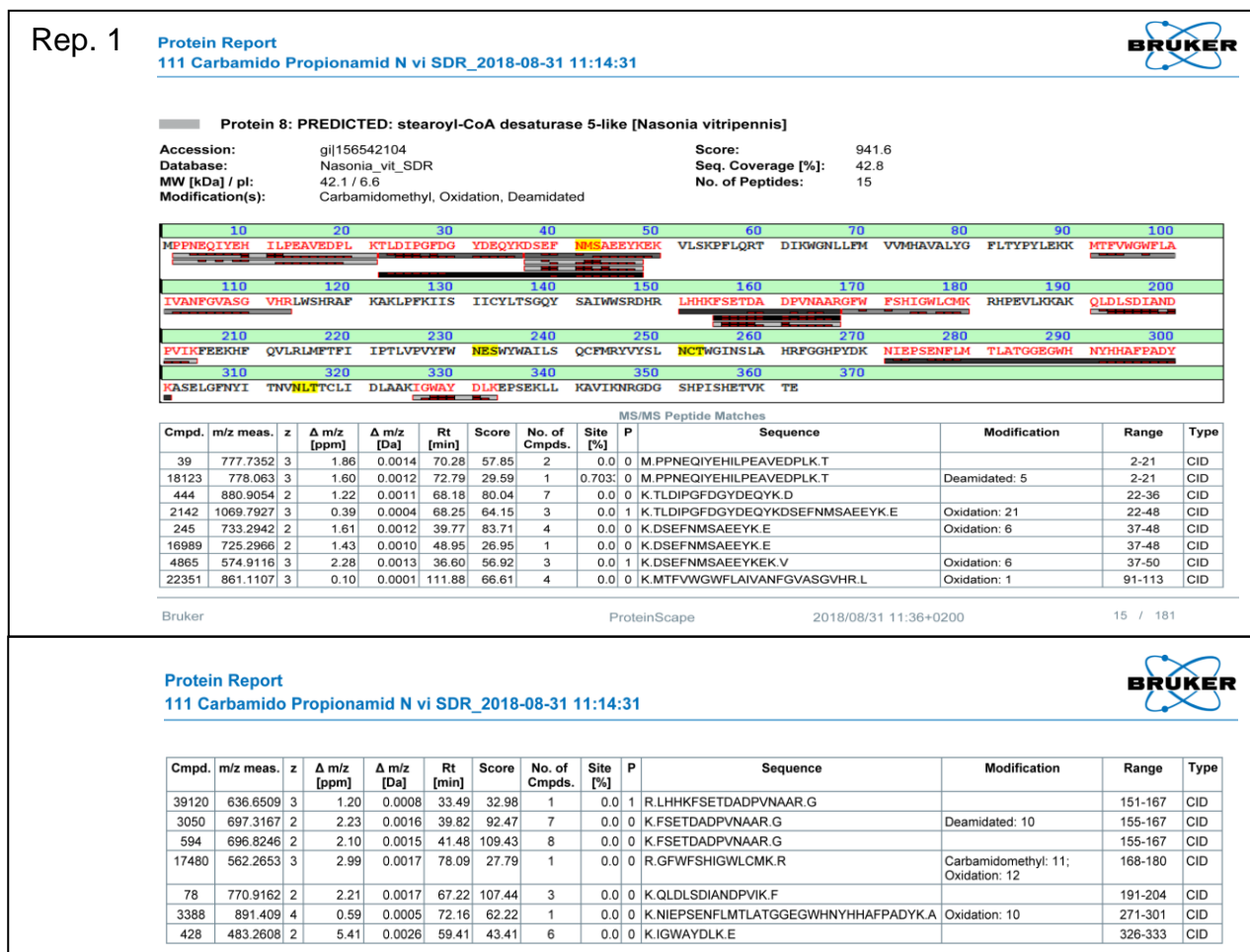**Fig. S2. Results the two replicates (Rep. 1 and Rep. 2) of the mass spectrometric analysis of the Δ12-desaturase Nvit_D12a in the rectal vesicles of male *N. vitripennis*.** Ten rectal vesicles of 1–2 day old wasps were pooled per replicate and subjected to SDS-PAGE. *In-gel* trypsin digested proteins 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)

