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Evidence for the plasticity of arthropod signal transduction pathways

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Abstract

Metazoans are known to contain a limited, yet highly conserved, set of signal transduction pathways that instruct early developmental patterning mechanisms. Genomic surveys that have compared gene conservation in signal transduction pathways between various insects and *Drosophila* support the conclusion that these pathways are conserved in evolution. However, the degree to which individual components of signal transduction pathways vary among more divergent arthropods is not known. Here, we report our results of a survey of the genome of the two-spotted spider mite *Tetranychus urticae*, using a set of 294 *Drosophila* orthologs of genes that function in signal transduction. We find a third of all genes surveyed absent from the spider mite genome. We also identify several novel duplications that have not been previously reported for a chelicerate. In comparison with previous insect surveys, *Tetranychus* contains a decrease in overall gene conservation, as well as an unusual ratio of ligands to receptors and other modifiers. These findings suggest that gene loss and duplication among components of signal transduction pathways are common among arthropods and suggest that signal transduction pathways in arthropods are more evolutionarily labile than previously hypothesized.

Keywords

Arthropod comparative genomics; Chelicerate; Evolution; Development; Signal transduction

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Author contributions R.M.P. designed the study, performed the data analyses, and wrote the first draft manuscript. M.G. and L.M.N. participated in study design and data discussion. L.M.N. and P.C.E contributed to data analyses and writing the final version of the manuscript. All authors approved the final version of the manuscript.

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Introduction

A limited set of signal transduction pathways are known to pattern developing embryos throughout the metazoans. In model systems, where signal transduction pathways have been extensively studied, any given signaling pathway is composed of extracellular ligands, modifiers of these ligands, receptors and co-receptors, and varying numbers of cytoplasmic signal transducers. Ligand diversification for some pathways has been well studied. Surprisingly, little effort has been made toward a comparison of gene conservation within these signal transduction pathways across diverse taxa despite an increased number of metazoan genome sequences. The most comprehensive genomic surveys of signal transduction pathways are attributed to arthropods, with a focus on higher insects and conservation of key genes known to pattern *Drosophila melanogaster* during embryogenesis (Dearden et al. 2006; Shigenobu et al. 2010; Behura et al. 2011). As might be expected, based on the phylogenetic relationship between *Drosophila* and the species sampled, the majority of genes surveyed are conserved. Instances of lineage-specific gene duplications and losses were identified, demonstrating a perhaps unexpected degree of variation among signaling pathways. These results suggest that examination of the degree of conservation of *Drosophila* orthologs in the other major arthropod clades (Crustacea, Myriapoda, and Chelicerata) might reveal additional variation, as well as trends within signal transduction pathways during arthropod evolution.

The two-spotted spider mite *Tetranychus urticae* is one of several noninsect arthropods to have a recently completed genome project (Grbić et al. 2011). As a member of the phylogenetically basal group of arthropods, Chelicerata, *Tetranychus* is of taxonomic interest for comparative study. For example, *Tetranychus* forms its body segments sequentially like most arthropods, unlike the derived mode of development utilized by *Drosophila* in which all body segments form at once. In addition, *Tetranychus* contains several derived morphological features, including a reduced body plan achieved through a loss of posterior segments, a developmental delay of the fourth pair of walking legs, and silk spinnerets located on the anterior body tagma. With a sequenced genome (Grbić et al. 2011) and available methods for testing gene expression (Dearden et al. 2003) and embryonic gene silencing through RNA interference (RNAi) (Khila and Grbić 2007), *Tetranychus* represents a novel system to study evolution of developmental regulatory networks, including signal transduction pathways. As estimates place the Pancrustacea-Chelicerata time of divergence at ~725 million years ago (Pisani et al. 2004), it might be expected that there will be key differences in conservation of signal transduction pathways and other developmental processes between *Tetranychus*, *Drosophila*, and other insects.

