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Identification of ion channel genes in the *Acyrthosiphon pisum* genome

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Abstract

Aphids are major pests of crops, causing hundreds of millions of dollars worth of damage annually. Ion channel proteins are often the targets of modern insecticides and mutations in ion channel genes can lead to resistance to many leading classes of insecticides. The sequencing of the pea aphid, *Acyrthosiphon pisum,* genome has now allowed detailed *in silico* analysis of the aphid ion channels. The study has revealed significant differences in the composition of the ion channel families between the aphid and other insects. For example *A. pisum* does not appear to contain a homologue of the nACh receptor alpha 5 gene whilst the calcium channel beta subunit has been duplicated. These variations could result in differences in function or sensitivity to insecticides. The genome sequence will allow the study of aphid ion channels to be accelerated, leading to a better understanding of the function of these economically important channels. The potential for identifying novel insecticide targets within the aphid is now a step closer.

Keywords: Aphid, ion channel, genome.

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Introduction

Many aphid species are important crop pests causing estimated losses worth hundreds of millions of dollars annually. For example the peach-potato aphid, *Myzus persicae*, infests a large number of commercially important food plants including sugar beet, potatoes, lettuce, sweet pepper and tomato, whereas cereal crops are attacked by other species such as the Russian wheat aphid, *Diuraphis noxia*. Aphids cause both direct feeding damage and transmit pathogenic plant viruses.

Ion channels are responsible for the movement of ions across cell membranes and are essential components of the nervous system. They are also involved in numerous critical functions within the insect such as muscle contraction and ion homeostasis. There are several families of ion channels that have different specificities for the ions transported. Ion channels can be activated by voltage-sensing or by ligand binding, allowing the essential fine control of ion channel activity. In insects, the ion channel proteins are the target of many natural venoms and toxins used by predators to render the insect immobile. Many insecticides act by targeting ion channels (Raymond-Delpech *et al.*, 2005), thereby interfering with nervous system function, leading to the death of the insect. There are relatively few chemical classes of insecticide available for use against aphids, the main ones being neonicotinoids, which target nicotinic acetylcholine (nACh) receptors, and pyrethroids, which target voltage-gated sodium channels. Aphids resistant to these classes of chemical are increasing, as seen with the spread of kdr (knockdown resistance) to pyrethroids (Martinez-Torres *et al.*, 1999), leading to an urgent need to develop new insecticides with differing modes of action. Recently, a new class of insecticides was launched, targeting ryanodine receptors of lepidopteran and sucking pests. This class includes

Cyazypyr™ and Rynaxypyr® (Sattelle *et al.*, 2008) and these compounds will enable aphid control in previously resistant populations.

The sequencing of the pea aphid, *Acyrthosiphon pisum,* genome has provided an opportunity to study the complete set of genes encoding aphid ion channels. Here we report the genes present and compare them with their counterparts in other insects. It is hoped that the identification of these genes will lead to a better understanding of aphid ion channels, and highlighting the differences between insect channels will lead to an improvement in knowledge of the differences in efficacy of current insecticides among aphids and other insects. In addition, the genome sequence may facilitate the design of aphid-specific insecticides to better control these important pests.

Herein we follow the tradition of distinguishing between voltage-gated and ligand-gated channels, although this is of course an oversimplification.

Results and discussion

Voltage-gated sodium and calcium channels

Voltage-gated sodium channel (para). Voltage-gated sodium channel (VGSC) α-subunits are integral membrane proteins that conduct sodium ions through a cell's plasma membrane. In electrically excitable cells, they are responsible for the rising phase of action potentials. The genomes of insects for which sequence data is currently available contain a single identifiable VGSC gene named '*para*' (Davies *et al.*, 2007a). A putative *para* homologue has been identified in the *A. pisum* genome (Supplementary Table S1). The predicted protein has: the expected four repeat domains, each containing 6 transmembrane (TM)-spanning segments (S1–S6); positively charged S4 regions which act as the channel's voltage sensor; and extracellular/cytoplasmic pore regions formed by the 'P-loops' (located between S5 and S6 of the four domains and the S5 and S6 transmembrane regions, respectively). Surprisingly, the channels sodium 'selectivity filter' is made up of amino acids D, E, N and S (located on the P-loops of each domain) rather than the classic DEKA filter (Catterall, 2000), which normally defines a VGSC. Point mutations within VGSCs have been implicated in kdr and super-kdr-like resistance to the pyrethroid insecticides used in aphid control (reviewed in Davies *et al.*, 2007b).

Insect VGSCs are presumed to gain heterogeneity and functional diversity by extensive alternative splicing (Olson *et al.*, 2008). Nine such splicing sites have been identified in *para* and its orthologues to date; seven exons, a, b, i, j, e, f, h, are optional and form parts of the intracellular linkers of mature channel proteins, whereas exons c/d and l/k are mutually exclusive and are situated in the transmembrane regions. Exons l/k have been implicated in differential sensitivity to pyrethroid insecticides (Du *et al.*, 2006). Exons l/k are evolutionarily highly conserved, and are present in the *A. pisum* sequence, as are exons a, b. Given the absence of some splice sites in the aphid gene, it seems that fewer variants of this channel will be present in the pea aphid compared with, for example, *Drosophila melanogaster*, although there may be other unidentified splice sites.

Voltage-gated sodium channel auxillary subunits. High level expression of insect VGSCs, with maximal current and optimal kinetics, requires a small auxillary subunit, *TipE* (Warmke *et al.*, 1997). Four additional *TipE*-like proteins (*TEH 1-4*) have been identified in *D. melanogaster* (Derst *et al.*, 2006). We have identified genes encoding all five of these auxiliary subunits in *A. pisum* on a ~123 kb genome tract (Supplementary Table S1).

Sodium channel 60E. Like the VGSC α-subunit genes, the *D. melanogaster* sodium channel 1 (*DSC1*) gene (Salkoff *et al.*, 1987) and the orthologous *Blatella germanica BSC1* gene (Liu *et al.*, 2001) belong to the 4 × 6 TM domain family. Although originally classified as sodium channels based on their similarity to *para*, they actually encode calcium-selective channels (Zhou *et al.*, 2004). The partially reduced expression of *DSC1* in a *smell-impaired D. melanogaster* mutant *smi60E* correlates with certain olfactory defects. (Kulkarni *et al.*, 2002), suggesting a role in olfactory avoidance response. A putative homologue has been identified in the *A. pisum* genome (Supplementary Table S1).

Pickpocket (ENaC/DEG) channels. The epithelial sodium channel (ENaC)/degenerin (DEG) gene family encode membrane-bound, voltage-insensitive ion channels (Kellenberger & Schild, 2002). Individual subunits assemble as homotrimers or heterotrimers to form functional channels (Jasti *et al.*, 2007). There are more than thirty predicted ENaC/DEG subunits in *D. melanogaster* (Littleton & Ganetzky, 2000) but only a handful have been characterized (Supplementary Table S1). Pickpocket *(ppk),* the first subunit to be identified, is involved in locomotive behaviour in fruit fly larvae. Other *ppk* genes have been implicated in fluid re-distribution (Adams *et al.*, 1998; Liu *et al.*, 2003a), gustatory salt perception (Liu *et al.*, 2003b), and male response to female pheromones (Lin *et al.*, 2005). Preliminary analysis of the pea aphid genome has indicated that there are between 23 and 26 *ppk* genes present with conserved structural features, but low overall sequence similarity.

Calcium channels. Calcium channels are formed from heteromeric units containing a pore-forming α subunit, a β subunit and $\alpha_2\delta$ subunits, combinations of which allow distinct channel types to be formed. *D. melanogaster* has four α subunits, three of which correspond to the mammalian L, N and T channels and one which is related to a calcium channel found in *Caenorhabditis elegans* (Littleton & Ganetzky, 2000). In *A. pisum* we have identified a single gene for the L-, N- and T-type α subunits, however, no match to the *C. elegans*-related CG13762 gene was found (see Supplementary Fig. S1).

The auxiliary subunits of calcium channels (β and $\alpha_2\delta$ subunits) have a role in targeting the α subunit to the plasma membrane and also in aligning the α subunit into the correct conformation. We identified two orthologues of the β subunit in *A. pisum* using the *D. melanogaster* β subunit gene as the target. These proteins, ACYPI005770 and ACYPI008838, show 71% and 47% similarity to the *D. melanogaster* orthologue, respectively. *D. melanogaster* also has three $\alpha_2\delta$ subunits, which are post-translationally cleaved into two peptides (α_2 and δ) (Dickman *et al.*, 2008), and the analysis of the *A. pisum* genome reveals four orthologues of these genes with the $\alpha_2\delta$ 1 subunit being duplicated (Supplementary Table S2).