MPPNEQIHEHVLPEAVEDPLKTLDIPGFNDYDEEHKDSEFNMSAEEYNEKVINKPFLQRT
MPPNEQIYEHILPEAVEDPLKTLDIPGFDGYDEQYKDSEFNMSAEEYKEKVLSKPFLQRT
******:**:*****************:.***::*************:***:.*******

DIKWGNLYFMVVLHAIALYGFLTYPYFEKKMTFVWGWFLAIVANFGVAGGVHRLWSHRAF
DIKWGNLLFMVVMHAVALYGFLTYPYLEKKMTFVWGWFLAIVANFGVASGVHRLWSHRAF
*******  ****:**:**********:*:*********************.**********

KAKVPLKIIFILSYLTSGQYSAIWWARDHRIHHKFSETDADPVNAARGFWFSHVGWLCMK
KAKLPFKIISIICYLTSGQYSAIWWSRDHRLHHKFSETDADPVNAARGFWFSHIGWLCMK
***:*:*** *:.************:****:***********************:******

RHPEVLKKAKQLDLSDILNDPVIKFEEKHFQVLRLIFAFIIPTLVPVYFWNESWYWAILS
RHPEVLKKAKQLDLSDIANDPVIKFEEKHFQVLRLMFTFIIPTLVPVYFWNESWYWAILS
*****************  *****************:*:**********************

QCFMRYAYSLNCTWGINSFAHMFGNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPADY
QCFMRYVYSLNCTWGINSLAHRFGGHPYDKNIEPSENFLMTLATGGEGWHNYHHAFPADY
******.************:** **.*****:***:***:***************.*****

KASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHPISHEPVK
KASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHPISHETVK
*********************************************************.**