Here, we present the first comprehensive characterization of six major signal transduction pathways (e.g., transforming growth factor-β (TGF-β), Wnt, Notch, Janus kinase/signal transducers and activators of transcription (JAK-STAT), receptor tyrosine kinase, and Hedgehog) in a chelicerate with a sequenced genome. The patterns of conservation of *Drosophila* orthologs within the insects, the unique morphology of *Tetranychus*, and the time of divergence between insects and chelicerates led us to hypothesize that conservation of similar pathway components in *Tetranychus* would differ from previous surveys in insects.

Our results show that *Tetranychus* contains a reduced number of *Drosophila* orthologs, an expansion of lineage-specific gene duplications, and suggest that conservation of signal transduction pathway genes used during development in arthropods may be more plastic than previously understood.

Materials and methods

Identification and annotation of T. urticae signal transduction pathway genes

Using the genome of *T. urticae* (Sanger sequencing, 8.05× coverage) (Grbić et al. 2011), BLAST (blastn, blastp) analysis was performed using the bioinformatics suite located at online resource for community annotation of eukaryotes (OrcAE) (Sterck et al. 2012). For the list of genes, GI sequences, BLAST scores, and *e*-values, please see Supplemental Table 1. Signal transduction pathway genes were determined from previous surveys (Dearden et al. 2006; Shigenobu et al. 2010), gene ontology annotations from FlyBase (http://www.flybase.org) (St Pierre et al. 2014), the Kyoto Encyclopedia of Gene and Genomes (http://www.genome.jp/kegg/pathway.html) (Kanehisa and Goto 2000), and the Interactive Fly (http://www.sdbonline.org/fly/aimain/1aahome.htm) (Brody 1999). Sequences were then curated from the National Center for Biotechnology Information (NCBI) database. We used an *e*-value requirement of ≤0.001 for our initial BLAST. All gene orthology calls were corroborated by reciprocal BLAST analyses against the NCBI nr and *D. melanogaster* protein databases, to identify the top *D. melanogaster* sequence. Protein sequences for phylogenies were initially aligned with MUSCLE, followed by Gblocks to eliminate poorly aligned regions (except where noted), with the following parameters allowing for smaller final blocks, gap positions in final alignment, and less strict flanking positions (Castresana 2000; Talavera and Castresana 2007). Phylogenetic trees were constructed with PhyML (Guindon et al. 2010). The following parameters were used: amino acid substitution model=WAG; proportion of invariable sites—estimated; number of categories of substitution rate=4. Statistical support for phylogenetic grouping was assessed by approximate likelihood ratio tests based on a Shimodaira-Hasegawa-like procedure (SH-aLRT) with scores shown in the tree. In the case where no homologous sequences could be found, *e*-values were decreased and/or tblastn or tblastx was used to confirm absences from the genomic database. Gene annotations were entered manually using OrcAE. Accession numbers for *D. melanogaster* and *T. urticae* genes are provided in Supplemental Table 1.

Results

Gene and major signal transduction pathways that are expected conserved

Many cellular signal transduction pathways are expected conserved in *Tetranychus* based on their fundamental role in development in arthropods and throughout the Metazoa. However, little is known about the variation within signaling pathways in arthropods. To test this, we examined the *Tetranychus* genome for the presence and absence of genes from six major *Drosophila* signal transduction pathways (TGF-β, Wnt, Notch, receptor tyrosine kinase (RTK), JAK-STAT, and Hedgehog) and compared their conservation to the honeybee *Apis mellifera* and the pea aphid *Acyrthosiphon pisum*. In addition, in specific cases, we

compared gene conservation in *Tetranychus* to the red flour beetle *Tribolium castaneum* and the deer tick *Ixodes scapularis*.

TGF-β—The TGF-β signaling pathway acts through a concentration gradient of secreted morphogens to regulate cell growth, proliferation, and differentiation during embryogenesis. Among the higher ordered developmental processes that TGF-β signaling regulates are dorsal-ventral polarity, patterning and growth of developing organs, and neuronal patterning.