Narrow abdomen *or Dma1U.* Dma1U in *D. melanogaster* (Nash *et al.*, 2002) belongs to a unique family of putative 4×6 TM ion channels related to voltage-gated sodium and calcium channels. These channels diverge from voltage-gated sodium/calcium channels by virtue of a unique selectivity filter sequence and a reduced number of positively charged amino acids (arginine or lysine) in the voltage-sensing fourth transmembrane domains, suggesting that they may form channels with distinctive properties. Dma1U corresponds to the *narrow abdomen* (*na*) gene, a locus for point mutations associated with changes in abdominal morphology, locomotor behaviour and day/night circadian rhythms (Lear *et al.*, 2005). We have identified a gene corresponding to *na* in *A. pisum* (Supplementary Table S1); its function is likely to be similar to that of the *D. melanogaster* channel since the sequence is highly conserved (~80% identity).

Potassium channels

Potassium channels are the largest family of ion channels in invertebrates and have a wide range of roles, including the maintenance of the membrane potential in cells, maintenance of the circadian rhythm and potassium homeostasis. There are several distinct families of potassium channels, both voltage-gated and non-voltage-gated and each family is responsible for various different currents. In this study, we have identified thirty-two potassium channel genes in *A. pisum* (Supplementary Fig. S2 and Supplementary Table S3).

Voltage-gated potassium channels:
Shaker potassium channels

The Shaker family of potassium channels control membrane potential in a variety of cells and have six transmembrane domains, S1-S6. Mutations in the potassium channel Shaker in *D. melanogaster* give rise to disturbances of the transient outward potassium (iA) current. Other members of the Shaker family include ShaI, ShaB, ShaW and ShaWI which contribute to the transient and delayed rectifier currents in neurons and muscles. *D. melanogaster* has a single gene for ShaB and ShaI, whilst ShaW is encoded by two genes, ShaW and ShaWI (Littleton & Ganetzky, 2000). We have identified an orthologue of each of these genes in *A. pisum*. The aphid ShaB protein model is highly divergent from *D. melanogaster* at the N and C terminals and is much shorter at 558 amino acids compared with 985aa for *D. melanogaster*. ShaW and Shaker are extremely well conserved throughout, whereas ShaI and ShaWI are more divergent in the C-terminal regions and are again shorter in length than the *D. melanogaster* protein.

KCNQ channels

The KCNQ potassium channel is encoded by a single gene in *D. melanogaster* and is responsible for the M-current (Littleton & Ganetzky, 2000). P-element insertion lines in the KCNQ gene have shown that the KCNQ channel is involved in control of heart rhythm and the knockout line showed increased arrhythmias compared with wild-type controls (Ocorr *et al.*, 2007). *A. pisum* contains a single KCNQ gene, hmm111514, which is currently modelled as a pseudogene as it contains a premature stop codon. The aphid protein model shows low homology, 24%, to the *D. melanogaster* protein and is especially divergent at the C-terminal end.

Eag potassium channels

There are three sub-families of the Eag potassium channels; Eag, Egl and Erg. Although their basic structure is similar to the voltage-gated potassium channels with six transmembrane domains and a pore domain, the Eag channels also encode a C-terminal sequence with a cyclic-nucleotide binding domain (Guy *et al.*, 1991). *D. melanogaster Eag* mutants display a phenotype of leg-shaking, caused by repetitive firing and enhanced neurotransmitter release in the motor neurons, whereas homozygous mutations in the *D. melanogaster erg* (*Seizure*) gene result in paralysis (Warmke *et al.*, 1994; Wang *et al.*, 1997a). We have identified three Eag-type channels in *A. pisum*. The aphid Eag and Egl proteins are shorter than the *D. melanogaster* orthologues, having much shorter N- and C-terminal sequence. The aphid Erg protein, however, has a longer C terminus but shorter N terminus.

Non-voltage gated potassium channels:
Calcium-activated potassium channels
This group of potassium channels are heavily involved in the control of cell excitability (Derst *et al*., 2003). The group is divided into large (BK), intermediate (IK) and small-conductance (SK) channels that are regulated by both voltage gating and calcium ions. A C-terminal calcium-binding motif known as the 'calcium bowl' is encoded by the amino acid sequence DDDDD (Bian *et al*., 2001). In *D. melanogaster* a single gene, *Slowpoke* (*pSlo*), is responsible for the large-conductance current and associates with a second gene, *Slack*, to form the IK channel. The SK channel is also encoded by a single gene. We have a single homologue of each of these genes in *A. pisum*. The pSlo protein is especially well conserved with 82% identity between the aphid and *D. melanogaster* sequences. The current aphid Slack protein model shows good conservation with *D. melanogaster*, with the exception of two large deletions (250aa and 200aa) in the central section of the protein.
Inward-rectifying potassium channels
Three inward-rectifying potassium (*irk*) channel genes have been identified in *D. melanogaster*, *dIr*, *dIrk2* and *dIrk3*.These are involved in neuronal excitability and maintenance of potassium levels. It has been shown that renal tissues have high expression of all three *irk* genes, indicating a role in fluid secretion (Evans *et al*., 2005). We have identified two genes in *A. pisum*, which show close homology to *dIr* and *dIrk2*.
Two-pore, weakly inward rectifying potassium channels (TWIK)
TWIK channels, characterized by four transmembrane domains and two potassium selective pores, are involved in a diverse range of processes including mediating resting potential and mechanosensation (Buckingham *et al*., 2005). We have identified 16 putative *TWIK* genes in *A. pisum*, all containing the potassium ion selectivity motif GYGD. One of these genes, *ACYPIG684244*, is currently modelled as a pseudogene.

Chloride channels

Voltage-gated chloride channels Voltage-gated chloride channels are involved in several biological and physiological functions such as pH regulation, volume homeostasis and organic solute transport. The channels are characterized by the presence of twelve transmembrane domains. Mammals have ten genes encoding voltage-gated chloride channels whereas only three were found in *D. melanogaster* (Littleton & Ganetzky, 2000). We have found five in *A. pisum* (Supplementary Table S4), with three of them being similar to *D. melanogaster CG8594* gene.

Ionotropic glycine and γ-aminobutyric acid and glycine receptors. Ionotropic glycine and γ-aminobutyric acid (GABA) receptors are chloride channels mediating rapid, mostly inhibitory, synaptic transmission. Both GABA and glycine receptors belong to the dicysteine-loop ('Cys-loop') superfamily of neurotransmitter receptors (Grenningloh *et al*., 1987; Schofield *et al*., 1987). These chloride channels are pentameric (Nayeem *et al*., 1994) and they are the target of cyclodiene and phenylpyrazole insecticides.

In vertebrates, these GABA receptors have been divided into two families: $GABA_A$, characterized by bicuculline sensitivity (Sieghart, 1995), and bicuculline-insensitive $GABA_C$ receptors (Woodward *et al*., 1993). Several *$GABA_A$* (*α 1-6, β 1-4, γ1-4, δ, ε, θ* and *π 1-3*) and five *$GABA_C$* (ρ1-5) genes have been identified with the $GABA_A$ receptors having two α, two β and one of the other types of subunits (δ, ε, θ or π) (reviewed in Kash *et al*., 2004). The glycine receptors are composed of three α and two β subunits and in humans, four α (*α1–α4*) and one *β* genes have been identified (Bowery & Smart, 2006).

Both ionotropic GABA and glycine receptor subunits have large N-terminal and short C-terminal extracellular domains and four hydrophobic transmembrane domains (M1–M4) with a large intracellular loop of variable length between M3 and M4. In *D. melanogaster*, ten different genes encoding proteins with similarity to GABA/glycine subunits have been identified (Littleton & Ganetzky, 2000). The GABA receptors are bicuculline-insensitive but are regulated by some though not all of the allosteric modulators of $GABA_A$ receptors. Splice variants have been identified in some of these genes (Hosie *et al*., 2001). In *A. pisum,* we have identified ten genes encoding proteins with similarity to GABA/Glycine subunits (Supplementary Table S4) and interestingly, two of the genes show similarity to the *D. melanogaster Resistant to dieldrin* (*Rdl*) gene and two to *D. melanogaster pH-sensitive chloride Channel* (*pHCl*).