TK
TE
*:
```

**Fig. S3. Sequence alignment of the two Δ12-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 Δ12-desaturases).
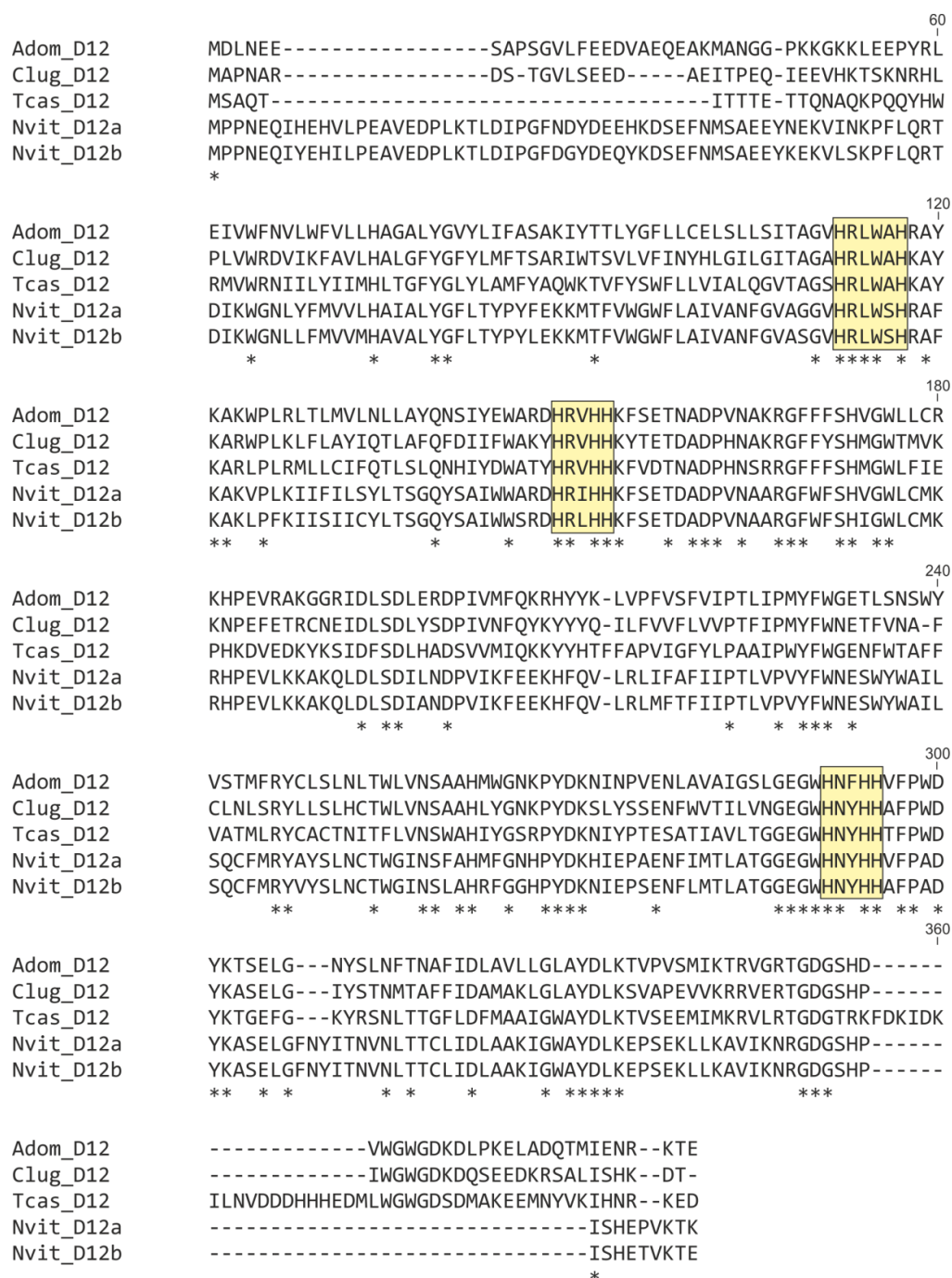
```
                                                                    60
                                                                    |
Adom_D12    MDLNEE----------------SAPSGVLFEEDVAEQEAKMANGG-PKKGKKLEEPYRL
Clug_D12    MAPNAR----------------DS-TGVLSEED-----AEITPEQ-IEEVHKTSKNRHL
Tcas_D12    MSAQT-------------------------------ITTTE-TTQNAQKPQQYHW
Nvit_D12a   MPPNEQIHEHVLPEAVEDPLKTLDIPGFNDYDEEHKDSEFNMSAEEYNEKVINKPFLQRT
Nvit_D12b   MPPNEQIYEHILPEAVEDPLKTLDIPGFDGYDEQYKDSEFNMSAEEYKEKVLSKPFLQRT
                 *

                                                                    120
                                                                    |
Adom_D12    EIVWFNVLWFVLLHAGALYGVYLIFASAKIYTTLYGFLLCELSLLSITAGVHRLWAHRAY
Clug_D12    PLVWRDVIKFAVLHALGFYGFYLMFTSARIWTSVLVFINYHLGILGITAGAHRLWAHKAY
Tcas_D12    RMVWRNIILYIIMHLTGFYGLYLAMFYAQWKTVFYSWFLLVIALQGVTAGSHRLWAHKAY
Nvit_D12a   DIKWGNLYFMVVLHAIALYGFLTYPYFEKKMTFVWGWFLAIVANFGVAGGVHRLWSHRAF
Nvit_D12b   DIKWGNLLFMVVMHAVALYGFLTYPYLEKKMTFVWGWFLAIVANFGVASGVHRLWSHRAF
               *         *     **         *              * **** * *

