Article:
Audsley, N, Down, RE and Isaac, RE (2013) Genomic and peptidomic analyses of the neuropeptides from the emerging pest, Drosophila suzukii. Peptides, 68. pp. 33-42. ISSN 0196-9781

https://doi.org/10.1016/j.peptides.2014.08.006

Genomic and peptidomic analyses of the neuropeptides from the emerging pest, Drosophila suzukii

Neil Audsley[a*], Rachel E. Down[a] and R. Elwyn Isaac[b]

[a] The Food and Environmental Research Agency, Sand Hutton, York, YO41 1LZ, UK.

[b] Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK.

Corresponding author: Neil Audsley, The Food and Environmental Research Agency, Sand Hutton, York, YO41 1LZ, UK.

E mail: neil.audsley@fera.gsi.gov.uk

Tel: +44 (0)1904 462628

Highlights

- In silico analysis identified 28 peptide precursors
- Peptidome analysis of adult brain, corpus cardiacum, thoracico-abdominal ganglion identified peptides from 15 different families.
- Myosuppressin was present in all tissue analysed, and was the only peptide identified in the crop nerve bundle

Abstract

Drosophila suzukii is a highly polyphagous invasive pest which has been recently introduced into Europe and North America, where it is causing severe economic losses through larval infestations of stone and berry fruits. The peptidome of the selected nervous tissues of adult D. suzukii was investigated as a first step in identifying potential targets for the development of novel insecticides.

Through in silico analyses of the D. suzukii genome databases 28 neuropeptide families, comprising more than 72 predicted peptides were identified. Using a combination of liquid chromatography and mass spectrometry of tissue extracts, 33 predicted peptides, representing 13 different peptide families were identified by their molecular masses and a total of 17 peptide sequences were confirmed by ion fragmentation.

A comparison between the peptides and precursors of D. suzukii and D. melanogaster shows they are highly conserved, with differences only identified in the amino acid sequences of the peptides encoded in the FMRFamide, hugin and ecydysis triggering hormone precursors. All other peptides predicted and identified from D. suzukii appear to be identical to those previously characterized from D. melanogaster.

Adipokinetic hormone was only identified in the corpus cardiacum, other peptides present included short neuropeptide F, a pyrokinin and myosuppressin, the latter of which was the only peptide identified from the crop nerve bundle. Peptides present in extracts of the brain and/or thoracico-abdominal ganglion included allatostatins, cardioacceleratory peptide 2b, corazonin, extended FMRFamides, pyrokinins, myoinihibitory peptides, neuropeptide-like precursor 1, SIFamide, short neuropeptide F, kinin, sulfakinins and tachykinin related peptides.

1. Introduction

Drosophila suzukii Matsumura, is a vinegar fly native to Asia. It is a highly polyphagous invasive pest which has been recently introduced into Europe and North America, where it is commonly known as the spotted winged Drosophila [14].

In contrast to most other fruit flies which infest overripe or decaying fruit, a prominent serrated ovipositor permits female D. suzukii to penetrate and lay eggs in unripe fruit (predominantly berry and stone fruit). This results in severe economic losses through larval infestations and damage, often leading to secondary infection by pathogens [18, 54].

Since its reported introduction into Europe and North America in 2008, the biology, ecology and management of this invasive pest has received much attention [18, 54], but knowledge in other areas, such as its behavior and physiology, is lacking. However, the recent publication of the genome and transcriptome of D. suzukii from an Italian alpine population [42] and the sequencing and annotation of the genome from a North American strain [15] will facilitate genomic and functional studies. These will yield insights into the evolution and adaptation of this pest as well as comparative analyses with other Drosophila and insect species. This genomic data also contains the information of all proteins and peptides (as gene precursors) and hence virtually all biochemical and physiological processes that occur, which will aid in the identification of new insecticide targets.

Neuropeptides and their cognate receptors, which have a central role in the regulation of physiological and behavioral processes in insects, are considered important targets for the development of novel pesticides [25, 49]. A variety of insect neuropeptides and neuropeptide analogues have been shown to be insecticidal. These include the PISCF allatostatins (ASTs) and insect kinins which, when fed to aphids, cause significant mortality [23, 38]. Others such as FGLa/ASTs and short neuropeptide F have been shown to regulate feeding and foraging behavior [33, 55]. Furthermore, the down regulation of G protein-coupled receptors involved in larval growth, moulting and metamorphosis in Tribolium castaneum were found to be lethal, thus identifying targets for novel pesticides [11].

There are numerous reviews and reports on the neuropeptides, peptide and protein hormones, and their receptors from insects. This is particularly evident for Drosophila melanogaster [28, 39, 50, 53], the first insect for which its genome was sequenced and annotated [2]. Furthermore, various insect genomes have been sequenced, and in June 2011 the i5k Initiative was launched aimed at sequencing the genomes of 5,000 insects and other arthropods that are considered to be important world-wide for agriculture, food security, medicine and energy production, over a five year period [1]. The availability of sequenced and annotated insect genomes has considerably assisted the identification of peptides and proteins from insect tissues by mass spectrometric techniques. Moreover, proteomics together with techniques such as functional genomics, RNA interference and mutations, are providing information on essential physiological and behavioral processes that could be exploited for novel pest control strategies, reviewed by Boerjan et al [13].

As a first step in identifying insecticidal targets, the neuropeptide precursors from the D. suzukii genome database and the corresponding peptides present in the central nervous system (CNS), have been investigated and identified.

2. Materials and Methods

2.1. Genome analyses

To identify peptide sequences in the D. suzukii genome, the nucleotide sequences of D. melanogaster peptide precursors, acquired from flybase (http://flybase.org/) were used to search database whole-genome shotgun contigs using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The annotated genome database spottedwingflybase (http://spottedwingflybase.oregonstate.edu) was also searched for peptide precursors. To identify the precursor for ion transport peptide, the open reading frame for D. melanogaster reported by Dircksen et al. [22] was used to search for the D. suzukii annotated transcript sequence.

2.2. Insects

Drosophila suzukii were obtained from a culture maintained at the Food and Environment Research Agency, originating from Italy and were reared on D. melanogaster medium (Blades Biological Ltd, UK). Adults (1-2 weeks post-eclosion) of both sexes were used in this study.

2.3. Tissue extraction and liquid chromatography

One hundred brains were dissected from adult D. suzukii and placed in Eppendorf tubes containing ice-cold acidic methanol (87% methanol, 5% glacial acetic acid) and infused on ice for 30 minutes. The extraction medium was removed after centrifugation (4°C, 12,000 x g for 20 minutes) and diluted 20-fold with 0.1 % trifluoroacetic acid (TFA) for high performance liquid chromatography, performed using a Beckman System gold chromatography system (Beckman Coulter (UK) Ltd). The diluted sample was loaded onto a Jupiter C_{18} 10µm 300Å reversed-phase column (250 x 2.1 mm i.d.; Phenomenex, Macclesfield, UK). The column was eluted with a linear gradient of 5-60% acetonitrile/0.1% TFA over 55 minutes at a flow rate of 200 µl/minute, and elution monitored at 214 nm. One minute (200 µl) fractions were collected and concentrated to c. 10µl by centrifugal evaporation using a Savant Speed Vac concentrator (Thermo Electron, Basingstoke, UK) for mass analyses as previously described [3 -5].

Single tissues of corpus cardiacum (CC), crop nerve bundle (CNB) and thoracico-abdominal ganglion (TAG) were dissected and analysed directly (see below).

2.4. Mass and sequence analyses

Aliquots of HPLC fractions were mixed 1µl:1µl with matrix solution (α-Cyano-4-hydroxycinnamic acid; 10 mg/ml in 70% acetonitrile 0.1% TFA), 1 µl was then pipetted onto a MALDI sample plate and air dried. Dissected single tissues of the CC, CNB or TAG were transferred directly into 0.5 µl of HPLC-grade water on the MALDI sample plate. The water was immediately removed by blotting with filter paper and approximately 0.5µl of methanol/matrix solution (1:1) added and allowed to dry.

Mass spectra were acquired using a Voyager DE STR MALDI TOF mass spectrometer (applied Biosystems, Warrington, UK) or a Bruker ultraflex mass spectrometer (Bruker Daltronic GmbH, Bremen, Germany) in positive reflectron mode over the mass range m/z 500 –5000 Daltons [5]. Results are the mean of three independent MS measurements for each sample and all masses are shown as monoisotopic average masses $[M+H]^+$.

External calibration was conducted using a calibration mixture containing des-Arg-bradykinin, angiotensin 1, Glu-fibrinopeptide B and neurotensin (Applied Biosystems) or angiotensin I, angiotensin II, substance P, bombesin, ACTH clip 1-17, ACTH clip 18-39, and somatostatin 28 (Bruker Daltronic).

Fragmentation of selected mass ions for sequence analyses was achieved on the Bruker ultraflex using LIFT™ technology and data was analysed by FlexAnalysis software. Sequences of peptides were determined manually and/or by comparing the fragmentation patterns with those predicted for known and predicted peptides using Protein Prospector (University of California, San Francisco, USA).

3. Results

3.1. Genome data

Nucleotide Blast searches of the D. suzukii genome databases and gene queries of the annotated spottedwingflybase revealed the precursor sequences of 27 neuropeptide families, comprising more than 70 putative peptides (supplementary data figure 1). Peptides and their calculated monoisotopic masses ($[M+H]^+$) were predicted from these precursors (Table 1).

3.2. Mass and sequence analyses

The peptides with identical monoisotopic masses ($[M+H]^+$) to those of predicted peptides from precursor sequences by direct analyses of single CC, CNB or TAG tissues or of aliquots of HPLC fractions from an extract of 100 brains are shown in Table 1. Peptides of masses > 5000 MW were not measured in this study, and masses > 2500 Da did not correspond to any of the predicted peptides. Peptide sequences determined by fragmentation of the parent ions present in HPLC fractions of brain extracts are also identified in Table 1.