In total, we identified 45 of 63 *Drosophila* TGF-β signaling pathway components in the *Tetranychus* genome (Table 1). We identified four of seven *Drosophila* TGF-β ligands, including *decapentaplegic* (*dpp*), *myoglianin* (*myo*), *activin-beta*, and *glass bottom boat*, with duplications of the latter two (Fig. 1). The ligands *maverick*, *Dawdle* (*Daw/ALP23*), and *screw* are missing, with the absence of screw expected as it is derived in the lineage leading to *Drosophila* (Van der Zee et al. 2008). Two additional TGF-β ligands (BMP-10 and *anti-dorsalizing morphogenetic protein*) found in *Tribolium*, *Apis*, and vertebrates (Shigenobu et al. 2010; Van der Zee et al. 2008) are not conserved in *Tetranychus*. Four of five *Drosophila* TGF-β receptors were identified: *baboon* (type I), *saxophone* (type I), and duplications of *thickveins* (type I) and *punt* (type II). We were unable to find the type II receptor *wishful thinking* (*wit*), also absent from the *Acyrthosiphon* genome (Shigenobu et al. 2010). Orthologs of *wit* are found in *Ixodes*, *Apis*, *Tribolium*, and *Drosophila*, where they cluster most closely with mouse BMP type II receptor (Van der Zee et al. 2008) (Fig. 2), suggesting *wit* has been independently lost in *Tetranychus* and *Acyrthosiphon*. We found Smad family cytoplasmic transducers *Mothers against dpp*, *Smad on X*, and *Medea*, but not the anti-Smad *daughters against dpp*. We found several extracellular modifiers known to modulate TGF-β signal activity through interactions with both ligand receptors, including the *dpp*-inhibitor *short gastrulation* (*sog*) (Holley et al. 1996; reviewed in Massagué and Chen 2000) and the activin-inhibitor *follistatin*. Additional extracellular modifiers that counteract ligand inhibitors and enhance the TGF-β signal were also searched for including, *crossveinless* (*cv1*/*tsg2*), the *sog* cleavage protein *tolloid* (*tld*), and its relative *tolloid-related* (*tlk*). While *cv1* and *tlk* are present, we did not find a homolog for *tld*.

Wnt—The Wnts are a highly conserved family of secreted growth factors involved in early developmental patterning, including embryonic induction, cell fate, polarity, and death. Wnt ligands have been implicated in arthropods such as *Tribolium* and the spider *Achaearanea* in the establishment of posterior segments (Bolognesi et al. 2008; McGregor et al. 2008).

We identified 60 of 84 *Drosophila* Wnt signal transduction pathway components (Table 1). In Wnt-producing cells, the growth factors are glycosylated and lipid-modified for proper secretion and signal activity (Willert et al. 2003; Zhai 2004). Except for *wnt8*/*D* (Ching et al. 2008), palmitoylation by the ER membrane-bound protein *porcupine* is required for recognition of *Drosophila* Wnts by *Wntless*, a second membrane-bound protein essential for further transport and secretion (Bartscherer et al. 2006; Bänziger et al. 2006; Herr and Basler 2012). Both ligand modifiers required for proper Wnt secretion were found. Conservation of Wnt ligand subfamilies in arthropods varies. Insects have been found to contain as few as six in *Anopheles* and *Acyrthosiphon*, on up to nine in *Tribolium*. Both crustaceans and chelicerates have been found to contain 12 (Janssen et al. 2010).

We identified eight sequences in *Tetranychus* with conserved Wnt domains, belonging to six subfamilies—*Wnt4*, *Wnt5*, *Wnt6*, *Wnt8*, *Wnt16*, and *WntA*, with three copies of *Wnt6* (Fig. 3). Surprisingly, we did not find an ortholog of the *wingless* (*wg*/*Wnt1*) ligand that is conserved throughout Arthropoda and thus represents the first reported absence in an arthropod. Duplications of Wnt ligands are rare, but lineage-specific duplications have been reported in the cnidarian *Nematostella*, vertebrates, and the spider *Achaearanea* (Janssen et al. 2010). The three copies of *Wnt6* present in *Tetranychus* represent the largest paralog group identified in an arthropod genome. Once secreted, Wnts bind to members from the frizzled family of transmembrane receptors and the LDL-receptor-related protein *arrow*. We found five of six *Drosophila* Wnt receptors, with additional duplications of the receptors *Van gogh*, *frizzled*, *frizzled-2*, and *arrow* (Fig. 4). Absence of ligand-receptor activity results in a complex of proteins (Axin, adenomatous polyposis coli (APC), and shaggy) phosphorylating and thus targeting the cytoplasmic transducer/transcription factor armadillo/β-catenin (arm) for degradation. Once the receptor binds to its respective ligand, the intracellular effector Disheveled is activated and blocks shaggy from phosphorylating arm. This allows hypophosphorylated arm to accumulate in the cytoplasm and promotes its translocation to the nucleus to regulate transcription with co-regulators, principally from the TCF/ LEF family of transcription factors. We found two copies of *disheveled* (*Dsh*), and three copies of *armadillo*/*β-catenin*. Of the three proteins that form the core β-catenin destruction complex, APC and shaggy homologs were present, but an Axin homolog is missing.