                                                                    180
                                                                    |
Adom_D12    KAKWPLRLTLMVLNLLAYQNSIYEWARDHRVHHKFSETNADPVNAKRGFFFSHVGWLLCR
Clug_D12    KARWPLKLFLAYIQTLAFQFDIIFWAKYHRVHHKYTETDADPHNAKRGFFYSHMGWTMVK
Tcas_D12    KARLPLRMLLCIFQTLSLQNHIYDWATYHRVHHKFVDTNADPHNSRRGFFFSHMGWLFIE
Nvit_D12a   KAKVPLKIIFILSYLTSGQYSAIWWARDHRIHHKFSETDADPVNAARGFWFSHVGWLCMK
Nvit_D12b   KAKLPFKIISIICYLTSGQYSAIWWSRDHRLHHKFSETDADPVNAARGFWFSHIGWLCMK
            **  *               *       ** ***   * *** *   *** ** **

                                                                    240
                                                                    |
Adom_D12    KHPEVRAKGGRIDLSDLERDPIVMFQKRHYYK-LVPFVSFVIPTLIPMYFWGETLSNSWY
Clug_D12    KNPEFETRCNEIDLSDLYSDPIVNFQYKYYYQ-ILFVVFLVVPTFIPMYFWNETFVNA-F
Tcas_D12    PHKDVEDKYKSIDFSDLHADSVVMIQKKYYHTFFAPVIGFYLPAAIPWYFWGENFWTAFF
Nvit_D12a   RHPEVLKKAKQLDLSDILNDPVIKFEEKHFQV-LRLIFAFIIPTLVPVYFWNESWYWAIL
Nvit_D12b   RHPEVLKKAKQLDLSDIANDPVIKFEEKHFQV-LRLMFTFIIPTLVPVYFWNESWYWAIL
                        * **     *                  *    * *** *

                                                                    300
                                                                    |
Adom_D12    VSTMFRYCLSLNLTWLVNSAAHMWGNKPYDKNINPVENLAVAIGSLGEGWHNFHHVFPWD
Clug_D12    CLNLSRYLLSLHCTWLVNSAAHLYGNKPYDKSLYSSENFWVTILVNGEGWHNYHHAFPWD
Tcas_D12    VATMLRYCACTNITFLVNSWAHIYGSRPYDKNIYPTESATIAVLTGGEGWHNYHHTFPWD
Nvit_D12a   SQCFMRYAYSLNCTWGINSFAHMFGNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPAD
Nvit_D12b   SQCFMRYVYSLNCTWGINSLAHRFGGHPYDKNIEPSENFLMTLATGGEGWHNYHHAFPAD
                   **         *    ** **   *  ****        *  ****** ** ** *
                                                                    360
                                                                    |
Adom_D12    YKTSELG---NYSLNFTNAFIDLAVLLGLAYDLKTVPVSMIKTRVGRTGDGSHD------
Clug_D12    YKASELG---IYSTNMTAFFIDAMAKLGLAYDLKSVAPEVVKRRVERTGDGSHP------
Tcas_D12    YKTGEFG---KYRSNLTTGFLDFMAAIGWAYDLKTVSEEMIMKRVLRTGDGTRKFDKIDK
Nvit_D12a   YKASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHP------
Nvit_D12b   YKASELGFNYITNVNLTTCLIDLAAKIGWAYDLKEPSEKLLKAVIKNRGDGSHP------
            **  * *          *  *       *      * *****              ***