There are five genes in *D. melanogaster*, present in other insect species, with no identifiable orthologues in *A. pisum* (Supplementary Table S4). Two of the genes identified in *A. pisum* encode subunits not present in *D. melanogaster* and are most similar to human Glycine receptor α3.

Two other putative chloride receptors genes have been identified in *A. pisum*, a *histamine-gated chloride channel subunit 1* (*HisCl1*) and *glutamate-gated chloride channel* (*GluClα*) (Supplementary Table S4).

Cyclic nucleotide-gated ion channels

Cyclic nucleotide-gated (CNG) ion channels are activated by cyclic nucleotides, cAMP or cGMP, and play an impor-

tant role in vision and olfaction. They are tetramers, with each subunit having an intracellular N- and C-terminal region and six transmembrane spanning domains with a pore-loop domain between the fifth and sixth transmembrane domain. The cyclic nucleotides bind the C-terminal domain. Four CNG genes have been identified in *D. melanogaster* (Littleton & Ganetzky, 2000). The search of orthologous genes in the *A. pisum* genome yielded five putative genes: one putative orthologue for each one of the *D. melanogaster* genes (Supplementary Table S5). There are two putative genes encoding the *D. melanogaster CG7779*, but these are incomplete and probably represent two halves of the same gene.

Glutamate receptors

The ionotropic glutamate receptor family mediates most forms of excitatory synaptic transmission in the mammalian CNS and they play an important role in learning and memory. In mammals, four categories have been identified according to the agonist response: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate, N-Methyl-D-Aspartate (NMDA) and δ. All of the receptor subunits are thought to adopt the same membrane topology and each subunit is composed of an extracellular N-terminal domain, three transmembrane domains, a single 'P-loop' membrane domain and an intracellular C-terminal domain that interacts with scaffolding proteins (Hollmann *et al*., 1994; Roche *et al*., 1994; Bennett & Dingledine, 1995). Approximately 30 ionotropic glutamate receptor genes were identified in *D. melanogaster* (Littleton & Ganetzky, 2000). Invertebrates have additional subtypes, absent in mammals, which have low similarity to the *Arabidopsis thaliana* Glur5 glutamate receptor. Some glutamate receptors are the target of the avermectin family of insecticides.

In mammals, NMDA receptors are heterotetramers and seven subunits have been identified encoded by the genes *NR1, NR2A, NR2B, NR2C, NR2D, NR3A* and *NR3B*. A functioning receptor requires at least two subunits: NR1 and NR2 (Stephenson *et al*., 2008) and diversity is generated by the association of the obligatory NR1 subunit with different NR2 subunits.

In *D. melanogaster,* two NMDA receptor genes have been identified (*NMDAR1* and *NMDAR2*) and our analysis of the *A. pisum* genome shows the presence of one copy of each one of these genes. One copy of *NMDAR3* was also identified (Supplementary Table S6 and Supplementary Fig. S3), a gene which is absent from *D. melanogaster* but present in other insects such as *Anopheles gambiae*, *Tribolium castaneum*, *Apis mellifera* and *Nasonia vitripennis*. Two other genes identified in *D. melanogaster* and described as showing NMDA selective glutamate activity in Flybase, CG3814 and CG30379, could not be identified in *A. pisum*; these genes have only been identified in other *Drosophila* species.

AMPA and kainate receptors mediate fast synaptic transmission in the CNS and both are described as ionotropic, although there is evidence that they can be metabotropic, inducing the activation of G proteins and signalling cascades (Wang *et al*., 1997b; Huettner, 2003; Lerma, 2003). Kainate receptors are tetramers. Five different subunits have been identified, GLU_{K5}, GLU_{K6}, GLU_{K7}, GLU_{K1} and GLU_{K2} (Hollmann & Heinemann, 1994), divided into two families based on sequence similarity.

Ten genes encoding kainate selective glutamate receptors were identified in *D. melanogaster.* We have identified seven putative genes in the *A. pisum* genome (Supplementary Fig. S4 and Supplementary Table S6) but were unable to identify genes homologous to *KaiRIA, GluRIIa, GluRIIB, GluRIIC* and *GluRIIE*, present in members of the Drosophilidae family. The gene *CG5621* also seems to be absent from *A. pisum* despite being present in *Aedes aegypti*, *An. gambiae* and *Culex quinquefasciatus*. Interestingly in *A. pisum,* there is a cluster of kainate receptor genes located in the same scaffold, with three genes with similarity to *CG3822* and one gene similar to *clumsy.* There is another gene similar to *CG3822* located in another cluster.

The AMPA receptors, have four types of subunits, GluR1, GluR2, GluR3, and GluR4 (also called GluRA-D), and are usually heterotetrameric, consisting of symmetric 'dimer of dimers' of GluR2 and either GluR1, GluR3 or GluR4. Only two AMPA receptors have been described in *D. melanogaster*, GluRI and GluRIB. We have found two genes in the *A. pisum* genome, both similar to *GluRIB* that might be two halves of the same gene (Supplementary Fig. S4 and Supplementary Table S6). In addition to these receptors, several novel subtypes have been identified in *D. melanogaster* with no mammalian orthologues: CG32704, CG15627, CG7385, CG6185, CG2657 and CG42315. One orthologue of each has been identified in *A. pisum* (Supplementary Fig. S5 and Supplementary Table S6). Another ionotropic channel, CG14076, has six copies in *A. pisum*, two located in the same scaffold (Supplementary Fig. S5 and Supplementary Table S6). Several other ionotropic receptors present in *D. melanogaster* are not found in *A. pisum*.

Nicotinic acetylcholine receptors

Nicotinic acetylcholine (nACh) receptors are prototypical members of the Cys-loop ligand-gated ion channel (cysLGIC) superfamily, consisting of five subunits arranged around a central ion channel. In insects, nACh receptors mediate the fast actions of acetylcholine in the nervous system and are of particular interest as they are targets of a major group of insecticides, the neonicoti-