3.2.1. Single tissue analysis

i. Corpus cardiacum

A representative mass spectrum of a single CC is shown in Figure 1A. The measured masses identical to the calculated monoisotopic masses of predicted D. suzukii peptides were identified.

The precursor for adipokinetic hormone (AKH) predicts an 8 amino acid peptide amidated at its C-terminus (QLTFSPDWamide). Post translational modification to the N-terminus converts the glutamine residue (Q) to pyroglutamic acid (pE). Mass ions corresponding to the sodiated ($[M+Na]^+$; 997.5) and potassiated ($[M+K]^+$; 1013.5) adducts of AKH (pELTFSPDWamide) were detected in CC samples, as well as AKH that is extended with GK and GKR at its C-terminus giving a monoisotopic masses of 1161.6 and 1317.7 respectivley. The sodiated and potassiated forms of the GK extended peptide were also observed. Fragmentation of the sodiated adduct confirmed the sequence of AKH.

The mass ion 1329.7 corresponds to the short neuropeptide F (sNPF; AQRSPSLRLRFamide). However, the most prominent mass ion (974.6) is in agreement with a truncated form of this peptide (SPSLRLRFamide), suggesting the occurrence of alternative prohormone processing.

Myosuppressin (TDVDHVFLRFamide) was also identified by its monoisotopic mass (1247.6) and the monoisotopic mass 1430.7 is in agreement with pyrokinin (PK) $1^{2\text{-}15}$ (GPSASSGLWFGPRLamide). Additional mass ions present did not correspond to the masses of any predicted peptide shown in Table 1, and remain unassigned.

ii. Crop nerve bundle

Only one peptide, myosuppressin, was identified by its monoisotopic mass (1247.6) from the CNB (Figure 1B), corresponding to the peptide predicted in the myosuppressin precursor. No other mass ions were measured in any of the single CNB tissues analysed.

iii. Thoracico-abdominal ganglion

Figure 1C shows the mass spectra for a single TAG, and 15 mass ions corresponding to predicted peptides are labelled. The peptides assigned are 7 FMRFamide-like peptides, a sNPF, FGL/AST-4, 3 CAPA pepitdes (CAP_{2b}-1 and CAP_{2b}-2 and PK-1), kinin, SK-2 and Nplp1-3. The sequences of two of the FMRFamide -like peptides (DPKQDFMRFa and SPSEDFMRFa) and the CAPA peptides were confirmed by fragmentation of their parent ions.

3.2.2. Analyses of brain HPLC fractions

Fractionation of an extract of 100 D. suzukii brains by RP-HPLC showed that the peptide profiles of tissue samples are complex (Figure 2) and provided separation of peaks with close molecular masses, enabling sequence analysis by fragmentation of the parent ions. Mass analyses of HPLC fractions produced 29 ions that were in agreement with predicted peptides from D. suzukii and a total of 12 peptide sequences were confirmed by ion fragmentation, representing 13 different peptide families.

Representative mass spectra of two separate HPLC fractions that contain a variety of mass ions corresponding to predicted D. suzukii peptides are shown in figure 2. Other peptides that were identified through their corresponding masses in different HPLC fractions are identified in Table 1.

i. Allatostatins

Four FGLa/allatostatin (AST) peptides are encoded in the FGLa/AST precursor. Mass analyses of HPLC fractions identified four masses which corresponded to the predicted peptides (Figure 2, Table 1), and the sequences of three of these peptides, SRYPSFGLamide (figure 3A), VERYSFGLamide and TTRPQPFNFGLamide (Table 1) were confirmed by fragmentation of their parent ions. The sequence of the largest FGLa/AST (AST-2) was not confirmed by fragmentation.

Two masses (1921.9 and 1904.9) corresponding to the predicted PISCF/AST (QVRYRQCYFNPISCF) and the post-translational modified peptide (Q to pE at the N-terminus) were measured, but no sequence data was obtained.

No mass ion for the sequence of allatostatin CC (ASTCC), predicted from the partial precursor, was detected in any tissue sample or HPLC fraction.

ii. FMRFamide-like peptides

A comparison of the D. suzukii and the D. melanogaster FMRFamide-like peptide precursors is shown in Figure 4. Differences in their sequences are shown by boxes and predicted peptides are highlighted by shading. The predicted peptides in the D. suzukii precursor are listed in Table 1 and those that differ from D. melanogaster FMRFamide-like peptides are highlighted in bold. The FMRFamide-like peptide precursor contains 11 peptides with the C-terminal FMRFamide motif, including 6 copies of a single peptide, DPKQDFRMFamide (Figure 4). The masses 925.4, 1128.5 and 1181.6, in agreement with three of these peptides, were measured in HPLC fractions from an extract of D. suzukii brains. Five other peptides are predicted by the FMRFamide-like peptide precursor, NAVVLHFQKHa, SLQDNFMHFamide, ASMDRYamide, MDSNFIRFamide and SAPQDFVRSamide, and a mass ion in agreement with the latter was identified. The sequence of PDNFMRFamide was confirmed by fragmentation of its parent ion.

iii. Myosuppressin

Myosuppressin was identified in a single HPLC fraction of the brain extract from its calculated monoisotopic mass (1247.6). Fragmentation of this ion was used to confirm its sequence (Table 1).

iv. Sulfakinin

The sulfakinin precursor encodes two RFamide peptides; FDDYGHMRFamide and GGDDQFDDYGHMRFamide, but only the latter was identified by its monoisotopic mass. In addition, a third peptide is predicted within this precursor (NQKIVGFamide; 804.5), flanked by

processing sites for N-terminal cleavage (RR) and C-terminal amidation (GRR), but its presence in tissues extracts was not confirmed by mass analysis.

v. Short neuropeptide F

The precursor for short neuropeptide F (sNPF) predicts four amidated peptides; AQRSPSLRLRFamide, WFGDVNQKPIRSPSLRLRFamide, PQRLRWamide, PMRLRWamide, and two non-amidated peptides; SDPDMLNNIVE and DPNLPQM. In brain HPLC fractions, monoisotopic masses in agreement with AQRSPSLRLRFamide (1329.8) and SPSLRLRFamide (974.6; Figure 2), a cleavage product of the former, were identified, but not the other predicted molecules.

vi. CAPA and Hugin peptides

The CAPA gene contains two CAP_{2b} peptides (GANMGLYAFPRVamide and ASGLVAFPRVamide) and a pyrokinin (TGPSASSGLWFGPRLamide). All three peptides were detected in HPLC fractions of D. suzukii brain extracts (Figure 2) and their sequences were confirmed by fragmentation of their parent ions. In addition, a mass ion corresponding to $PK1^{2-15}$ was also detected and sequenced (Table 1).

The partial D. suzukii hugin precursor contains two peptides, PK-2 (SVPKPRLamide) which was identified in brain extracts by its monoisotopic mass, and hug γ. The partial D. suzukii hugin precursor is identical to the corresponding region of the D. melanogaster hugin precursor, except for 5 amino acids at its N-terminus, which may be part of the hug γ peptide (Figure 5). The sequence for hug γ was not verified.

vii. Myoinhibitory peptides

The precursor for the myoinhibitory peptides (MIP) contains 5 peptides, but only one MIP (DQWQKLHGGWamide) was found in the HPLC fractions analysed.

viii. Tachykinin related peptides

Six tachykinin-related peptides (TRPs) are present in the D. suzukii tachykinin precursor (supplementary data Figure 1), four masses measured in the brain HPLC fractions are in agreement with these peptides. The sequence of APVNSFVGMRa was confirmed by fragmentation of its parent ion (Table 1). Three other putative peptides are also predicted. The sequences and monoisotopic masses are: DVSHQHY (885.4), AALSEFWHNFF (1368.6) and SYDLRa (652.3); however, no corresponding mass ions were detected.

ix. SIFamide

The SIFamide precursor encodes for a single peptide, AYRKPPFNGSIFamide, with a corresponding monoisotopic mass of 1395.7. This peptide was detected in the brain, and its sequence confirmed by fragmentation (figure 3B).

x. Neuropeptide like peptides

Nine peptides are present in the neuropeptide-like peptide 1 (Nplp1) precursor. Masses in agreement with three of these peptides were detected in brain HPLC fractions, and Nplp1-4 (NVGTLARDFQLPIPNamide) was sequenced by fragmentation of its parent ion (Figure 3C).

xi. Corazonin

A mass ion corresponding to corazonin (1369.6; pETFQYSRGWTNamide) calculated from the peptide predicted by its precursor and modification of the C-terminal (Q to pE), was present in a brain HPLC fraction.

3.2.3. Peptide families not identified by mass spectrometry

The precursors for a variety of peptide families were identified through in silico analyses of the D. suzukii genome databases (supplementary Figure 1), but mass ions for the corresponding predicted peptides were not identified by mass spectrometry of tissue extracts. These include Drosophila-kinin, cardioacceleratory peptide, proctolin and pigment dispersing factor, whose precursor genes encode for single peptides (Table 1).

The partial precursor for the D. suzukii ecdysis triggering hormone is compared to that of D. melanogaster in figure 6. Two amidated peptides are predicted; DDSPGFFLKITKNVPRLamide which differs from the D. melanogaster peptide by the omission of a Ser residue at position 4, and GESFTMKNLKTIPRIamide which differs in amino acids at positions 3, 5 and 6. Neither of these peptides was detected by mass analysis of tissue extracts.

The D. suzukii precursor genes for CCH1 and CCH2 have been annotated, however, only the CCH2 precursor encodes for an amidated peptide (Table 1, supplementary data Figure 1).