Notch—Notch and its ligands *Delta* and *Serrate* are transmembrane proteins that provide direct cell-cell communication. Notch signaling is required during neurogenesis in vertebrates and invertebrates and likely has an ancestral role in the establishment and maintenance of posterior segments in arthropods (Chesebro et al. 2013; Chipman and Akam 2008; Pueyo et al. 2008; Schoppmeier and Damen 2005; Stollewerk et al. 2003), a function similar to its role during vertebrate somitogenesis (Dequeant et al. 2006; Palmeirim et al. 1997).

A majority of the core *Drosophila* Notch signaling pathway is present in the *Tetranychus* genome (42/66) (Table 1). There are four copies of the *Notch* receptor and single copies of the ligands *Delta* and *Serrate* (Fig. 5). During trafficking to the cell membrane and upon ligand binding, Notch undergoes a tripartite series of cleavages (S1, S2, and S3) that results in activation and release from the cell membrane to the cytosol. The cleaved portion, or Notch intracellular domain, then translocates into the nucleus to regulate transcription. Among the proteases that participate in Notch cleavage, we found duplications of the S1 protease *furin-1* and the S3 γ-secretase component *anterior pharynx defective 1* (*APH-1*). Of the metalloproteases that participate in S2 extracellular cleavage of Notch, *Kuzbanian* (*Kuz*) is present, but not TNF-α converting enzyme (*TACE*). The loss of *TACE* appears to be lineage-specific as a homolog is present in *I. scapularis* (XP_002405453) and may be attributed to its redundancy in canonical Notch signaling. For example, *Kuz* and *TACE* have been reported both genetically and biochemically to have partially redundant functions in ecdysozoans (Brou et al. 2000; Mumm et al. 2000). In addition, *Drosophila TACE* does not appear to be required for most Notch-mediated cell decisions as loss of *Kuz*

leads to associated Notch phenotypes (Pan and Rubin 1997; Rooke et al. 1996), and overexpression of *TACE* is insufficient for restoring appropriate Notch S2 cleavage (Lieber 2002). Several other Notch protein regulators such as *deltex* and *mastermind* are also missing and have been reported absent in several other metazoan genomes suggesting Notch signaling functions in their absence (Gazave et al. 2009; Maier 2006; Shigenobu et al. 2010). The absence of *deltex* appears to be lineage-specific, as a homolog has been recorded for *Ixodes* (IscW_ISCW003605), and also suggests that *Tetranychus* lacks this noncanonical Notch signaling pathway. Additional missing regulators (Table 1) appear to be derived in *Drosophila* and other higher insects and have been expected absent in more basally branching arthropods. For example, the Notch antagonist *hairless* to date has only been found within dipterans and hymenopterans (Maier 2006).