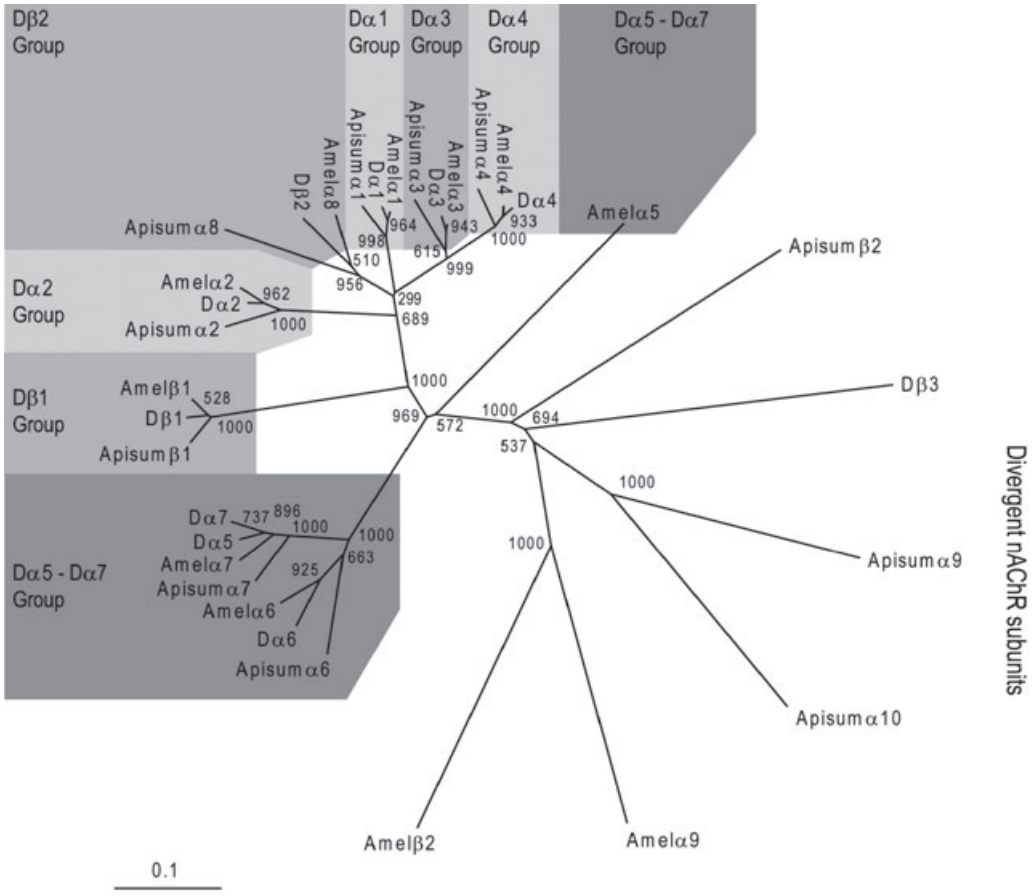

Figure 1. Tree showing relationships of *Acyrthosiphon pisum*, *Apis mellifera* and *Drosophila melanogaster* nAChR subunit protein sequences. Numbers at each node signify bootstrap values with 1000 replicates and the scale bar represents substitutions per site. The nAChR subunits shown in the tree (as well as GenBank accession numbers) are as follows: Da1 (CAA30172), Da2 (CAA36517), Da3 (CAA75688), Da4 (CAB77445), Da5 (AAM13390), Da6 (AAM13392), Da7 (AAK67257), Db1 (CAA27641), Db2 (CAA39211), Db3 (CAC48166), Amela1 (DQ026031), Amela2 (AY540846), Amela3 (DQ026032), Amela4 (DQ026033), Amela5 (AY569781), Amela6 (DQ026035), Amela7 (AY500239), Amela8 (AF514804), Amela9 (DQ026037), Amelb1 (DQ026038), Amelb2 (DQ026039). The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) available with the CLUSTALX program (Thompson *et al*., 1997) and displayed using TreeView (Page, 1996).

noids, which have been the fastest-growing class of insecticides in modern crop protection (Jeschke & Nauen, 2008). The *A. pisum* genome contains 11 genes encoding putative nACh receptor subunits (Fig. 1), a similar number to that found in *An. gambiae*, *Ap. mellifera*, *Bombyx mori*, *D. melanogaster* and *T. castaneum,* all with between 10 and 12 subunits. Eight of the aphid subunits fall into core groups (Dα1 Group, Dα2 Group, etc.), which are highly conserved (over 60% amino acid identity) between different insect species (Jones *et al*., 2007). As in other insects, *A. pisum* also has divergent subunits, which show particularly low sequence identity (20% or less) with other nACh receptors and may perform species-specific roles. The complement of *A. pisum* divergent subunits, comprising one β (Apisumβ2) and two α (Apisum α9 and Apisum α10) subunits, is currently unique to the aphid. *Apisumα4* and *Apisumα6* have alternative splicing of exons 4 and 8, respectively, which is also seen in orthologous subunits of other insect species (Supplementary Fig. S6). Such splicing effectively diversifies receptor function and generates species-specific isoforms by introducing variations in (1) the Cys-loop that is involved in receptor assembly (Lansdell & Millar, 2000), (2) loops E and B that contribute to ligand binding, and (3) the second transmembrane domain that forms the ion channel (Grauso *et al*., 2002).

We have found features particular to the *A. pisum* nACh receptor gene family. For example, the aphid is the first insect that appears not to possess a Dα5 orthologue (Fig. 1). In this regard, it is worth noting that the closely related Apisumα7 subunit has unusual tandem duplications of exons 6 and 7, which likely represent alternatively spliced exons (Supplementary Fig. S6). It is interesting to speculate that the genome duplication events seen in *Apisumα7* may represent the early steps of diversification leading to the generation of a separate gene identified as *α5* in more highly evolved insects. This finding would

Table 1. Cys-loop sequences of divergent insect nAChR subunits

Subunit	Cys-loop sequence	Reference
Apisumα9	CQADMTYWPYDTETG	This report
Apisumα10	CKTETTNWPHDTETG	This report
Apisumβ2	CDVDLTNWPFGEHTC	This report
Amelα9	CATDFSSWPYDTHRC	(Jones *et al*., 2006)
Amelβ2	CEYDHTWWPYDILNC	(Jones *et al*., 2006)
Tcasα9	CVADLTKYPFDTHNC	(Jones & Sattelle, 2007)
Tcasα10	CNLNLKYWPFDTERC	(Jones & Sattelle, 2007)
Bmα9	CSTSLRRWPYDTENC	(Shao *et al*., 2007)
Bmβ2	CRVDLRNWPLDVEQC	(Shao *et al*., 2007)
Bmβ3	CVPDLTDWPYDRETC	(Shao *et al*., 2007)
Agamα9	CELNMRLWPFDYEKC	(Jones *et al*., 2005)
Mdβ3	CNLNLKMWPYDQETC	(Gao *et al*., 2007)
Dβ3	CELNMLNWPHDKESC	(Lansdell & Millar, 2002)

The cysteine residues forming the cys-loop are highlighted in black shading, while grey shading indicates conserved residues.

imply that *α5* is the newest member of the insect core group of subunits which is perhaps subjected to fewer functional constraints, because Amelα5, Tcasα5 and Bmα5 show considerably more divergence in amino acid sequence than any other core subunit orthologues (Jones *et al*., 2006; Jones & Sattelle, 2007; Shao *et al*., 2007). The two aphid divergent subunits, Apisumα9 and Apisumα10, are the first examples of insect nACh receptor subunits without both cysteine residues of the characteristic Cys-loop motif (Table 1). Instead, both subunits have the first cysteine while the second is replaced by a glycine residue. The Cys-loop plays a key role in receptor assembly (Green & Wanamaker, 1997) and removal of one cysteine from the *Torpedo californica* α subunit altered the response to ACh (Sumikawa & Gehle, 1992). Thus, Apisumα9 and Apisumα10 may show distinct functional properties. As neonicotinoid insecticides are frequently used to combat outbreaks of aphid infestation of crops (Jeschke & Nauen, 2008), knowledge of the receptor family targeted by such chemicals will accelerate our understanding of the molecular basis of aphid control.

Transient Receptor Potential channels

Transient Receptor Potential (TRP) proteins have six transmembrane domains and form calcium-permeable cation channels. The first description of a TRP gene was in *D. melanogaster* but there have been relatively few comparative studies on the TRP genes in insects. *D. melanogaster* has 13 well described genes, in seven subfamilies (Supplementary Table S7) (Montell, 2005). Most were identified from mutant flies which display aberrant sensory perception phenotypes and many TRP channels do seem to be involved in various forms of sensation. In contrast, *A. pisum* seems to have only eight or nine *trp* genes, with no representatives of the TRPML and TRPP sub-families (Supplementary Fig. S7 and Supplementary Table S7). Interestingly, these two sub-families are the most distantly related members of the whole gene superfamily and have been referred to as the group-2 subfamilies. As aphids are primarily parthenogenetic, it is perhaps not surprising that a gene, the TRPP *Pkd-2*, with a role in sperm motility, is absent. The aphid genome, then, only has representatives of the group 1 *trp* sub-families.

The best characterized of these subfamilies is TRPC, or classical TRP, which is involved in photoreception (Hardie & Minke, 1992). TRPL (TRP-like) and TRP-γ form heteromultimeric complexes with TRP (Phillips *et al*., 1992; Xu *et al*., 2000). Together, these three genes make up the TRPC subfamily. In *D. melanogaster*, these channels are held in a signalling complex involving the scaffolding protein InaD (Georgiev *et al*., 2005). Only one member of the TRPC sub-family was found in the aphid genome and InaD is not obviously present, suggesting that the physiology of visual signalling is markedly different in the aphid.

As in *D. melanogaster*, there are two members of the TRPV subfamily, one each related to *nanchung* and *inactive*, though the aphid equivalent of *inactive* is more similar to that of *A. mellifera* than to *D. melanogaster*. In flies, these genes are involved in sound perception (Kim *et al*., 2003) and the responses to humidity (Liu *et al*., 2007) and heat (Chentsova *et al*., 2002). The single insect member of the TRPM family is also conserved in the aphid. The function of this gene has not been determined, although it is known not to play a role in larval thermotaxis (Rosenzweig *et al*., 2005). *Nompc* is the sole member of the TRPN subfamily and mutations in this gene produce a phenotype of defective mechanoreception (Kernan *et al*., 1994; Walker *et al*., 2000). There is a clear orthologue of *NompC* in the aphid genome (ACYPIT949557).