The precursors for peptides such as bursicon, corticotropin releasing factor-like and calcitonin-like diuretic hormones, ion transport peptide and neuropeptide F were also identified, but contained peptides whose masses were greater than the upper range of detection used for mass spectrometry in this study (3000 Da).

No precursor genes for eclosion hormone, allatotropin, the AKH/corazonin-related peptide, IMFamide, neuroparsin, orcokinin, and vasopressin-like peptide were identified in the D. suzukii genome databases.

4. Discussion

Since the sequencing and annotation of the D. melanogaster genome in 2000 (Adams et al 2010), the peptidome of this insect has been repeatedly investigated by in silico and mass spectrometric analyses resulting in a comprehensive catalogue of the peptides present in this insect [8, 9, 28, 43, 56]. Similar evaluations of various invertebrates have since been facilitated by the availability of other genome databases [4, 16, 17, 27, 29, 57], and the recent publication of the genome of D. suzukii has now enabled a similar evaluation of the peptidome of this invasive pest. A comparison between the peptides and precursors of D. suzukii and D. melanogaster show they are highly conserved.

Adipokinetic hormone is synthesized in the glandular lobe of the CC, reviewed by Nassel and Winther [29], and consistent with this, AKH was identified from direct analyses of single CC by mass spectrometry. Furthermore, incompletely processed forms of AKH, extended at its C-terminus with the amidation and cleavage sites (GK and GKR), were also present in the CC. Sodiated and potassiated rather than protonated forms of the fully processed AKH are common for this peptide using MALDI-TOF MS, as is detection of the extended peptide [3, 43].

Three different peptide families, derived from different genes, have been designated as allatostatins in various insects but their inhibitory (allatostatic) activity on juvenile hormone synthesis in the corpus allatum is species dependent. Furthermore, the nomenclature of these peptides is inconsistent, the most recent suggested by Coast and Schooley [20] classifies them as the FGLa/AST and PISCF/AST based on their C-terminal peptide sequences, and the myoinhibitory peptides (MIPs) based on their original name. The FGLa/ASTs have been shown to inhibit JH biosynthesis in blattodean and orthopteran species whereas the PISCF/AST regulates JH biosynthesis only in Lepidoptera, reviewed by Weaver and Audsley [57] and Bendena and Tobe [10]. Allatostatins are pleitropic peptides and

although there is no evidence to date that these peptides regulate JH biosynthesis in D. melanogaster the FGLa/ASTs influence foraging and feeding behavior [55] and the PISCF/AST is myoinhibitory on the pupal heart [45]. The FGLa/AST and PISCF/AST identified in D.suzukii are identical to those of D. melanogaster. Furthermore, a peptide with homology to PISCF/AST, first identified by Veenstra [51] and called allatostatin CC (ASTCC), has also been identified. Audsley et al [6] showed that the Tribolium castaneum ASTCC activated the PISCF/AST receptor in this insect suggesting that these two peptides may have similar functions, but its role in fruit flies has not been determined.

The allatostatic activity of MIPs has only been demonstrated in the cricket Gryllus bimaculatus [34] and the beetle Tenebrio molitor, and in other insects they inhibit visceral muscle contractions and prothoracicostatic activity, reviewed by Bendena and Tobe [10]. Furthermore, the MIPs in D.melanogaster have been shown to have a role in ecdysis behavior [31] and be ligands for a receptor that is identical to that for the sex peptide, even though they share little structural homology. However, unlike sex peptide, MIPs are not expressed in the male reproductive accessory glands and cannot trigger the same post-mating responses when injected into female flies [32].

In D.melanogaster a variety of peptides are involved in ecdysis behavior, reviewed by Nassel and Winther [39], including ecdysis triggering hormone (ETH), eclosion hormone (EH) and bursicon. Two peptides are present in the ETH gene of both D. melanogaster and D. suzukii, but the predicted peptides are not identical. The sequences of the ETH peptides from D. melanogaster have been confirmed by mass spectrometry [58], but not for D. suzukii. Bursicon is a heterodimer of c. 30kD molecular weight, consisting of two cystine knot polypeptides, bursicon α and bursicon β and is involved in tanning and wing inflation in D. melanogaster [35]. Although homologous polypeptides were identified from the spottedwingedflybase, masses corresponding to either bursicon sub-unit were not measured. In contrast, the EH precursor was not identified from D. suzukii.

There are variations in the peptides encoded in the FMRFamide precursors between the two Drosophila species. The precursor of D. suzukii contains six copies of DPKQDFMRFamide, whereas the D. melanogaster precursor contains only five copies, and additionally there are minor differences in the sequences of three other predicted peptides. The tetrapeptide FMRFamide is a molluscan cardio-acceleratory peptide originally characterized from the clam Macrocallista nimbosa [45]. This tetrapeptide itself is not present in insects, only N-terminally extended FMRFamides have been identified to date. Furthermore, although a variable number of peptides are predicted from the FMRFamide-like peptide precursors of insects, it is only in flies [5, 46] and aphids [16, 29] where peptides with a C-terminal FMRFamide motif have been identified. In extracts of nervous tissues from D. suzukii, FMRFamides were only found in the brain and TAG, and do not appear to be present in the CC of this insect. Similarly, previous reports identified FMRFamide-like peptides throughout the CNS of the cabbage root fly (Delia radicum), the house fly (Musca domestica), the sheep blowfly (Lucilia cuprina), the blue bottle (Calliphora vomitoria) and the grey flesh fly (Sarcophaga bullata), and were not detected in the CC [5, 46, 52].

The D. melanogaster FMRFamide-like peptide precursor also contains predicted peptides with FVRSamide, FIRFamide, FMHFamide and MDRYamide C-terminal motifs [48]. Mass spectrometry has only confirmed the expression of the FVRSamide, FIRFamide peptides in the CNS of this insect [43]. Similar peptides are also present in the D. suzukii precursor, and all but the MDRYamide peptide were assigned by mass spectrometry in the present study. An additional peptide sequence (NAVVLHFQKHamide) is predicted in the Drosophila FMRFamide precursors, but analyses of extracts of both D. melanogaster and D. suzukii nervous tissue by mass spectrometry did not identify a corresponding mass ion. Hence it is unclear whether this peptide is present or has a biological function.

The precursor for myosuppressin contains a single copy of this peptide, the sequence of which is conserved in dipterans investigated [5, 44, 47, 50]. Myosuppressin has been shown to inhibit contractions in various visceral muscles, and has been localised throughout the CNS of D. melanogaster [40]. Consistent with its localisation in the crop nerve bundle, myosuppressin has been shown to be myoinhibitory on the crop of D. melanogaster [25].

Four neuropeptides 6-19 amino acids in length and terminating in RLRFamide or RLRWamide are present in the D.suzukii sNPF precursor, and are identical to those previously identified from D. melanogaster [50]. All these peptides have potent activity on the D. melanogaster sNPF receptor [37] and have been implicated in the regulation of feeding [33]. Two other peptides, contained between cleavage sites, are also predicted, but these peptides have not been identified in insect tissues and hence any putative biological activity has not been investigated. Neuropeptide F, a 36 amino acid peptide with an RVRFamide C-terminus, is encoded on a separate gene to the sNPFs, but they have similar functions such as the regulation of feeding and foraging in D. melanogaster. The encoded peptides on the NPF precursors of D. suzukii and D. melanogaster are identical.

Two pyrokinins, characterized by the FXPRLamide C-terminus, are encoded on two different genes, the capability (capa) gene (PK1) and the hugin gene (PK2), in D. melanogaster and D. suzukii. A second peptide, hug γ, which has a similar C-terminus to PKs (RTPRLamide) and which also activates the PK receptors [41] is also encoded in the hugin precursor. These peptides have been implicated in the control of growth and metabolism (hug γ) and food intake (PK) in D. melanogaster [35]. Pyrokinin-$1^{2\text{-}15}$ has been found in the nervous tissue from several insect species and is probably formed from the action of an aminopeptidase removing the N-terminal residue from PK-1. Further attack by aminopeptidase activity is prevented by the presence of a Pro in position 2, which because the imino ring structure of Pro prevents free rotation around the αC-N peptide bond [30]. Proline is also present at position 2 in all the Drosophila tachykinins and in many of the FMRFamide peptides, suggesting that this strategy is frequently used for blocking N-terminal degradation of insect peptides. Only a partial hugin gene was identified form the D. suzukii genome database, and hence can only be used to predict a putative hug γ peptide sequence, and may explain why its N-terminus differs from that of D.melanogaster. The capa gene also encodes for two cardioactive (CAP_{2b}) peptides, characterized by PRVamide C-terminus, which have been shown to stimulate fluid secretion D. melanogaster, reviewed by Beyenbach [12].

The control of fluid secretion by the Malpighian tubules of D. melanogaster is well characterized and in addition to CAP_{2b} is stimulated by Drosophila kinin, calcitonin (CT)-like diuretic hormone and corticotropin releasing factor (CRF)-like diuretic hormone [19, 24]. These peptides have no sequence homology and all act via distinct receptors and drive fluid secretion via different mechanisms [19]. The precursors for all four types have been identified from the D. suzukii genome but only the CAP_{2b} peptides were identified by mass spectrometry. The sizes of the CT-like and CRF-like DHs, 31 and 44 amino acids respectively, precluded their identification by mass spectrometry in this study.

The precursor for ion transport peptide (ITP), which regulates ion and fluid transport in the ileum of the desert locust, Schistocerca gregaria, and the related peptides (ITP-L1 and ITP-L2), which have no known function, were identified from the D. suzukii genome and the encoded peptides were identical to those of D. melanogaster. The peptides were not identified in tissue extracts due to their molecular masses being greater than the upper detection limit of the mass analyses methods used. Tissue distribution in the brain, clock neurones, abdominal ganglion and neurones innervating the heart in D. melanogaster suggests multiple function for this peptide [21, 22], but its role in osmoregulation in fruit flies has yet to be established. ITP is one of the few neuropeptides for which the receptor has not been identified, but appears to act via both cyclic AMP and cyclic GMP in Schistocerca gregaria, implying two different receptors are involved in mediating its actions [7].