Adom_D12    -------------VWGWGDKDLPKELADQTMIENR--KTE
Clug_D12    -------------IWGWGDKDQSEEDKRSALISHK--DT-
Tcas_D12    ILNVDDDHHHEDMLWGWGDSDMAKEEMNYVKIHNR--KED
Nvit_D12a   -----------------------------ISHEPVKTK
Nvit_D12b   -----------------------------ISHETVKTE
                                         *
```

**Fig. S4. Multiple sequence alignment of functionally characterised insect Δ12-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 *Bsa*I are underlined and restriction sites are bold.

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## Nucleotide sequences

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### *Nvit_D12a* (XP_001599836.1)

5'AAAAAA<u>GGTCTCA</u>**CATG**CCACCAAACGAACAAATCCATGAACATGTTTTGCCAGAAGCTGTT
GAGGATCCATTGAAAACTTTGGATATTCCAGGTTTCAACGACTACGATGAAGAACATAAGGAC
TCCGAATTCAACATGTCTGCTGAAGAGTACAACGAAAAGGTTATCAACAAGCCATTCTTGCAA
AGAACCGATATTAAGTGGGGTAACTTGTACTTCATGGTTGTCTTGCATGCTATTGCCTTGTAC
GGTTTTTTGACTTACCCATACTTCGAGAAGAAGATGACTTTTGTTTGGGGTTGGTTTTTGGCTA
TCGTTGCTAATTTTGGTGTTGCTGGTGGTGTTCATAGATTGTGGTCACATAGAGCTTTTAAGG
CTAAGGTTCCACTGAAGATCATTTTCATCTTGTCCTACTTGACCTCCGGTCAATATTCTGCTAT
TTGGTGGGCTAGAGATCATAGAATCCATCACAAGTTCTCTGAAACTGATGCTGATCCAGTTAA
TGCTGCTAGAGGTTTTTGGTTTTCTCATGTTGGTTGGTTGTGCATGAAGAGACATCCAGAAGT
TTTGAAGAAGGCCAAGCAATTGGATTTGTCCGATATTTTGAATGACCCCGTCATCAAGTTTGA
AGAAAAGCACTTTCAAGTCCTGAGATTGATTTTCGCCTTCATCATTCCAACTTTGGTTCCAGTT
TACTTCTGGAACGAATCTTGGTATTGGGCCATTTTGTCTCAATGCTTTATGAGATACGCCTACT
CTTTGAACTGTACCTGGGGTATTAACTCTTTCGCTCATATGTTTGGTAACCATCCATACGATAA
GCACATTGAACCAGCCGAAAACTTCATTATGACTTTGGCTACTGGTGGTGAAGGTTGGCATAA
TTACCATCATGTTTTTTCCAGCTGATTACAAGGCTTCTGAATTGGGTTTCAACTACATCACCAAC
GTTAACTTGACCACCTGTTTGATTGATTTGGCTGCTAAAATTGGTTGGGCCTACGATTTGAAA
GAACCATCTGAAAAGTTGTTGAAGGCCGTGATTAAGAATAGAGGTGATGGTTCTCATCCAATC
TCTCATGAACCAGTTAAGACCAAG**TAAG**A<u>GAGACC</u>AAAAAA3'

### *Nvit_D12b* (XP_001599873.1)

5'AAAAAA<u>GGTCTCA</u>**CATG**CCACCAAACGAACAAATCTACGAACATATTTTGCCAGAAGCCGTT
GAAGATCCATTGAAAACTTTGGATATTCCAGGTTTCGATGGTTACGATGAACAGTACAAAGAC
TCCGAATTCAACATGTCTGCTGAAGAGTACAAAGAGAAGGTTTTGTCTAAGCCATTCTTGCAA
AGAACCGATATTAAGTGGGGTAACTTGTTGTTCATGGTTGTTATGCATGCTGTTGCCTTGTAC
GGTTTTTTGACTTATCCATACTTGGAGAAGAAGATGACTTTTGTTTGGGGGTTGGTTTTTGGCTA