The TRPA subfamily is the largest and most diverse in both *D. melanogaster* and *A. pisum*. Though the genomes of both species seem to contain four members of this subfamily, it has proved very difficult, based on sequence data, to deduce a gene-for-gene relationship between the two TRPA subfamilies. The one exception is *TrpA1*, which has a clear aphid equivalent (ACYPIT433926). In flies, this gene is involved in larval thermotaxis (Rosenzweig *et al*., 2005). The other three Drosophila TRPA genes, *pyrexia*, *waterwitch* (CG31284) and *painless* (*painless* is somewhat divergent) are involved in responses to heat (Lee *et al*., 2005), humidity (Liu *et al*., 2007) and in mechanosensation/nociception (Goodman, 2003; Tracey *et al*., 2003), respectively. The best candidate for an orthologue to any of these genes in the aphid is for *waterwitch* where a putative orthologue seems to have been duplicated, with two copies adjacent to each other in the inverse orientation. There is no obvious aphid equivalent of *pyrexia.* There is a gene distantly related to *painless*, ACYPIT907328, but the sequence divergence is sufficient to suggest that this may be a novel member of the TRP family, rather than an orthologue.

Ryanodine receptors

Ryanodine receptors (so named due to their interaction with an insecticidal alkaloid ryanodine) are ligand-gated calcium channels that mediate the release of calcium ions from intracellular stores. They are located in the sarcoplasmic reticulum of skeletal muscle, where calcium release is important in muscle contraction, and also in the endoplasmic reticulum of neurons and other cell types, where they are heavily involved in calcium signalling (Sattelle *et al.*, 2008). Each receptor is composed of a large cytoplasmic domain at the N terminus and a small transmembrane domain at the C terminus (Hamilton, 2005). Functional channels present as large homomeric tetramers. The *A. pisum* receptor (Supplementary Table S2) is highly conserved with 77.7% identity/85.8% similarity with the *D. melanogaster* receptor. The sequence conservation within aphids is even more striking, with > 98% amino acid identity between *A. pisum*, *M. persicae* and *Aphis gossypii* channels. Compared with the *D. melanogaster* gene which is composed of twenty-six exons, with exons 9a/b being mutually exclusive and exon 13 having an optional splice site, the *A. pisum* receptor has an estimated 98 exons and no equivalent splice sites. The recently commercialized synthetic insecticide Cyazypyr™, which targets the ryanodine receptors of aphids, acts as a channel activator and binds at a site distinct from the ryanodine binding site (Sattelle *et al.*, 2008).

Inositol 1,4,5-triphosphate (IP3) receptor

A second receptor that also releases calcium from intracellular stores is the inositol 1,4,5-triphosphate (IP3) receptor. The receptor is activated by binding of the IP3 ligand and is involved in numerous cellular response pathways and sensory mechanisms (Hasan and Rosbash, 1992). The IP3 receptor protein is highly conserved between *D. melanogaster* and *A. pisum* with amino acid identity of 62% and similarity of 72% (Supplementary Table S2).

General discussion

The sequencing of the *A. pisum* genome is an important milestone in basic insect and insecticide research. This sequencing effort and the uncovering of ion channel genes will play an important part in the development of the next generation of chemicals to control these pests in three ways. Firstly, the genome will allow an understanding of the broad architecture of aphid ion channels and will reveal the role of these genes in aphid biology. Important differences in ion channel composition between aphids and other insect species have already been identified in this study. A major finding is that aphids are the only insect to date without an orthologue of the Drosophila Dα5 subunit. Interestingly, it does have orthologues of insect subunits shown to be targeted by imidacloprid (*Apisumα2*) (Matsuda *et al.*, 1998) and spinosad (*Apisumα6*) (Perry *et al.*, 2007). Also, of considerable interest is the finding that two of the divergent nACh receptor subunits *Apisumα9* and *Apisumα10* are the only insect nAChRs known to have incomplete signature Cys-loop sequences, likely to profoundly modify their assembly and functional properties. Another example of the role of ion channels in differences in aphid biology is seen with the TRP genes. The TRP channels that are involved in vision are reduced in *A. pisum* when compared with *D. melanogaster.* In terms of sensory process, the best conserved genes between the two species appear to be those involved in detecting humidity and sound/mechanical stimuli. The genes involved in larval thermotaxis and heat avoidance are less well conserved. It is tempting to speculate that these differences reflect the relative importance of the senses in the two species.

This study has shown that the pea aphid is divergent from other insects in the number of auxiliary calcium channel subunits it encodes. The aphid has a second beta subunit and a duplicated $\alpha_2\delta$ subunit 1. These genes may act in an aphid-specific manner or be expressed in alternative tissues to give rise to novel calcium currents (Grabner *et al.*, 1994). It is also seen that the pea aphid contains a duplicated GABA *rdl* subunit and the duplicated gene contains the A302S allele as is the case with *Myzus persicae*. The presence of this allele, however, does not confer resistance to insecticides that act at GABA chloride channels, such as cyclodienes (Anthony *et al.*, 1998). The presence of the second gene in *A. pisum* would indicate that the duplication is likely to be present throughout aphid species.

The second important role of the genomic information will be in the design and development of new insecticides to control aphid infestations. The genome data is likely to include novel target proteins that insecticides can act upon either as agonists or antagonists. The ion channel amino acid sequences will also be of use to researchers trying to identify how chemicals bind to these receptors and what domains are involved in the binding. The genome data will allow the development of better tools such as specific cell lines for binding studies and in this way accelerate research into new chemistries. An important example of this will be in the better understanding of how the new ryanodine receptor-inhibiting chemicals bind to the target and what motifs in the protein sequence are responsible for the aphid selectivity.

The third use of the genomic data will be in the area of insecticide resistance research. Through knowledge of the sequences of the target genes of insecticides, gene sequencing of suspected resistant aphids can proceed rapidly without the complication of lengthy gene cloning

steps. This will allow novel mutations causing insecticide resistance to be identified and quickly characterized, and diagnostic tests to be developed to enable early screening of aphid populations. In this way, the problem of resistance will be easier to contain and manage in resistance management programs.

Some of the current gene models produced through the *in silico* analysis of the genome will need to be confirmed through expressed sequence tag (EST) data. Currently, this level of EST coverage is not available for *A. pisum* but it is expected to be generated within 12 months. Once this information is available then the current models will no doubt be refined and extra detail added such as splice variation. Future research on the aphid ion channels may focus on understanding which subunits combine together to form the channels, and in which tissues or developmental stages these are expressed. The roles of the novel aphid ion channel proteins will also need to be addressed.

Experimental procedures

To identify putative nAChR subunits, the *A. pisum* genome was screened with every member of the *Ap. mellifera*, *D. melanogaster* and *T. castaneum* nAChR gene families, using the TBLASTN algorithm. Candidate aphid nAChR subunits were identified based on their considerable sequence homology with previously characterized subunits, particularly in the N-terminal ligand-binding domain and the four transmembrane regions.

Sequences of the TRP gene family from *D. melanogaste*r were used to perform reciprocal BLAST searches of the genome sequence database of *A. pisum*. Translated *Drosophila* sequences were used to search the aphid genome sequences using the TBLASTN algorithm. Sequences which showed a significant match to the *Drosophila* sequence used in the initial query were then used to search the NCBI sequence database, using the TBLASTX algorithm, in order to ascertain whether they were likely to be true orthologues of the *Drosophila* gene in question. In cases where the reciprocal BLAST criteria were not fully met, further searches of the aphid genome were performed using the most similar sequence found in the database, in order to assemble fragments of any aphid TRP gene sequences with no clear *Drosophila* orthologue.

For sodium channels, potassium channels, calcium channels and the ryanodine receptor, TBLASTN and BlastP searches of the *A. pisum* genome were carried out using *D. melanogaster* orthologues. The Genome Informatics Laboratory of Indiana University Biology Department, which provides computed annotations for the first draft genome sequence release (Acyr_1.0) of *A. pisum* (http://insects.eugenes.org/aphid/), was also queried. Softberry protein-based gene predictions software FGENESH+ (http://linux1.softberry.com/berry.phtml) was used to refine gene sequence data.

Previously identified members of the Glutamate receptor, Cyclic nucleotide-gated ion channel and chloride receptor families were retrieved for *D. melanogaster* and *Homo sapiens*. These sequences were uploaded into TBLASTN with *A. pisum* specified as the search set. High probability hits were compared with the Gnomon prediction in AphidBase (http://www.aphidbase.com, 2008 release). Where available, EST data were used to verify the coding sequence of each candidate. To control for possible erroneous annotation, multiple sequence alignments were performed and clustering patterns on individual phylogenetic trees were noted. Gene names were assigned following the closest *Drosophila* orthologue.