We also identified several well-known transcriptional targets of Notch, including genes from the achaete-scute (AS-C) and enhancer of split complexes (E(spl)-C) (Schlatter and Maier 2005). The *Drosophila* AS-C is a 40-kb complex of four basic helix-loop-helix (bHLH) transcription factors, *achaete*, *scute*, *lethal of scute*, *and asense* (reviewed in García-Bellido and de Celis 2009). We found apparent duplications of both *achaete* and *scute* on multiple contigs (Supplemental Table 1) but did not find homologs of *lethal of scute* and *asense*. The E(spl)-C of *Drosophila* spans a 45-kb region and contains a group of four bearded family and seven bHLH transcription factors thought to have been duplicated from a pair of ancestral genes (reviewed in Dearden and Duncan 2010). We found two classes of bHLH members in tandem (tetur01g03010 and tetur01g03020; tetur08g02740 and tetur08g02780). Our phylogenetic analysis suggests that these may be part of an ancestral E(spl)-C or that these genes likely represent a lineage-specific duplication of a single ancestral bHLH gene (Supplemental Fig. 1). These findings parallel previous pancrustacean surveys that revealed similar reductions in the number of E(spl)-C genes, and none have been found in *Ixodes* (Dearden and Duncan 2010; Dearden et al. 2006; Schlatter and Maier 2005).

RTK (EGF/FGF/Sevenless)—RTK signaling contributes to changes in cell shape and terminal and dorsal patterning, among several other developmental mechanisms that all rely on intracellular MAP kinase activity. We searched for 50 *Drosophila* genes among three well-known RTK pathways: epidermal growth factor (EGF), fibroblast growth factor (FGF), and Sevenless. These pathways share a MAPK signal cascade that includes *Sos*, *Ras1*, *Raf1*, *Dsor1*, and *rolled*; all of which are conserved in *Tetranychus* (Table 1).

EGF signaling—Fourteen of 30 EGF signaling components were identified, with the core pathway conserved (Table 1). We found two of four ligands: *spitz* and *vein*. Of the four receptors searched for, we found single copies of epidermal growth factor receptor and *kekkon-1*, along with two *kekkon-3* orthologs. We found two copies of the extracellular EGFR inhibitor *argos* (*giant lens*), also found with four copies in *Acyrthosiphon*.

FGF signaling—The FGF signaling pathway appears highly modified in *Tetranychus* (Table 1). Three FGF ligands (*branchless*, *pyramus*, and *thisbe*) and two FGF receptors (*breathless* and *heartless*) have been identified in *Drosophila*. We did not find any ligands and only found a single FGF receptor, *heartless*. Similar modifications of FGF signaling has been reported for the genomes of three mosquito species, with *Aedes aegypti* missing the

ligand *branchless*, and all three mosquitoes missing the receptor *breathless*, and the *heartless* ligands *pyramus* and *thisbe*.

Sevenless signaling—Except for the absence of the canonical ligand *boss of Sevenless* (*boss*), the Sevenless signaling pathway is conserved in *Tetranychus* (Table 1). A review of the *Ixodes* genome also shows *boss* to be missing. The absence of *boss* is striking due to its role in determining cell fate during fly retina development (Krämer et al. 1991). Further inquiry into the developmental genetic mechanisms that underlie chelicerate eye development may provide further insight into whether *Tetranychus* is likely to have a functioning Sevenless pathway in the absence of this canonical ligand.

JAK/STAT—The JAK/STAT signaling pathway is a conserved, pleiotropic cell signaling pathway involved in cytokine transduction and growth factor signaling (Behura et al. 2011; Darnell 1997; Rawlings et al. 2004; Shigenobu et al. 2010; Zeidler et al. 2000) (Table 1). The pathway involves stimulation of a variety of receptors, each having a characteristic JAK tyrosine kinase attached. This results in the transphosphorylation of JAKs, which in turn phosphorylate STATs, a family of otherwise inactive transcription factors that reside in the cytoplasm. STATs then enter the nucleus and control expression of JAK/STAT target genes. We were unable to identify any previously identified JAK/STAT ligands (*os*, *upd2*, *upd3*). Interestingly, *Tetranychus* contains duplications of both *hopscotch* and *Stat92E*, which are classified as canonical JAK- and STAT-like proteins in *Drosophila* (Zeidler et al. 2000). *Stat92E* duplications are not unique to *Tetranychus* as three copies have been reported in the mosquito *Culex quinquefasciatus* (Behura:2011dv). Additional effectors of the JAK/STAT signaling pathway include protein families such as STAMs and SOCs, which activate and repress different pathway components, respectively (Rawlings et al. 2004). Orthologs of *Socs36E* and *Socs44A* were not found in our search of the genome, while *Socs16D* has duplicated. The JAK/STAT inhibitor phosphatidylinositol 3-kinase was also found present in multiple copies.