D. melanogaster does not appear to have a full complement of all neuropeptides identified in insects; the genes for the AKH/corazonin-releated peptide, allatotropin, IMFamide, neuroparsin, orcokinin, and vasopressin-like peptide have not been identified [39]. The same may also be true for D. suzukii (this study) It is not unusual for some neuropeptide genes to be missing from insect genomes suggesting that some species do not require their regulatory function, or that their roles are mediated through other signalling molecules [39].

In conclusion, the vast majority of neuropeptides identified from the CNS of D. suzukii are identical to their homologues in D. melanogaster. This information, together with the vast array of functional studies on peptides and their receptors in D. melanogaster, will help identify suitable targets for the development of novel pest control strategies against this invasive pest.

Acknowledgements

Funding for this work was provided by Defra seedcorn (UK), from Africa College, University of Leeds, UK. This work has also received funding from the European Union's seventh framework programme for research, technological development and demonstration under grant agreement number 613678 (DROPSA).

5. References

[1] http:/www.arthropodgenomes.org/wiki/i5K

[2] Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, George RA, Lewis SE, Richards S, Ashburner M, Henderson SN, Sutton GG, Wortman JR, Yandell MD, Zhang Q, Chen LX, Brandon RC, Rogers YH, Blazej RG, Champe M, Pfeiffer BD, Wan KH, Doyle C, Baxter EG, Helt G, Nelson CR, Gabor GL, Abril JF, Agbayani A, An HJ, Andrews-Pfannkoch C, Baldwin D, Ballew RM, Basu A, Baxendale J, Bayraktaroglu L, Beasley EM, Beeson KY, Benos PV, Berman BP, Bhandari D, Bolshakov S, Borkova D, Botchan MR, Bouck J, Brokstein P, Brottier P, Burtis KC, Busam DA, Butler H, Cadieu E, Center A, Chandra I, Cherry JM, Cawley S, Dahlke C, Davenport LB, Davies P, de Pablos B, Delcher A, Deng Z, Mays AD, Dew I, Dietz SM, Dodson K, Doup LE, Downes M, Dugan-Rocha S, Dunkov BC, Dunn P, Durbin KJ, Evangelista CC, Ferraz C, Ferriera S, Fleischmann W, Fosler, C, Gabrielian AE, Garg NS, Gelbart WM, Glasser K, Glodek A, Gong F, Gorrell JH, Gu Z, Guan P, Harris M, Harris NL, Harvey D, Heiman TJ, Hernandez JR, Houck J, Hostin D, Houston KA, Howland TJ, Wei MH, Ibegwam C, Jalali M, Kalush F, Karpen GH, Ke Z, Kennison JA, Ketchum KA, Kimmel BE, Kodira CD, Kraft C, Kravitz S, Kulp D, Lai Z, Lasko P, Lei Y, Levitsky AA, Li J, Li Z, Liang Y, Lin X, Liu X, Mattei B, McIntosh TC, McLeod MP, McPherson D, Merkulov G, Milshina NV, Mobarry C, Morris J, Moshrefi A, Mount SM, Moy M, Murphy B, Murphy L, Muzny DM, Nelson DL, Nelson DR, Nelson KA, Nixon K, Nusskern DR, Pacleb JM, Palazzolo M, Pittman GS, Pan S, Pollard J, Puri V, Reese MG, Reinert K, Remington K, Saunders RD, Scheeler F, Shen H, Shue BC, Siden-Kiamos I, Simpson M, Skupski MP, Smith T, Spier E, Spradling AC, Stapleton M, Strong R, Sun E, Svirskas R, Tector C, Turner R, Venter E, Wang AH, Wang X, Wang ZY, Wassarman DA, Weinstock GM, Weissenbach J, Williams SM, Woodage T, Worley KC, Wu D, Yang S, Yao QA, Ye J, Yeh RF, Zaveri JS, Zhan M, Zhang G, Zhao Q, Zheng L, Zheng XH, Zhong FN, Zhong W, Zhou X, Zhu S, Zhu X, Smith HO, Gibbs RA, Myers EW, Rubin GM, Venter JC. The genome sequence of Drosophila melanogaster. Science 200; 287: 2185–2195.

[3] Audsley N, Weaver RJ. A comparison of the neuropeptides from the retrocerebral complex of adult male and female Manduca sexta using MALDI-TOF mass spectrometry. Regul Peptides 2003; 116:127– 137.

[4] Audsley N, Weaver RJ. Analysis of peptides in the brain and corpora cardiaca–corpora allata of the honey bee, Apis mellifera using MALDI-TOF mass spectrometry. Peptides 2006; 27:512-520.

[5] Audsley N, Matthews HJ, Down RE, Weaver RJ. Neuropeptides associated with the central nervous system of the cabbage root fly, Delia radicum (L). Peptides 2011 32(3):434-440.

[6] Audsley N, Vandersmissen HP, Weaver R, Dani, Matthews J, Down R, Vuerinckx K, Kim Y-J, Vanden Broeck J. Characterisation and tissue distribution of the PISCF allatostatin receptor in the red flour beetle, Tribolium castaneum. Insect Biochem Molec Biol 2013; 43:65-74.

[7] Audsley N, Jansen D, Schooley DA. Signal transduction for Schistocerca gregaria ion transport peptide is mediated via both cyclic AMP and cyclic GMP. Peptides 2013; 41:74-80.

[8] Baggerman G, Cerstiaens A, De Loof A, Schoofs L. Peptidomics of the larval Drosophila melanogaster central nervous system. J Biol Chem 2002; 277:40368–40374.

[9] Baggerman G, Boonen K, Verleyen P, De Loof A, Schoofs L, Peptidomic analysis of the larval Drosophila melanogaster central nervous system by two dimensional capillary liquid chromatography quadrupole time-of-flight mass spectrometry. J Mass Spectrom 2005; 40:250–260.

[10] Bendena WG, tobe SS. Families of allatoregualtory sequences: a 2011 perspective. Can J Zool 2011; 90:521-544.

[11] Bai H, Zhu F, Shah F, Palli SR. Large-scale RNAi screen of G protein-coupled receptors involved in larval growth, molting and metamorphosis in the red flour beetle. BMC Genomics 2011; 12:388-399

[12] Beyenbach KW, Skaer H, Dow JA. The developmental, molecular, and transport biology of Malpighian tubules. Ann Rev Entomol 2010; 55(1):351-374.

[13] Boerjan B, Cardoen D, Verdonck R, Caers J, Schoofs L. Insect omics research coming of age. Can J Zool 2012; 90:440-455.

[14] Calabria G, Maca J, Bachli G, Serra L, Pascua M. First records of the potential pest species Drosophila suzukii (Diptera: Drosophilidae) in Europe. J App Entomol 2010; 136(1-2):139–147.

[15] Chiu JC, Jiang X, Zhao L, Hamm CA, Cridland JM, Saelao P, Hamby KA, Lee EK, Kwok RS, Zhang G, Zalom FG, Walton VM, Begun DJ. Genome of Drosophila suzukii, the Spotted Wing Drosophila. Genes, Genomes, Genetics, 2013; 3:2257-2271.

[16] Christie AE. In silico analyses of peptide paracrines/hormones in Aphidoidea. Gen. Comp. Endocrinol. 2008; 159:67–79.

[17] Christie AE. Neuropeptide discovery in Ixodoidea: an in silico investigation using publicly accessible expressed sequence tags. Gen Comp Endocrinol 2008; 157:174–185.

[18] Cini A, Iorriatti C, Anfora G. A review of the invasion of Drosophila suzukii in Europe and a draft research agenda for integrated pest management. Pest Manage Sci 2012; 65:149-160.

[19] Coast G. The endocrine control of salt balance in insects. Gen Comp Endocrinol 2007; 152:332–338.

[20] Coast GM, Schooley DA. Towards a concensus nomenclature for insect neuropeptide and peptide hormones. Peptides 2011; 32:620-631.

[21] Damulewicz M, Pyza E. The Clock Input to the First Optic Neuropil of Drosophila melanogaster Expressing Neuronal Circadian Plasticity. PLoS ONE 2011; 6(6):e21258.

[22] Dircksen H, Tesfai LK, Albus C, Nassel DR. Ion transport peptide splice forms in central and peripheral neurons throughout postembryogenesis of Drosophila melanogaster. J Comp Neurol 2008; 509: 23–41.

[23] Down RE, Matthews HJ, Audsley N. Effects of Manduca sexta allatostatin and an analogue on the pea aphid Acyrthosiphon pisum (Hemiptera: Aphididae) and degradation by enzymes from the aphid gut. Peptides 2010; 31:489-497.

[24] Dow JA, 2009. Insights into the Malpighian tubule from functional genomics. J Exp Biol 2009; 212:435–445.

[25] Duttlinger A, Berry K, Nichols R. The different effects of three Drosophila melanogaster dFMRFamide-containing peptides on crop contractions suggest these structurally related peptides do not play redundant functions in gut. Peptides 2002; 23:1953-1957.

[26] Gade G, Goldsworthy GJ. Insect peptide hormones: a selective review of their physiology and potential application for pest control. Pest Manage Sci 2003; 59:1063–75.

[27] Hauser F, Cazzamali G, Williamson M, Park Y, Li B, Tanaka Y, Predel R, Neupert S, Schachtner J, Verleyen P, Grimmelikhuijzen CJ. A genomewide inventory of neurohormone GPCRs in the red flour beetle Tribolium castaneum. Front Neuroendocrinol 2008; 29:142–165.