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References

Adams, C.M., Anderson, M.G., Motto, D.G., Price, M.P., Johnson, W.A. and Welsh, M.J. (1998) Ripped pocket and pickpocket, novel *Drosophila* DEG/ENaC subunits expressed in early development and in mechanosensory neurons. *J Cell Biol* **140**: 143–152.

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatideo, P.G. *et al.* (2000) The genome sequence of *Drosophila melanogaster*. *Science* **287**: 2185–2195.

Anthony, N., Unruh, T., Ganser, D. and Ffrench-Constant, R. (1998) Duplication of the Rdl GABA receptor subunit gene in an insecticide-resistant aphid, Myzus persicae. *Mol Gen Genet* **260**: 165–175.

Bennett, J.A. and Dingledine, R. (1995) Topology profile for a glutamate receptor: three transmembrane domains and a channel-lining reentrant membrane loop. *Neuron* **14**: 373–384.

Bian, S., Favre, I. and Moczydlowski, E. (2001) Ca2+-binding activity of a COOH-terminal fragment of the Drosophila BK channel involved in Ca2+-dependent activation. *Proc Natl Acad Sci U S A* **98**: 4776–4781.

Bowery, N.G. and Smart, T.G. (2006) GABA and glycine as neurotransmitters: a brief history. *Br J Pharmacol* **147**: S109–S119.

Buckingham, S.D., Kidd, J.F., Law, R.J., Franks, C.J. and Sattelle, D.B. (2005) Structure and function of two-pore-domain K+ channels: contributions from genetic model organisms. *Trends Pharmacol Sci* **26**: 361–367.

Catterall, W.A. (2000) From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* **26**: 13–25.

Chentsova, N.A., Gruntenko, N.E., Bogomolova, E.V., Adonyeva, N.V., Karpova, E.K. and Rauschenbach, I.Y. (2002) Stress response in *Drosophila melanogaster* strain inactive with decreased tyramine and octopamine contents. *J Comp Physiol [B]* **172**: 643–650.

Davies, T.G.E., Field, L.M., Usherwood, P.N.R. and Williamson, M.S. (2007a) A comparative study of voltage-gated sodium channels in the Insecta: implications for pyrethroid resistance in *Anopheline* and other Neopteran species. *Insect Mol Biol* **16**: 361–375.

Davies, T.G.E., Field, L.M., Usherwood, P.N.R. and Williamson, M.S. (2007b) DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life* **59**: 151–162.

Derst, C., Messutat, S., Walther, C., Eckert, M., Heinemann, S.H. and Wicher, D. (2003) The large conductance Ca^{2+}-activated potassium channel (pSlo) of the cockroach *Periplaneta americana*: structure, localization in neurons and electrophysiology. *Eur J Neurosci* **17**: 1197–1212.

Derst, C., Walther, C., Veh, R.W., Wicher, D. and Heinemann, S.H. (2006) Four novel sequences in *Drosophila melanogaster* homologous to the auxiliary Para sodium channel. *Biochem Biophys Res Commun* **339**: 938–948.

Dickman, D.K., Kurshan, P.T. and Schwarz, T.L. (2008) Mutations in a *Drosophila* alpha2delta voltage-gated calcium channel subunit reveal a crucial synaptic function. *J Neurosci* **28**: 31–38.

Du, Y., Liu, Z., Nomura, Y., Khambay, B. and Dong, K. (2006) An alanine in segment 3 of domain III (IIIS3) of the cockroach sodium channel contributes to the low pyrethroid sensitivity of an alternative splice variant. *Insect Biochem Mol Biol* **36**: 161–168.

Evans, J.M., Allan, A.K., Davies, S.A. and Dow, J.A. (2005) Sulphonylurea sensitivity and enriched expression implicate inward rectifier K+ channels in *Drosophila melanogaster* renal function. *J Exp Biol* **208**: 3771–3783.

Gao, J.R., Deacutis, J.M. and Scott, J.G. (2007) The nicotinic acetylcholine receptor subunits Mdalpha5 and Mdbeta3 on autosome 1 of *Musca domestica* are not involved in spinosad resistance. *Insect Mol Biol* **16**: 691–701.

Georgiev, P., Garcia-Murillas, I., Ulahannan, D., Hardie, R.C. and Raghu, P. (2005) Functional INAD complexes are required to mediate degeneration in photoreceptors of the *Drosophila* rdgA mutant. *J Cell Sci* **118**: 1373–1384.

Goodman, M.B. (2003) Sensation is painless. *Trends Neurosci* **26**: 643–645.

Grabner, M., Wang, Z., Mitterdorfer, J., Rosenthal, F., Charnet, P., Savchenko, A. *et al.* (1994) Cloning and functional expression of a neuronal calcium channel beta subunit from house fly (*Musca domestica*). *J Biol Chem* **269**: 23668–23674.

Grauso, M., Reenan, R.A., Culetto, E. and Sattelle, D.B. (2002) Novel putative nicotinic acetylcholine receptor subunit genes, Dalpha5, Dalpha6 and Dalpha7, in *Drosophila melanogaster* identify a new and highly conserved target of adenosine deaminase acting on RNA-mediated A-to-I pre-mRNA editing. *Genet* **160**: 1519–1533.

Green, W.N. and Wanamaker, C.P. (1997) The role of the cystine loop in acetylcholine receptor assembly. *J Biol Chem* **272**: 20945–20953.

Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K. *et al.* (1987) The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature* **328**: 215–220.

Guy, H.R., Durell, S.R., Warmke, J., Drysdale, R. and Ganetzky, B. (1991) Similarities in amino acid sequences of *Drosophila* eag and cyclic nucleotide-gated channels. *Science* **254**: 730.

Hamilton, S.L. (2005) Ryanodine receptors. *Cell Calcium* **38**: 253–260.

Hardie, R.C. and Minke, B. (1992) The trp gene is essential for a light-activated Ca^{2+} channel in Drosophila photoreceptors. *Neuron* **8**: 643–651.

Hasan, G. and Rosbash, M. (1992) Drosophila homologs of two mammalian Ca^{2+}-release channels: identification and expression patterns of the inositol 1,4,5-triphosphate and the ryanodine receptor genes. *Development* **116**: 967–975.

Hollmann, M. and Heinemann, S. (1994) Cloned glutamate receptors. *Annu Rev Neurosci* **17**: 31–108.

Hollmann, M., Maron, C. and Heinemann, S. (1994) N-Glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluR1. *Neuron* **13**: 1331–1343.

Hosie, A.M., Buckingham, S.D., Presnail, J.K. and Sattelle, D.B. (2001) Alternative splicing of a *Drosophila* GABA receptor subunit gene identifies determinants of agonist potency. *Neuroscience* **102**: 709–714.

Huettner, J.E. (2003) Kainate receptors and synaptic transmission. *Prog Neurobiol* **70**: 387–407.

Jasti, J., Furukawa, H., Gonzales, E.B. and Gouaux, E. (2007) Structure of acid-sensing ion channel 1 at 1.9A resolution and low pH. *Nature* **449**: 316–322.

Jeschke, P. and Nauen, R. (2008) Neonicotinoids-from zero to hero in insecticide chemistry. *Pest Manag Sci* **64**: 1084–1098.

Jones, A.K. and Sattelle, D.B. (2007) The cys-loop ligand-gated ion channel gene superfamily of the red flour beetle, *Tribolium castaneum*. *BMC Genomics* **8**: 327.

Jones, A.K., Grauso, M. and Sattelle, D.B. (2005) The nicotinic acetylcholine receptor gene family of the malaria mosquito, *Anopheles gambiae*. *Genomics* **85**: 176–187.

Jones, A.K., Raymond-Delpechm, V., Thany, S.H., Gauthier, M. and Sattelle, D.B. (2006) The nicotinic acetylcholine receptor gene family of the honey bee, *Apis mellifera*. *Genome Res* **16**: 1422–1430.

Jones, A.K., Brown, L.A. and Sattelle, D.B. (2007) Insect nicotinic acetylcholine receptor gene families: from genetic model organism to vector, pest and beneficial species. *Invert Neurosci* **7**: 67–73.

Kash, T.L., Trudell, J.R. and Harrison, N.L. (2004) Structural elements involved in activation of the gamma-aminobutyric acid type A (GABAA) receptor. *Biochem Soc Trans* **32**: 540–546.

Kellenberger, S. and Schild, L. (2002) Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. *Physiol Rev* **82**: 735–767.

Kernan, M., Cowan, D. and Zuker, C.S. (1994) Genetic dissection of mechanosensory transduction: mechanoreception-defective mutants of *Drosophila*. *Neuron* **12**: 1195–1206.