Hedgehog—The hedgehog (Hh) protein functions as a morphogen during development. The hedgehog ligand is highly lipid-modified; a cholesterol moiety is attached to the C-terminus, and the N-terminus undergoes palmitoylation. The covalent coupling of cholesterol to Hh acts to tether the ligand to the plasma membrane and regulate long-range signaling. High conservation of hedgehog signaling components has been observed in several higher insects, with each genome yielding a complete pathway (Dearden et al. 2006; Shigenobu et al. 2010). However, this trend does not hold for *Tetranychus*.

Eleven of 14 pathway components were found; the genome lacks *dispatched*, *costa*, and *fused* (Table 1). The absence of *dispatched* is striking as it is required for release of cholesterol anchored Hh from Hh-secreting cells (Burke et al. 1999). Curiously, *costa* and *fused* are essential components of the protein complex that, in unstimulated cells, collects the transcription factor *cubitus interruptus* (*ci*) in the cytoplasm (Alves et al. 1998; Farzana and Brown 2008; Sisson et al. 1997). This action encourages cleavage of the *ci* N-terminus before entering the nucleus, which conversely produces a repressive effect on targeted genes (Aza-Blanc et al. 1997). In stimulated cells, *smoothened* inhibits cleavage of *ci* and allows the full protein to enter the nucleus and activate target genes (Alexandre et al. 1996).

Without *costa* and *fused*, the *ci*-sequestering complex is incomplete, making the canonical cleavage of *ci* impossible in this system and allowing the constant transcription of Hh target genes. Reports of lineage-specific gene duplications are rare in this pathway. However, two copies of *supernumerary limbs* (*Slmb*) have been reported in the vector mosquito, *C. quinquefasciatus* (Behura:2011dv). *Tetranychus* also contains a duplication of *Slmb*, as well as a duplication of *Suppressor of fused*.

Comparison to previous insect surveys—Unique sets of genes from the six signaling pathways surveyed in *Tetranychus* have been previously surveyed in insects. Of the 294 *Drosophila* genes that we surveyed in the *Tetranychus* genome, 100 were surveyed in the hymenopteran honey bee *Apis mellifera* genome (Dearden et al. 2006), and 124 were surveyed in the more distantly related hemipteran pea aphid *Acyrthosiphon pisum* genome (Shigenobu et al. 2010) (Fig. 6). Sixty-two genes were found to overlap within all three sets (Fig. 6a, Supplemental Table 2). Of these genes, there appears to be a phylogenetic trend of conservation, with 73, 92, and 97 % conserved in *Tetranychus* (45/62), *Acyrthosiphon* (57/62), and *Apis* (60/62), respectively (Fig. 6b). Only two genes, *screw* and *gurken*, are missing from all three genomes, although these are expected absences as they are believed to have originated within Diptera (Dearden et al. 2006). *Acyrthosiphon* is missing three additional genes, of which one is shared with *Tetranychus* (*wnt10*). *Tetranychus* contains an additional 14 unique gene absences from the set of 62 genes shared among the three surveys. These results show that gene absences appear far more common in *Tetranychus*, both among the set of 62 genes shared among the three surveys and in the total number of genes surveyed.

In contrast, 33 of 124 genes surveyed in both *Acyrthosiphon* and *Tetranychus* genomes are found duplicated in *Acyrthosiphon* and *Tetranychus*, *Acyrthosiphon* alone, or *Tetranychus* alone (Table 2). Within this set of duplicated genes, *Tetranychus* contains almost twice the number of duplications as *Acyrthosiphon*. In total, however, these duplications represent less than half the number of duplications in signaling pathways found in the *Tetranychus* genome.