TCGTTGCTAATTTTGGTGTTGCCTCTGGTGTTCATAGATTGTGGTCACATAGAGCTTTTAAGG
CTAAGTTGCCATTCAAGATCATCTCCATTATTTGCTACTTGACCTCCGGTCAATATTCTGCTAT
TTGGTGGTCTAGAGATCACAGATTGCATCACAAATTCTCTGAAACTGATGCCGATCCAGTTAA
TGCTGCTAGAGGTTTTTGGTTCTCTCATATTGGTTGGTTGTGCATGAAGAGACATCCAGAAGT
TTTGAAGAAGGCCAAGCAATTGGATTTGTCCGATATTGCTAATGACCCAGTCATCAAGTTTGA
AGAAAAGCACTTCCAAGTCCTGAGATTGATGTTCACCTTCATTATCCCAACTTTGGTTCCAGTT
TACTTCTGGAATGAATCTTGGTACTGGGCCATTTTGTCTCAATGTTTCATGAGATACGTCTACT
CATTGAACTGTACCTGGGGTATTAACTCTTTGGCACATAGATTCGGTGGTCATCCATACGATA
AGAACATTGAACCATCCGAGAATTTCTTGATGACATTGGCTACTGGTGGTGAAGGTTGGCATA
ATTATCATCATGCTTTTCCAGCTGACTACAAGGCTTCTGAATTGGGTTTTAACTACATCACCAA
CGTTAACTTGACCACCTGTTTGATTGATTTGGCTGCTAAAATTGGTTGGGCCTACGATTTGAA
AGAACCCTCTGAAAAGTTGTTGAAGGCCGTTATTAAGAACAGAGGTGATGGTTCTCATCCAAT
TTCTCACGAAACTGTTAAGACCGAG**TAAG**A<u>GAGACC</u>AAAAAA 3'

## Amino acid sequences

### Nvit_D12a (XP_001599836.1)

MPPNEQIHEHVLPEAVEDPLKTLDIPGFNDYDEEHKDSEFNMSAEEYNEKVINKPFLQRTDIKWG
NLYFMVVLHAIALYGFLTYPYFEKKMTFVWGWFLAIVANFGVAGGVHRLWSHRAFKAKVPLKIIFIL
SYLTSGQYSAIWWARDHRIHHKFSETDADPVNAARGFWFSHVGWLCMKRHPEVLKKAKQLDLS
DILNDPVIKFEEKHFQVLRLIFAFIIPTLVPVYFWNESWYWAILSQCFMRYAYSLNCTWGINSFAHM
FGNHPYDKHIEPAENFIMTLATGGEGWHNYHHVFPADYKASELGFNYITNVNLTTCLIDLAAKIGW
AYDLKEPSEKLLKAVIKNRGDGSHPISHEPVKTK*

### Nvit_D12b (XP_001599873.1)

MPPNEQIYEHILPEAVEDPLKTLDIPGFDGYDEQYKDSEFNMSAEEYKEKVLSKPFLQRTDIKWGN
LLFMVVMHAVALYGFLTYPYLEKKMTFVWGWFLAIVANFGVASGVHRLWSHRAFKAKLPFKIISIIC
YLTSGQYSAIWWSRDHRLHHKFSETDADPVNAARGFWFSHIGWLCMKRHPEVLKKAKQLDLSDI
ANDPVIKFEEKHFQVLRLMFTFIIPTLVPVYFWNESWYWAILSQCFMRYVYSLNCTWGINSLAHRF
GGHPYDKNIEPSENFLMTLATGGEGWHNYHHAFPADYKASELGFNYITNVNLTTCLIDLAAKIGW
AYDLKEPSEKLLKAVIKNRGDGSHPISHETVKTE*

**Table S2. Conversion of externally supplied fully labelled $^{13}C18:1^{\Delta 9}$ into $^{13}C18:2^{\Delta 9,12}$ by *Urolepis rufipes.*** Single 2-d-old wasps (n = 12 for each sex) were treated with an acetone solution of $^{13}C18:1^{\Delta 9}$. 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 $^{13}C18:1^{\Delta 9}$ to $^{13}C18:2^{\Delta 9,12}$ 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 Δ12-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.