Kim, J., Chung, Y.D., Park, D.Y., Choi, S., Shin, D.W., Soh, H. *et al.* (2003) A TRPV family ion channel required for hearing in *Drosophila*. *Nature* **424**: 81–84.

Kulkarni, N.H., Yamamoto, A.H., Robinson, K.O., Mackay, T.F.C. and Anholt, R.R.H. (2002) The DSC1 channel, encoded by the smi60E locus, contributes to odor-guided behavior in *Drosophila melanogaster*. *Genet* **161**: 1507–1516.

Lansdell, S.J. and Millar, N.S. (2000) Cloning and heterologous expression of Dalpha4, a *Drosophila* neuronal nicotinic acetylcholine receptor subunit: identification of an alternative exon influencing the efficiency of subunit assembly. *Neuropharmacology* **39**: 2604–2614.

Lansdell, S.J. and Millar, N.S. (2002) Dbeta3, an atypical nicotinic acetylcholine receptor subunit from *Drosophila:* molecular

cloning, heterologous expression and coassembly. *J Neurochem* **80**: 1009–1018.

Lear, B.C., Lin, J.-M., Keath, J.R., McGill, J.J., Raman, I.M. and Allada, R. (2005) The ion channel narrow abdomen is critical for neural output of the Drosophila Circadian pacemaker. *Neuron* **48**: 965–976.

Lee, Y., Lee, Y., Lee, J., Bang, S., Hyun, S., Kang, J. *et al.* (2005) Pyrexia is a new thermal transient receptor potential channel endowing tolerance to high temperatures in *Drosophila melanogaster*. *Nat Genet* **37**: 305–310.

Lerma, J. (2003) Role and rules of kainate receptors in synaptic transmission. *Nat Rev Neurosci* **4**: 481–495.

Lin, H., Mann, K.J., Starostina, E., Kinser, R.D. and Pikielny, C.W. (2005) A *Drosophila* DEG/ENaC channel subunit is required for male response to female pheromones. *Proc Natl Acad Sci U S A* **102**: 12831–12836.

Littleton, J.T. and Ganetzky, B. (2000) Ion channels and synaptic organization: analysis of the *Drosophila* genome. *Neuron* **26**: 35–43.

Liu, L., Johnson, W.A. and Welsh, M.J. (2003a) *Drosophila* DEG/ENaC pickpocket genes are expressed in the tracheal system, where they may be involved in liquid clearance. *Proc Natl Acad Sci U S A* **100**: 2128–2133.

Liu, L., Leonard, A.S., Motto, D.G., Feller, M.A., Price, M.P., Johnson, W.A. *et al.* (2003b) Contribution of *Drosophila* DEG/ENaC genes to salt taste. *Neuron* **39**: 133–146.

Liu, L., Li, Y., Wang, R., Yin, C., Dong, Q., Hing, H. *et al.* (2007) *Drosophila* hygrosensation requires the TRP channels water witch and nanchung. *Nature* **450**: 294–298.

Liu, Z., Chung, I. and Dong, K. (2001) Alternative splicing of the BSC1 gene generates tissue-specific isoforms in the German cockroach. *Ins Biochem Mol Biol* **31**: 703–713.

Martinez-Torres, D., Foster, S.P., Field, L.M., Devonshire, A.L. and Williamson, M.S. (1999) A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Insect Mol Biol* **8**: 339–346.

Matsuda, K., Buckingham, S.D., Freeman, J.C., Squire, M.D., Baylis, H.A. and Sattelle, D.B. (1998) Effects of the α subunit on imidacloprid sensitivity of recombinant nicotinic acetylcholine receptors. *Br J Pharmacol* **123**: 518–524.

Montell, C. (2005) Drosophila TRP channels. *Pflugers Arch* **451**: 19–28.

Nash, H.A., Scott, R.L., Lear, B.C. and Allada, R. (2002) An unusual cation channel mediates photic control of locomotion in *Drosophila*. *Curr Biol* **12**: 2152–2158.

Nayeem, N., Green, T.P., Martin, I.L. and Barnard, E.A. (1994) Quaternary structure of the native GABAA receptor determined by electron microscopic image analysis. *J Neurochem* **62**: 815–818.

Ocorr, K., Reeves, N.L., Wessells, R.J., Fink, M., Chen, H.S., Akasaka, T. *et al.* (2007) KCNQ potassium channel mutations cause cardiac arrhythmias in Drosophila that mimic the effects of aging. *Proc Natl Acad Sci U S A* **104**: 3943–3948.

Olson, R.O., Liu, Z.Q., Nomura, Y., Song, W.Z. and Dong, K. (2008) Molecular and functional characterization of voltage-gated sodium channel variants from *Drosophila melanogaster*. *Insect Biochem Mol Biol* **38**: 604–610.

Page, R.D. (1996) TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**: 357–358.

Perry, T., McKenzie, J.A. and Batterham, P. (2007) A *Dα6* knockout strain of *Drosophila melanogaster* confers a high level of resistance to spinosad. *Insect Biochem Mol Biol* **37**: 184–188.

Phillips, A.M., Bull, A. and Kelly, L.E. (1992) Identification of a *Drosophila* gene encoding a calmodulin-binding protein with homology to the trp phototransduction gene. *Neuron* **8**: 631–642.

Raymond-Delpech, V., Matsuda, K., Sattelle, B.M., Rauh, J.J. and Sattelle, D.B. (2005) Ion channels: molecular targets of neuroactive insecticides. *Invert Neurosci* **5**: 119–133.

Roche, K.W., Raymond, L.A., Blackstone, C. and Huganir, R.L. (1994) Transmembrane topology of the glutamate receptor subunit GluR6. *J Biol Chem* **269**: 11682.

Rosenzweig, M., Brennan, K.M., Tayler, T.D., Phelps, P.O., Patapoutian, A. and Garrity, P.A. (2005) The Drosophila ortholog of vertebrate TRPA1 regulates thermotaxis. *Genes Dev* **19**: 419–424.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**: 406–425.

Salkoff, L., Butler, A., Wei, A., Scavarda, N., Giffen, K., Ifune, C. *et al.* (1987) Genomic organization and deduced amino acid sequence of a putative sodium channel gene in *Drosophila*. *Science* **237**: 744–748.

Sattelle, D.B., Cordova, E. and Cheek, T.R. (2008) Insect Ryanodine Receptors: molecular targets for novel pest control agents. *Invert Neurosci* **8**: 107–119.

Schofield, P.R., Darlison, M.G., Fujita, N., Burt, D.R., Stephenson, F.A., Rodriguez, H. *et al.* (1987) Sequence and functional expression of the GABAA receptor shows a ligand-gated receptor super-family. *Nature* **328**: 221–227.

Shao, Y.M., Dong, K. and Zhang, C.X. (2007) The nicotinic acetylcholine receptor gene family of the silkworm, *Bombyx mori*. *BMC Genomics* **8**: 324.

Sieghart, W. (1995) Structure and pharmacology of gamma-aminobutyric acid a receptor subtypes. *Pharmacol Rev* **47**: 181–234.

Stephenson, F.A., Cousins, S.L. and Kenny, A.V. (2008) Assembly and forward trafficking of NMDA receptors (Review). *Mol Membr Biol* **25**: 311–320.

Sumikawa, K. and Gehle, V.M. (1992) Assembly of mutant subunits of the nicotinic acetylcholine receptor lacking the conserved disulfide loop structure. *J Biol Chem* **267**: 6286–6290.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl Acids Res* **25**: 4876–4882.

Tracey, W.D., Wilson, R.I., Laurent, G. and Benzer, S. (2003) Painless, a *Drosophila* gene essential for nociception. *Cell* **113**: 261–273.

Walker, R.G., Willingham, A.T. and Zuker, C.S. (2000) A Drosophila mechanosensory transduction channel. *Science* **287**: 2229–2234.

Wang, X.J., Reynolds, E.R., Déak, P. and Hall, L.M. (1997a) The seizure locus encodes the *Drosophila* homolog of the HERG potassium channel. *J Neurosci* **17**: 882–890.

Wang, Y., Small, D.L., Stanimirovic.D.B., Morley, P. and Durkin, J.P. (1997b) AMPA receptor-mediated regulation of a G_i-protein in cortical neurons. *Nature* **389**: 502–504.

Warmke, J.W. and Ganetzky, B. (1994) A family of potassium channel genes related to eag in Drosophila and mammals. *Proc Natl Acad Sci U S A* **91**: 3438–3442.