To determine whether the duplications found in *Tetranychus* represent lineage-specific duplications or are more broadly representative of duplications within acarid chelicerates, we searched the tick *I. scapularis* draft genome for similar sets of paralogs. The gene duplications *Tetranychus* that shares with *Acyrthosiphon* (*Su(H)*, *argos*, *arm*, and *Stat*) appear to have arisen independently in both lineages as only single orthologs were found in *Ixodes*. Of the remaining *Tetranychus* duplications, only two were found duplicated in the *Ixodes* genome: *gro* and *kek-3*. Our findings suggest that gene duplication and losses within signal transduction pathways are prominent features of the *Tetranychus* genome.

Discussion

We have completed a genomic survey in the two-spotted spider mite *T. urticae* to determine the degree of conservation of the genes involved in several major signal transduction pathways in arthropods. We used a sequence homology-based approach to identify genes as present or absent in the *Tetranychus* genome. In specific cases, our approach identifies genes

missing in *Drosophila* but present in other arthropods but does not identify genes specific to *Tetranychus*. Additionally, as with all genome sequencing projects, gene absences should be treated as putative due to the incomplete nature of current genome sequencing, assembly methods, and limitations using sequence homology to identify orthology. However, genuine gene absences in *Tetranychus* may arise from several scenarios. The first being the gene is derived only within *Drosophila* and/or higher insects. The second being the gene is present in other chelicerates but was subsequently lost in *Tetranychus*. We have identified genes that fall into both categories.

While it is understood that signal transduction pathways are conserved throughout the Metazoa, explorations of how much variation characterizes signal transduction pathways in arthropods that group more phylogenetically basal has not been previously explored. Conservation of *Drosophila* signal transduction pathway orthologs in insects has been found to range from 82 % in *Acyrthosiphon* (109/124) (Shigenobu et al. 2010) to 99 % in *Apis* (99/100) (Dearden et al. 2006). This conservation is higher than the 66 % conservation we report here for *Tetranychus* (195/294). Although the sample size is small, these datasets suggest that as evolutionary distance from *Drosophila* increases, gene conservation decreases, with gene conservation following a phylogenetic trend. The observed loss of conservation is not due to an increase in the sample size, as limiting the comparison to only the 62 genes shared among the three surveys follows a similar trend. We expect that analyses of the recently sequenced genomes from the crustacean *Daphnia pulex* and myriapod *Strigamia maritima* will provide support for this trend.

While many of the gene absences appear to be specific to the acarid chelicerate lineage as half the genes absent from *Tetranychus* are also absent from the *Ixodes* genome (16/33) (Supplemental Table 3), *Tetranychus* contains unexpected gene losses in every pathway we investigated. In several cases, these absences are incongruous with finding the core pathway conserved. For example, a fifth of all absences are attributed to ligands. For JAK/STAT, FGF, and Sevenless, these pathways are left without canonical ligands. Several other absences are known to be essential to proper signal reception (e.g., *breathless*), processing (e.g., *dispatched*), or transduction (e.g., *Axin*). Functional and genomic data provide insight into some, but not all, of the putative gene absences. In general, absent genes fall into two categories: those with partially redundant function in *Drosophila* (e.g., *TACE*) and those believed derived or not essential for signal transduction (e.g., *deltex*, *mastermind*, *hairless*, and *E(spl)-C* homologs). Nonetheless, many missing genes are known to be essential for proper signaling in *Drosophila*. While no functional data exists for these pathways in *Tetranychus*, observations of developmental stage and feeding RNA-seq data confirm that core pathway components are expressed. This suggests that the pathways function in the absence of what we assume to be essential components. This could be a consequence of the co-option of novel components into the pathway. Based on the number of protein coding genes predicted in the *Tetranychus* genome (~16,000, compared to the ~14,000 in *Drosophila*), it is possible for novel genes to act in these signaling pathways that are unidentifiable by sequence homology alone. Further analyses using structural homology-based searches may be of value in the identification of additional pathway components.