[28] Hewes RS, Taghert PH. Neuropeptides and Neuropeptide Receptors in the Drosophila melanogaster Genome. Genome Res 2001; 11:1126-1142

[29] Huybrechts J, Bonhomme J, Minoli S, Prunier-Leterme N, Dombrovsky A, Abdel-Latief M, Robichon A, Veenstra JA, Tagu D. Neuropeptide and neurohormone precursors in the pea aphid, Acyrthosiphon pisum. Insect Molec Biol 2010; 19 (2):87–95.

[30] Isaac RE, Audsley N. Insect peptide hormones. In Amino acids, peptides and proteins in organic chemistry. Wiley-VCH Ed. Hughes AB. 2009; Vol 2 pp. 575-596.

[31] Kim Y-J, Zitnan D, Cho KH, Schooley DA, Mizoguchi A, Adams ME. Central peptidergic ensembles associated with organization of an innate behavior. Proc Natl Acad Sci USA 2006; 103:14211–14216.

[32] Kim Y-J, Bartalska K, Audsley N, Yamanaka N, Yapici N, Lee J-Y, Kim Y-C, Markovic M, Isaac E, Kataoka H, Dickson B. MIPs are Ancestral Ligands for the Sex Peptide Receptor. PNAS 2001; 107 (14):6520-6525.

[33] Lee KS, You KH, Choo JK, Han YM, Yu K. Drosophila short neuropeptide F regulates food intake and body size. J Biol Chem 2004; 279:50781–50789.

[34] Lorenz MW, Kellner R, Hoffmann KH. A family of neuropeptides that inhibit juvenile hormone biosynthesis in the cricket, Gryllus bimaculatus. J Biol Chem 1995; 270:21103–21108.

[35] Melcher C, Pankratz MJ. Candidate gustatory interneurons modulating feeding behavior in the Drosophila brain. PLoS Biol 2004; 3:e305.

[36] Luo C-W, Dewey EM, Sudo S, Ewer J, Hsu SY, Honegger H-W, Hsueh AJW. Bursicon, the insect cuticle-hardening hormone, is a heterodimeric cystine knot protein that activates G protein-coupled receptor LGR2. PNAS 2005; 102(8):2820-2815.

[37] Mertens I, Meeusen T, Huybrechts R, De Loof A, Schoofs L. Characterization of the short neuropeptide F receptor from Drosophila melanogaster. Biochem Biophys Res Commun 2002; 297:1140–1148.

[38] Nachman RJ, Hamshou M, Kaczmarek K, Zabrocki K, Smagghe G. Biostable and PEG polymer-conjugated insect pyrokinin analogs demonstrate antifeedant activity and induce high mortality in the pea aphid Acyrthosiphon pisum (Hemiptera: Aphidae). Peptides 2012; 34:266–273.

[39] Nassell DR, Winther AME. Drosophila neuropeptides in regulation of physiology and behaviour. Progress in Neurobiol 2010; 92:42–104.

[40] Nichols R, McCormick J, Lim I. Multiple antigenic peptides designed to structurally related Drosophila peptides. Peptides 1997; 18(1):41-45.

[41] Olsen SR, Wilson RI. Cracking neural circuits in a tiny brain: new approaches for understanding the neural circuitry of Drosophila. Trends Neurosci. 2008; 31:512–520.

[41] Ometto L, Cestaro A, Ramasamy S, Grassi A, Revadi S, Siozios S, Moretto M, Fontana P, Varotto C, Pisani D, Dekker T, Wrobel N, Viola R, Pertot I, Cavalieri D, Blaxter M, Anfora G, Rota-Stabelli O. Linking Genomics and Ecology to Investigate the Complex Evolution of an Invasive Drosophila Pest. Genome Biol Evol 2013; 5(4):745–757.

[43] Predel R, Wegener C, Russell WK, Tichy SE, Russell DH, Nachman RJ. Peptidomics of CNS-associated neurohemal systems of adult Drosophila melanogaster: a mass spectrometric survey of peptides from individual flies. J. Comp. Neurol. 2004; 474:379–392.

[44] Predel R, Neupert S, Garczynski SF, Crim JW, Brown MR, Russell WK, Kahnt J, Russell DH, Nachman RJ. Neuropeptidomics of the mosquito Aedes aegypti. J Proteome Res 2010; 9:2006-2015

[45] Price DA, Greenberg MJ. Structure of a molluscan cardioexcitatory neuropeptide. Science 1977; 197:670-671.

[46] Rahman MM, Fromm B, Neupert S, Kreusch S, Predel R. Extended FMRFamides in dipteran insects: Conservative expression in the neuroendocrine system is accompanied by rapid sequence evolution. Gen Comp Endocrinol 2009; 162:52–58.

[47] Riehle MA, Garczynski SF, Crim JW, Hill CA, Brown MR. Neuropeptides and peptide hormones in Anopheles gambiae. Science 2002; 298:172–175.

[48] Schneider LE, Taghert PH. Isolation and characterization of a Drosophila gene that encodes multiple neuropeptides related to Phe-Met-Arg-Phe-NH_2 (FMRFamide). Proc Natl Acad Sci USA 1988; 85:1993–1997.

[49] Van Hiel MB, Van Loy T, Poels J, Vandersmissen HP, Verlinden H, Badisco L, Vanden Broeck J. Neuropeptide Receptors as Possible Targets for Development of Insect Pest Control Agents. Neuropeptide Systems as Targets for Parasite and Pest Control, edited by Timothy G. Geary and Aaron G. Maule. © 2010 Landes Bioscience and Springer Science and Business Media. Chapter 11.

[50] Vanden Broeck J. Neuropeptides and their precursors in the fruitfly, Drosophila melanogaster. Peptides 2001; 22:241–254.

[51] Veenstra JA. Allatostatin C and its paralog allatostatin double C: The arthropod somatostatins. Insect Biochem Molec Biol 2009; 39;161–170.

[52] Verleyen P, Huybrechts J, Sas F, Clynen E, Baggerman G, De Loof A, Schoofs L. Neuropeptidomics of the grey flesh fly, Neobellieria bullata. Biochem Biophys Res Comm 2004; 316:763–770.

[53] Vogel KJ, Brown MR, Strand MR. Phylogenetic investigation of peptide hormone and growth factor receptors in five dipteran genomes. Frontiers Endocrinol 2013; 4: 193.

[54] Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee JC, Bruck DJ, Walton VM, O'Neal SD, Zalom FG, Drosophila suzukii (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. J Integ Pest Manage2011; 1:1-7.

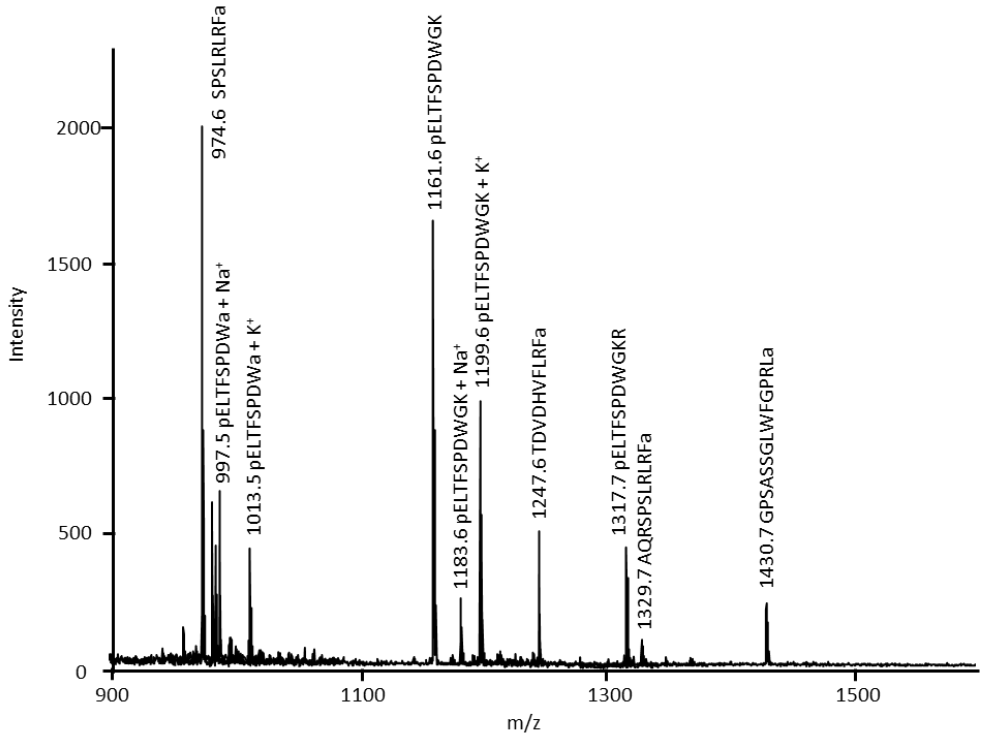
[55] Wang C, Chin-Sang I, Bendena WG. The FGLamide-Allatostatins Influence Foraging Behavior in Drosophila melanogaster. PLoS ONE 2012; 7(4):e36059.

[56] Weaver RJ, Audsley N. Neuropeptides of the beetle, Tenebrio molitor identified using MALDI-TOF mass spectrometry and deduced sequences from the Tribolium castaneum genome. Peptides 2008; 29:168-178.