Warmke, J.W., Reenan, R.A.G., Wang, P., Qian, S., Arena, J.P., Wang, J. *et al.* (1997) Functional expression of *Drosophila para* sodium channels: modulation by the membrane protein tipE and toxin Pharmacology. *J Gen Physiol* **110**: 119–133.

Woodward, R.M., Polenzani, L. and Miledi, R. (1993) Characterization of bicuculline/baclofen-insensitive (rho-like) gamma-aminobutyric acid receptors expressed in Xenopus oocytes. II. Pharmacology of gamma-aminobutyric acidA and gamma-aminobutyric acidB receptor agonists and antagonists. *Mol Pharmacol* **43**: 609–625.

Xu, X.Z., Chien, F., Butler, A., Salkoff, L. and Montell, C. (2000) TRPgamma, a Drosophila TRP-related subunit, forms a regulated cation channel with TRPL. *Neuron* **26**: 647–657.

Zhou, W., Chung, I., Liu, Z., Goldin, A. and Dong, K. (2004) A voltage gated calcium-selected channel encoded by a sodium channel-like gene. *Neuron* **42**: 101–112.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI 10.1111/j.1365-2583.2009.00975.x

Figure S1. Phylogenetic tree showing relationships of *Apis pisum* and *Drosophila melanogaster* calcium channel protein sequences. Numbers at each node signify bootstrap values with 1000 replicates and the scale bar represents substitutions per site. The proteins shown in the tree (as well as GenBank accession numbers) are as follows: Dmel Alpha 1T (NP_572296), Dmel Alpha 1D (NP_602305), Dmel Alpha Cacophony (NP_511133), Dmel Ca Beta 1 (NP_523546), Dmel CG12295 (NP_610902), Dmel CG4587 (NP_001097163), Dmel CG12455 (NP_609779) and Dmel CG13762 (NP_570013).The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) available with the CLUSTALX program (Thompson *et al*., 1997) and displayed using TreeView (Page, 1996).

Figure S2. Phylogenetic tree showing relationships of *Acythosiphon pisum* and *Drosophila melanogaster* potassium channel protein sequences. Numbers at each node signify bootstrap values with 1000 replicates and the scale bar represents substitutions per site. The proteins shown in the tree (as well as GenBank accession numbers) are as follows: Dmel Slo (NP_524486), Dmel Slack (NP_001097259), Dmel KCNQ (NP_788300), Dmel ShaWl (NP_001097131), Dmel ShaW (NP_476721), Dmel ShaB (NP_728783), Dmel Shaker (NP_728124), Dmel ShaI (NP_524159), Dmel Ih (NP_001033947),Dmel Seizure (NP_476713),Dmel Eag (NP_511158),Dmel Egl (NP_477009),Dmel SK (NP_726988),Dmel Irk1 (NP_651131), Dmel Irk2(NP_651149) and Dmel Irk3 (NP_609903). The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) available with the CLUSTALX program (Thompson *et al*., 1997) and displayed using TreeView (Page, 1996).

Figure S3. Phylogenetic tree of the NMDA receptors in *Drosophila melanogaster* and *Acyrthosiphon pisum.* Tree showing relationships of *A. pisum*, *Apis mellifera* and *D. melanogaster* NMDA receptors protein sequences. Numbers at each node signify bootstrap values with 1000 replicates and the scale bar represents substitutions per site. The proteins shown in the tree (as well as GenBank accession numbers) are as follows: DmelNmdar1 (NP_730940), DmelNmdar2 (NP_001014716), DmelCG3814 (NP_610824), DmelCG30379 (NP_724626), AmelNmdar1 (NP_001011573), AmelNmdar2 (XP_396271), Amel CG3814 (XP_391854), AmelNmdar3 (XP_624931). The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) available with the CLUSTALX program (Thompson *et al*., 1997) and displayed using MEGA.

Figure S4. Phylogenetic tree of the kainate receptors in *Drosophila melanogaster* and *Acyrthosiphon pisum.* Tree showing relationships of *A. pisum*, *Apis mellifera* and *D. melanogaster* kainate receptors protein sequences. Numbers at each node signify bootstrap values with 1000 replicates and the scale bar represents substitutions per site. The proteins shown in the tree (as well as GenBank accession numbers) are as follows: DmeCG5621 (NP_650927), Dmel KaiRIA (NP_651982), Dmel GluRIIE (NP_001036733), DmelCG3822 (NP_650925), Dmel CG9935 (NP_651931), Dmel CG11155 (NP_651941), Dmel Clumsy (NM_078886), Dmel GluRIIC (NP_608557), Dmel GluRIIA (NP_523484), Dmel GluRIIB (NP_523485), AmelCG3822-3 (XP_392185), AmelCG3822-1(XP_394265), AmelCG3822-2 (XP_394264). The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) available with the CLUSTALX program (Thompson *et al*., 1997) and displayed using MEGA.

Figure S5. Phylogenetic tree of the other ionotropic receptors in *Drosophila melanogaster* and *Acyrthosiphon pisum.* Tree showing relationships of *A. pisum*, *Apis mellifera* and *D. melanogaster* or other forms of ionotropic glutamate receptors protein sequences. Numbers at each node signify bootstrap values with 1000 replicates and the scale bar represents substitutions per site. The proteins shown in the tree (as well as GenBank accession numbers) are as follows: DmelCG32704 (NP_727328), DmelCG15627 (NP_608863), DmelCG42315 (NP_650924), DmelCG7385 (NP_649176), DmelCG2657 (NP_001097043), DmelCG6185 (NP_648455), DmelCG11775 (NP_649833), DmelCG17152 (NP_648548), DmelCG8533 (NP_649148), DmelCG33492 (NP_995744), DmelCG15732 (NP_572795), DmeCG15324 (NP_572411), DmelCG14076 (NP_649074), DmelCG10101 (NP_649720), DmelCG10633 (NP_647962), DmelCG31718 (NP_723585), DmelCG14586 (NP_649013), DmelCG14585 (NP_649012), AmelCG32704 (XP_393270), AmelCG15627 (XP_396400), AmelCG17274 (XP_624089), AmelCG7385 (XP_624096), AmelG6185 (XP_001123055), AmelCG7385 (XP_624096), AmelCG14076 (XP_001121834), AmelPlr2 (XP_395079). The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) available with the CLUSTALX program (Thompson *et al*., 1997) and displayed using MEGA.

Figure S6. Alternative splicing of exons in *Acyrthosiphon pisum* nAChR subunits. Comparison of alternative exons of *Drosophila melanogaster* and *A. pisum* α4 (a) and α6 (b) subunits. Aphid residues that differ from those of the orthologous Drosophila exon are underlined. The Cys-loop is marked by asterisks and loops involved in ligand binding (LpB and LpE) are indicated. (c) Putative splice variants of Apisumα7 which generate diversity in loop C (LpC) potentially affecting ACh binding and in transmembrane regions 1 and 2 (TM1 and TM2) potentially affecting ion channel properties.

Figure S7. Tree of sequence similarity showing the translated transient receptor potential sequences of *Acyrthosiphon pisum* and *Drosophila melanogaster.* A neighbour-joining tree was produced from a ClustalX alignment of the translated transient receptor potential sequences from *A. pisum* and *D. melanogaster*. *D. melanogaster* sequences were obtained from FlyBase (http://flybase.org), and are labelled according to their FlyBase gene symbol. Sequences from *A. pisum* were retrieved from the genome sequence reference assembly and putatively identified by a reciprocal TBLASTX search, using the *D. melanogaster* transient receptor potential sequences as the initial search queries. *A. pisum* sequences are labelled with the appropriate gene model reference number (with ACYPIT prefix) where available. Two additional *A. pisum* transient receptor potential sequences were found, and are labelled with the prefix Ap and an appropriate identity based on their similarity to *D. melanogaster* sequences. The partial translated sequences from the aphid genome were then aligned against the Transient Receptor Potential sequences from *Drosophila* using ClustalX (version 1.83). The ClustalX alignments were used to generate bootstrapped neighbour-joined trees, which were viewed using Treeview (version1.6.6) software.

Table S1. Sodium channels

Table S2. Calcium Channels

Table S3. Potassium channels

Table S4. Chloride channels

Table S5. Cyclic-gated nucleotide Channels

Table S6. Glutamate Receptors

Table S7. The Transient Receptor Potential channels of *Acyrthosiphon pisum* and *Drosophila melanogaster*.