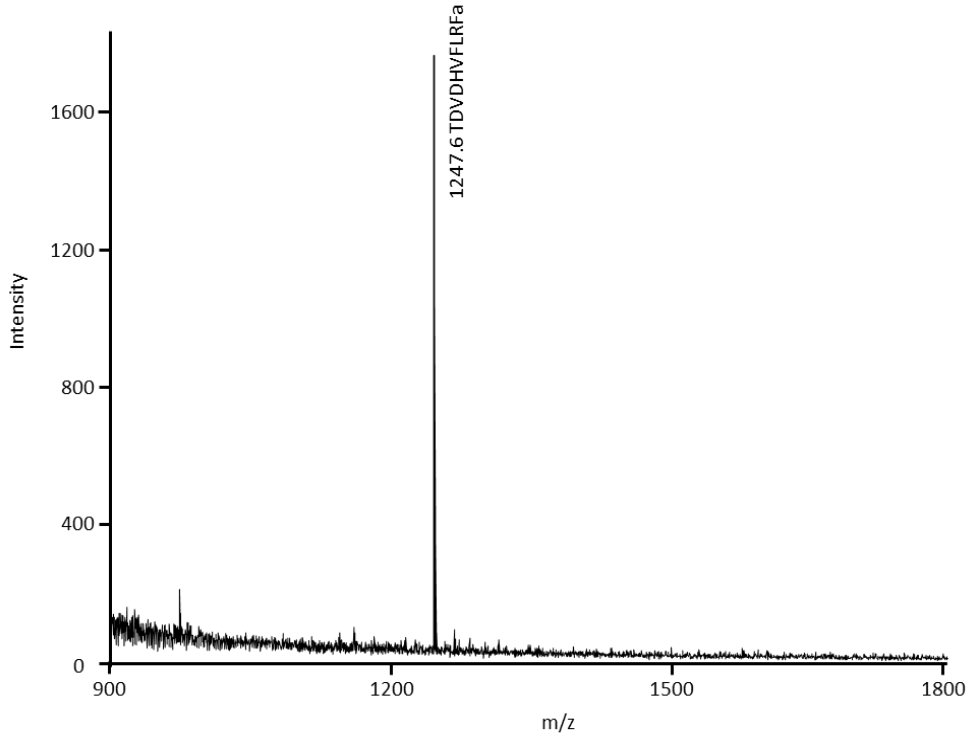
[57] Weaver RJ, Audsley N. Neuropeptide Regulators of Juvenile Hormone Synthesis Structures, Functions, Distribution, and Unanswered Questions. Trends in Comparative Endocrinology and Neurobiology: Ann NY Acad Sci 2009; 1163:316–329.

[58] Wegener C, Reinl T, Jansch L, Predel R. Direct mass spectrometric peptide profiling and fragmentation of larval peptide hormone release sites in Drosophila melanogaster reveals tagma-specific peptide expression and differential processing. J Neurochem 2006; 96:1362–1374.

A) Corpus cardiacum



B) Crop nerve bundle



C) Thoracico-abdominal ganglion

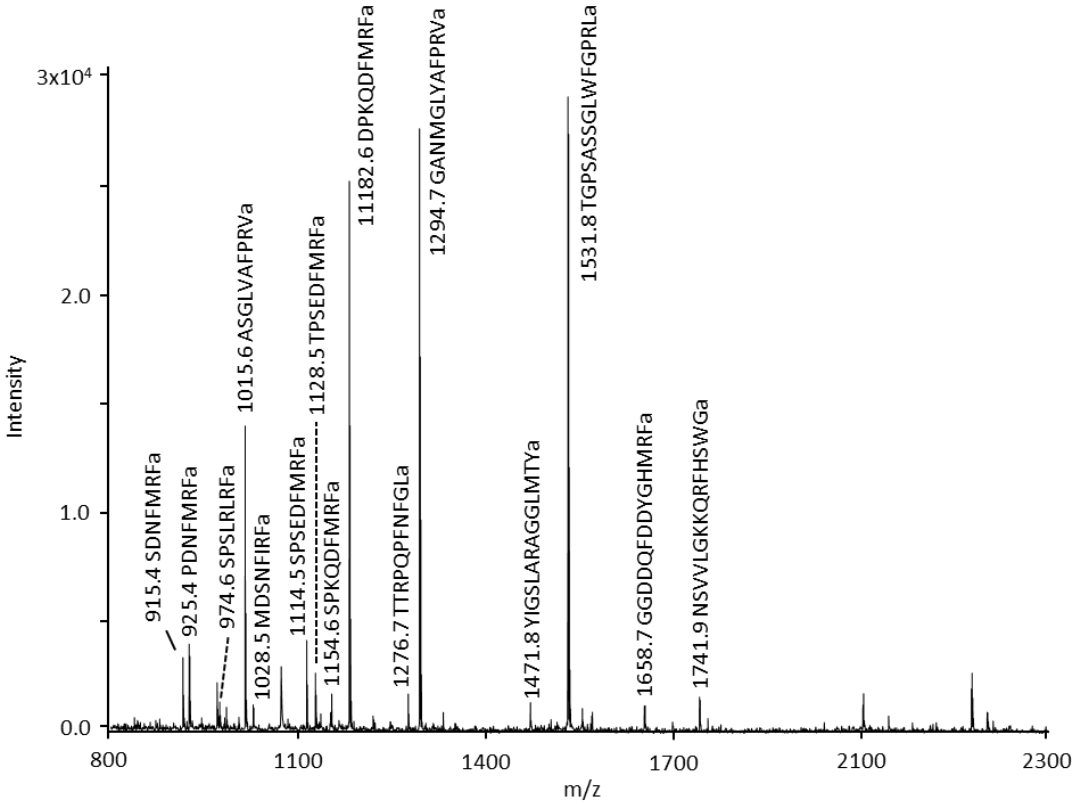
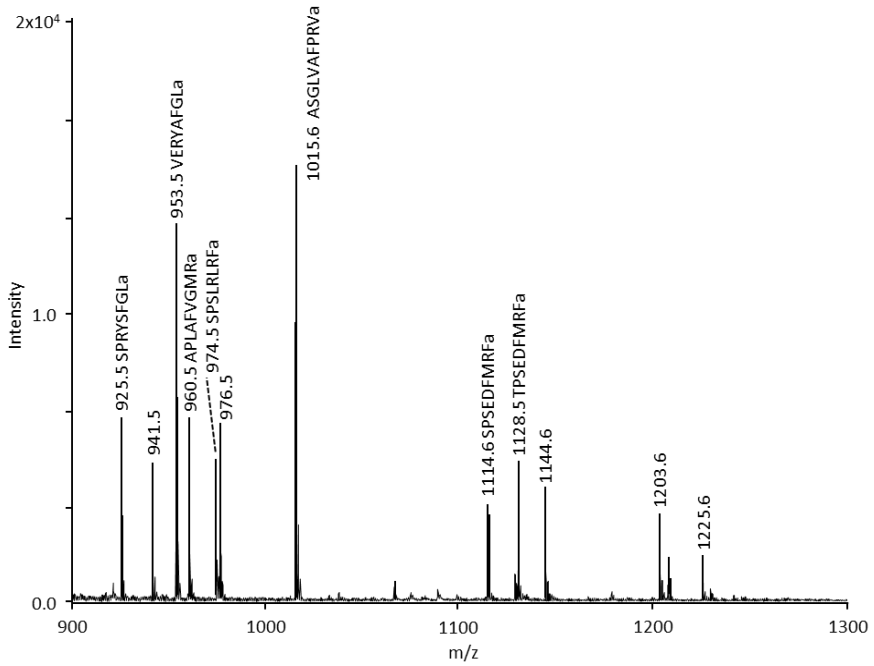


Figure 1. Mass spectra of direct analyses of single tissues of the corpus cardiacum (A) and the crop-nerve bundle (B) and thoracico-abdominal ganglion (C) from Drosophila suzukii.

A) Fraction 29



B) Fraction 31

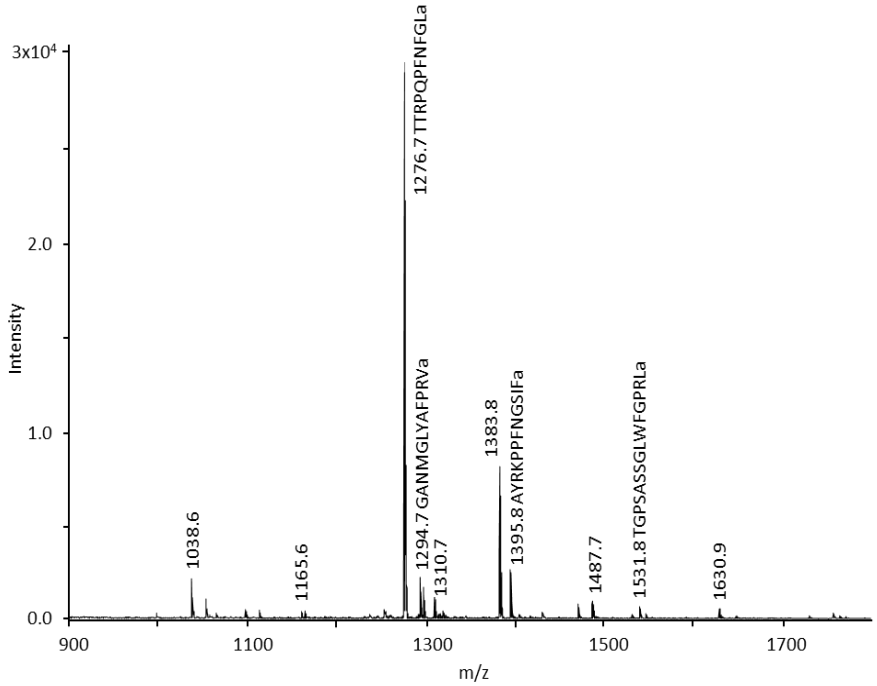
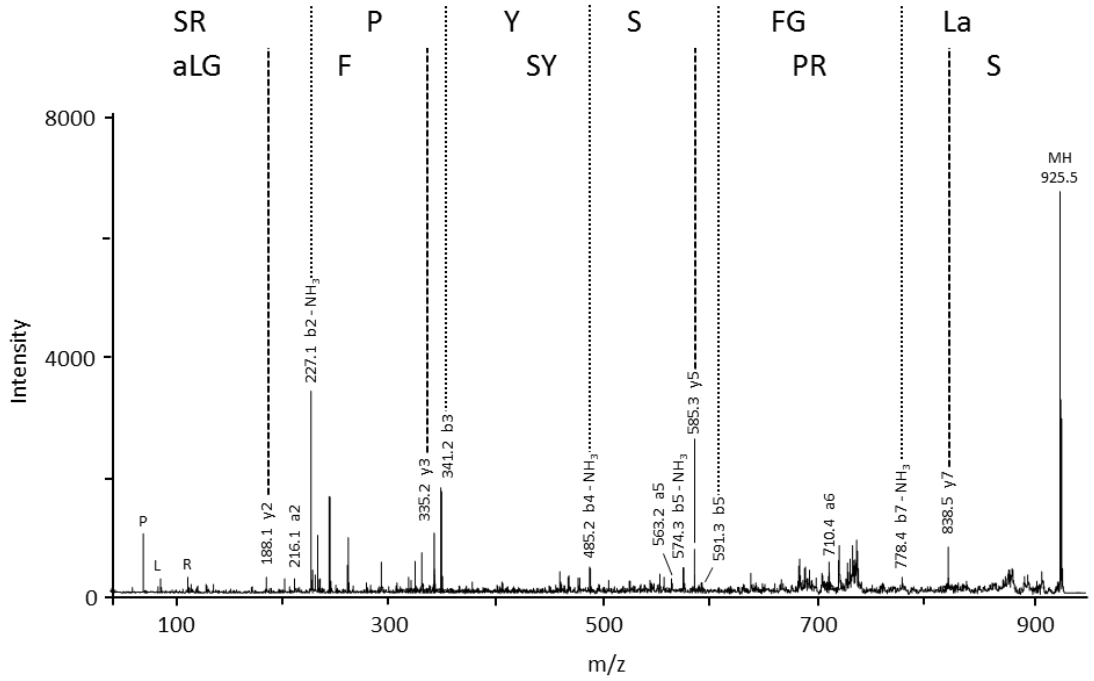
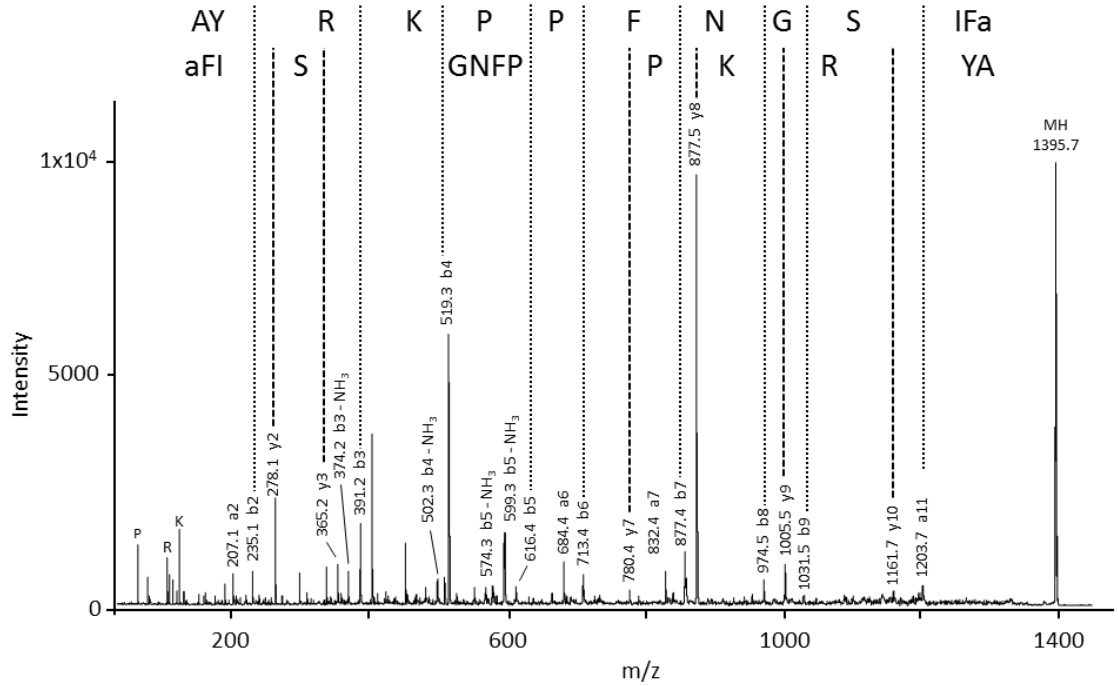


Figure 2. Mass spectra of aliquots of HPLC fraction 29 (A) and 31 (B) from extracts of 100 Drosophila suzukii brains.

A) FGL/Allatostatin 3



B) SIFamide



C) Neuropeptide-like precursor 1-4

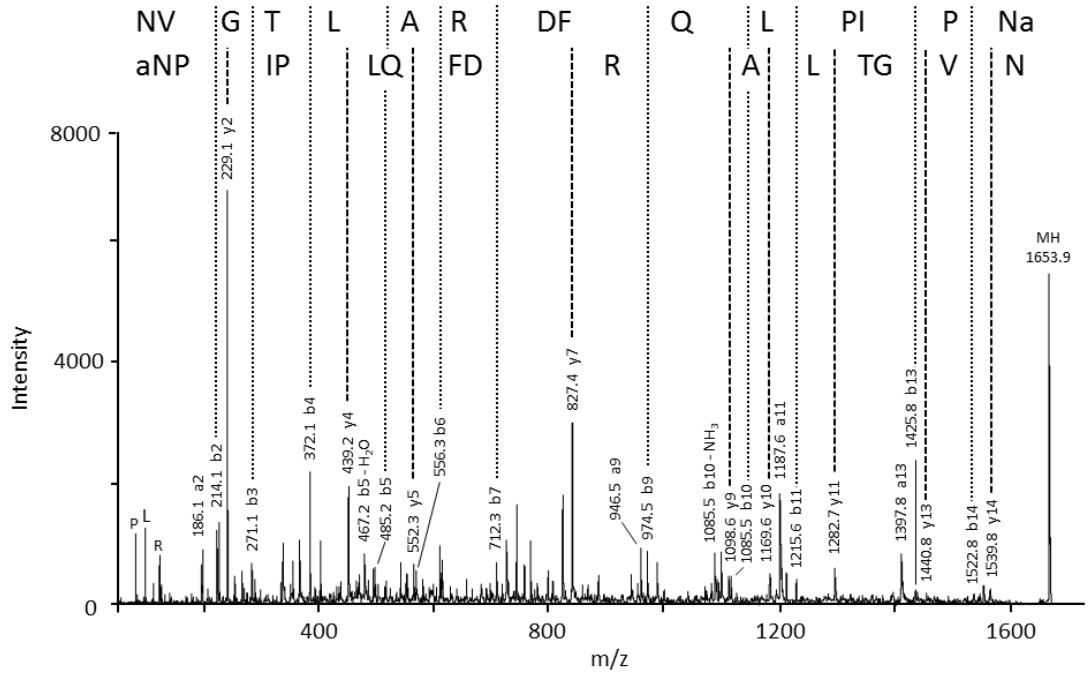


Figure 3. The MS/MS fragmentation spectra of the parent ions 925.5; FGL/allatostatin 3 (A), 1395.7; SIFamide (B) and 1653.9; Neuropeptide-like precursor 1-4 (C) from HPLC fractions of 100 Drosophila suzukii brains, acquired using LIFT™ technology. The measured fragment masses of a, b and y ions are labelled together with the corresponding amino acid sequence of the peptide.

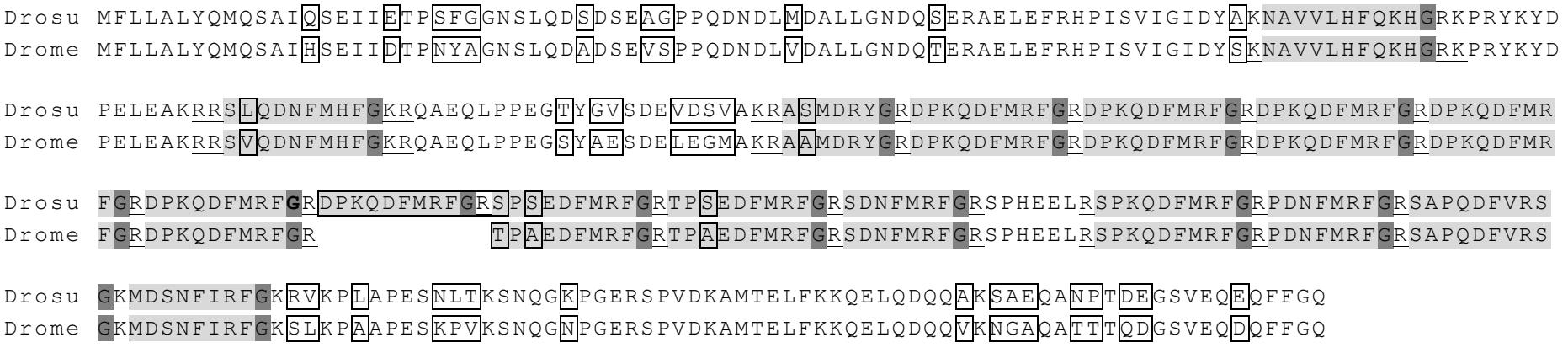


Figure 4. A comparison between the amino acid sequences of the Drosophila suzukii (Drosu) and Drosophila melanogaster (Drome) FMRFamide precursors identified from spottedwingflybase and flybase respectively. Differences in precursor sequences are identified by boxes. Predicted peptide sequences are highlighted in light grey, the amidation signals (G) are highlighted in dark grey, and potential cleavage sites (R and K) are underlined.

A. Drosophila suzukii

MLEQNVQSNGEPAYRVRTPRLGRSIDSWRILDGEGAPEESIGGQFVQRMA
KKSVPFKPRLGKRAQVCGGD

B. Drosophila melanogaster

MCGPSYCTLLLIAASCYILVCSHAKSLQGTSKLDLGNHISAGSARGSLSPASPALS
EARQKRAMGDYKELTDIIDELEENSLAQKASATMQVAAMPPQGQEFDLDTMPPL
TYYLLLQKLRQLQSNGEPAYRVRTPRLGRSIDSWRLLDAEGATGMAGGEEAIGG
QFMQRMVKKSVPFKPRLGKRAQVCGGD

Figure 5.

A comparison between the hugin precursors of (A) Drosophila suzukii (DS10_00011628) identified from spottedwingflybase and (B) Drosophila melanogaster (CG6371) using flybase. Predicted peptide sequences are highlighted in light grey and the amidation signal (G) in dark grey. Potential cleavage sites (R and K) are underlined. Differences between predicted peptide sequences are identified by boxes.

A. Drosophila suzukii

MRSLTVLAVSLLVTLVAVSQGDDSPGFFLKITKNVPRLGKRGESFTMKNLKTIPRIGRSD
QVSSSKKLSSRSAINLITNSRPL

A. Drosophila melanogaster

MRIITVLSVSLLVGLVAISQADDSSPGFFLKITKNVPRLGKRGENFAIKNLKTIPRIGRSEH
SSVTPLLAWLWDLETSPSKRRLPAGESPAKEQELNVVQPVNSNTLLELLDNNAIPSEQVK
FVHWKDFDRALQADADLYSKVIQLGRRPDQHLKQTLSFGSFVPIFGDEQNPDFMMYKN
NEDQELYGGGNRYDRQFLKYNIL

Figure 6. A comparison between the ecdysis triggering hormone precursors identified from (A) Drosophila suzukii (DS10_00003060) identified from spottedwingflybase and (B) Drosophila melanogaster (CG18105) using flybase. Predicted peptide sequences are highlighted in light grey and difference in peptide sequences are identified by boxes. The amidation signals (G) are highlighted in dark grey, and potential cleavage sites (R and K) are underlined.

Table 1. The neuropeptides identified from the Drosophila suzukii by genome and mass spectrometric analyses.

Peptide family	Sequence	Monoisotopic mass $[M+H]^+$	Analyses to identify		
			Genome	Mass (MS)	Sequence (MS/MS)
Adipokinetic hormone (AKH)	pELTFSPDWa	997.5	✓	✓[a]	✓
	pELTFSPDWGK	1161.5	✓	✓	✓
	pELTFSPDWGKR	1317.7	✓	✓	
FGL-allatostatins (AST)					
AST-1	VERYAFGLa	953.5	✓	✓	✓
AST-2	AYMYTNGGPGMKRLPVYNFGLa	2348.2	✓	✓	
AST-3	SRPYSFGLa	925.5	✓	✓	✓
AST-4	TTRPQPFNFGLa	1276.7	✓	✓	✓
Q^1 PISCF-allatostatin	QVRYRQCYFNPISCF	1923.9	✓	✓	
pE^1 PISCF-allatostatin	pEVRYRQCYFNPISCF	1906.9		✓	
Allatostatin-CC	IQPSGSGGGRAYWRCYFNAVSCF	2524.1	✓		
Allatotropin	No precursor identified				
Crustacean cardioactive peptide (CCAP)	PFCNAFTGCa	956.4	✓		
CAPA peptides					
Cardioacceleratory peptide (CAP) $CAP_{2b}1$	GANMGLYAFPRVa	1294.7	✓	✓	✓
$CAP_{2b}2$	ASGLVAFPRVa	1015.6	✓	✓	✓
Pyrokinin (PK) PK-1	TGPSASSGLWFGPRLa	1531.8	✓	✓	✓
$PK\text{-}1^{2\text{-}15}$	GPSASSGLWFGPRLa	1430.7		✓	✓
CCH-1	?				
CCH-2	GCQAYGHVCYGGHa	1350.5	✓		
Corazonin	pETFQYSRGWTNa	1369.7	✓	✓	
Calcitonin (CT)-like diuretic hormone (DH)	TVDFGLARGYSGTQEAKHRMGL AAANFAGGPa	3149.6	✓		
Corticotropin releasing factor (CRF)-like DH	NKPSLSIVNPLDVLRQRLLLEIARR QMKENSRQVELNRAILKNVa	5164.0	✓		
Ecdysis triggering hormone (ETH)	**DDSPGFFLKITKNVPRLa**	1946.1	✓		
	GESFTMKNLKTIPRIa	1734.0	✓		
Eclosion hormone	No precursor identified				

FMRFamide-like peptides					
FMRFamide-1	**SLQDNFMHFa**	1137.5	✓		
FMRFamide-2	DPKQDFMRFa	1182.6	✓	✓	✓
FMRFamide-3	**SPSEDFMRFa**	1114.5	✓	✓	✓
FMRFamide-4	**TPSEDFMRFa**	1128.5	✓	✓	
FMRFamide-5	SDNFMRFa	915.4	✓	✓	
FMRFamide-6	SPKQDFMRFa	1154.6	✓	✓	
FMRFamide-7	PDNFMRFa	925.4	✓	✓	✓
FMRFamide-8	SAPQDFMRFa	1097.5	✓		
FMRFamide-9	SAPQDFVRSa	1005.5	✓	✓	
FMRFamide-10	MDSNFIRFa	1028.5	✓	✓	
	ASMDRYa	741.3	✓		
	NAVVLHFQKHa	1191.7	✓		
Hugin peptides					
Hug γ	**EQNVQSNGEPAYRVRTPRLa**[b]	2313.2	✓	✓	
PK-2	SVPFKPRLa	942.6	✓		
Ion transport peptide	SNFFDLECKGIFNKTMFFRLDRICEDC YQLFRETSIHRLCKQECKQECFGSPFF NACIEALQLHEEMDKYNEWRDTLGa	9398.4	✓		
Ion transport peptide-L1	SNFFDLECKGIFNKTMFFRLDRICEDC YQLFRETSIHRLCKANCFVHETFGDC LKVLLIDDEEISQLQHYLKVINGSPYP FHKPIYH	10418.1	✓		
Ion transport peptide-L2	SNFFDLECKGIFNKTMFFRLDRICEDC YQLFRETSIHRLCKKDCFDSKWFGEC LKVLLIPEEEISNLQHFLRVVNGSPISF NMGPQT	10313.0	✓		
Drosophila-kinin	NSVVLGKKQRFHSWGa	1741.9	✓	✓	
Myoinhibitory peptides					
(MIPs) MIP1	AWQSLQSSWa	1091.5	✓		
MIP2	AWKSMNVAWa	1091.5	✓		
MIP3	RQAQGWNKFRGAWa	1603.8	✓		
MIP4	EPTWNNLKGMWa	1374.7	✓		
MIP5	DQWQKLHGGWa	1253.6	✓	✓	
Myosuppressin	TDVDHVFLRFa	1247.6	✓	✓	✓
Neuropeptide F	NDVNTMADAYKFLQDLDTYYGD RARVRFa	3356.6			

Short neuropeptide F (sNPF)	AQRSPSLRLRFa	1329.8	✓	✓	
	SPSLRLRFa	974.6	✓	✓	✓
	WFGDVNQKPIRSPSLRLRFa	2315.3	✓		
	SDPDMLNNIVE	1246.6	✓		
	DPNLPQM	814.4	✓		
	PQRLRWa	855.5	✓		
	PMRLRWa	858.5			
Neuropeptide-like precursor 1 (Nplp 1)					
Nplp1-1	SVAALAAQGLLNAP	1295.7	✓		
Nplp1-2	SLATLAKNGQLPTAEPGEDYADADSGEPSEQ	3161.4	✓		
Nplp1-3	YIGSLARAGGLMTYa	1471.8	✓	✓	
Nplp1-4	NVGTLARDFQLPIPNa	1653.9	✓	✓	✓
Nplp1-5	NLATMARLQSAPSTHREQP	2108.1	✓		
Nplp1-6	NVAAVARYNSQQSHNQRAGAE	2271.1	✓	✓	
Nplp1-7	NLGALKSSPVHGVQQ	1534.8	✓		
Nplp1-8	EDEEMLLPAAAPDYADPMQSYWWYPSYAGYADLDWNDY	4521.9	✓		
Nplp1-9	FLDTSKDPELFGIEHGNDATAVADEADEEPDTEQLPSPQ	4227.9	✓		
Pigment dispersing factor	NSELINSLLSLPKNMNDAa	1972.0	✓		
Proctolin	RYLPT	649.3	✓		
SIF amide	AYRKPPFNGSIFa	1395.7	✓	✓	✓
Sulfakinin (SK)					
SK-1	FDDYGHMRFa	1186.5	✓		
SK-2	GGDDQFDDYGHMRFa	1658.7	✓	✓	
Tachykinin-related peptides (TRP)					
TRP-1	APTSSFIGMRa	1065.5	✓	✓	✓
TRP-2	APLAFVGMRa	960.5	✓	✓	
TRP-3	APTGFTGMRa	936.5	✓	✓	
TRP-4	APVNSFVGMRa	1076.6	✓	✓	
TRP-5	QQRFADFNSKFVAVRa	1811.9	✓		
TRP-6	APNGFLGMRa	961.5	✓		

Bold highlight identifies peptides not identical to those from D. melanogaster

Abbreviations: single letter amino acid code, pE = pyroglutamic acid, a = amide

$✓^{6}$ = 6 copies of peptide in precursor

[a] sodium and potassium adducts of AKH

[b] putative sequence from partial Hugin precursor (not confirmed).