Also of particular interest is the absence of the Wnt signaling ligand *wg* from the *Tetranychus* genome. In *Drosophila*, *wg* functions in multiple developmental events including limb development (Cohen et al. 1993; Simcox et al. 1989), midgut morphogenesis (Mathies et al. 1994), and segmentation where it acts as a segment polarity gene (Nüsslein-Volhard et al. 1984). Loss of *wg* could therefore have dramatic consequences on developmental patterning and may be reflected in the reduced posterior segmentation of *Tetranychus*. However, functional analyses of *wg* from several insects suggest that unlike the segment polarity gene engrailed, which has a conserved function throughout Arthropoda, the role of *wg* within insects is evolving despite retaining a segmental expression pattern (Kraft and Jäckle 1994; Nagy and Carroll 1994). For example, RNAi depletion of *wg* in several insects that form posterior segments from a growth zone have shown it to have little to no effect on posterior segmentation (Bolognesi et al. 2008; Miyawaki et al. 2004; Ober and Jockusch 2006). In these arthropods, another Wnt ligand, *Wnt8*, of which a single copy is found in *Tetranychus*, appears to be important for segmentation. Functional data from *Tribolium* and the spider *Achaearanea tepidariorum* have shown *Wnt8* to be involved in establishing and maintaining segments as RNAi-treated samples lack a growth zone and posterior segments (Bolognesi et al. 2008; McGregor et al. 2008). While more diverse arthropod taxa await a functional analysis of *Wnt8*, it remains to be determined if this is merely a matter of functional convergence or if it is representative of an ancestral function in posterior segmentation. However, if *Wnt8* is involved in posterior segmentation in more phylogenetically basal arthropods, it is expected that changes in its regulation may contribute to the reduced posterior segmentation in *Tetranychus*.

In contrast with the number of genes absent, we also found *Tetranychus* signaling pathways to be characterized by unique duplications of paralogs within a given gene family. In general, gene duplication has played a prominent role during the evolution of signal transduction pathways. Many components from the signal transduction pathways present in extant arthropods are the result of ancestral duplications (e.g., the 12 arthropod Wnt ligand subfamilies (Janssen et al. 2010)). There are also cases of lineage-specific duplications in arthropod signaling pathways (Shigenobu et al. 2010; Van der Zee et al. 2008). Both *Tribolium* and *Drosophila* contain lineage-specific duplications of the TGF-β ligand *glass bottom boat*. The 11 and 4 members of the E(spl)-C from *Drosophila* and *Apis*, respectively, are believed to have arisen from an ancestral pair of genes found conserved in *Anopheles* (Dearden and Duncan 2010; Schlatter and Maier 2005). Eighteen signal transduction pathway genes are believed to have arose from lineage-specific duplications in *Acyrthosiphon* (Shigenobu et al. 2010). However, *Tetranychus* contains an increased number of unique genes present in multiple copies when directly compared against duplications identified from surveys in *Acyrthosiphon* and *Apis*. In *Tetranychus*, 30 genes are present with at least two copies. An additional six genes are present with three copies, and three genes are present with four copies (Supplemental Table 1). For example, receptors from each pathway are observed to have undergone duplications, and several pathways have multiple receptors duplicated. Likewise, duplications can be found for ligand modifiers, transducers, and transcription factors in each pathway. Similar, albeit larger in number, expansions in plant defense and pesticide resistance genes have occurred in *Tetranychus* (Grbić et al. 2011). In addition, the unusual ratio of ligand to receptors and modifiers may function

in pathway specificity. As signaling pathways rely on these proteins for specificity, gene duplication may provide a substrate for the expansion or refinement of pathway specificity through differences in ligand-receptor kinetics, combinatorial interactions, and novel targets of activity.

Conclusion

Our data provide additional evidence for the labile nature of arthropod signal transduction pathways and complement previous surveys by showing that gene loss and duplication are common themes during arthropod evolution. At the same time, our data is in contrast with the general view that the conservation of complete signal transduction pathways is fundamental to cellular and developmental processes in metazoans (Carroll et al. 2005; Pires-daSilva and Sommer 2003; Wilkins 2002). The lack of overall conservation of signal transduction pathway components and the amount of lineage-specific duplications suggests that novel genes may participate in these pathways. These components may not share sequence identity, instead sharing structural homologies, and are likely to be discovered as emerging arthropod models are better characterized at the developmental genetic level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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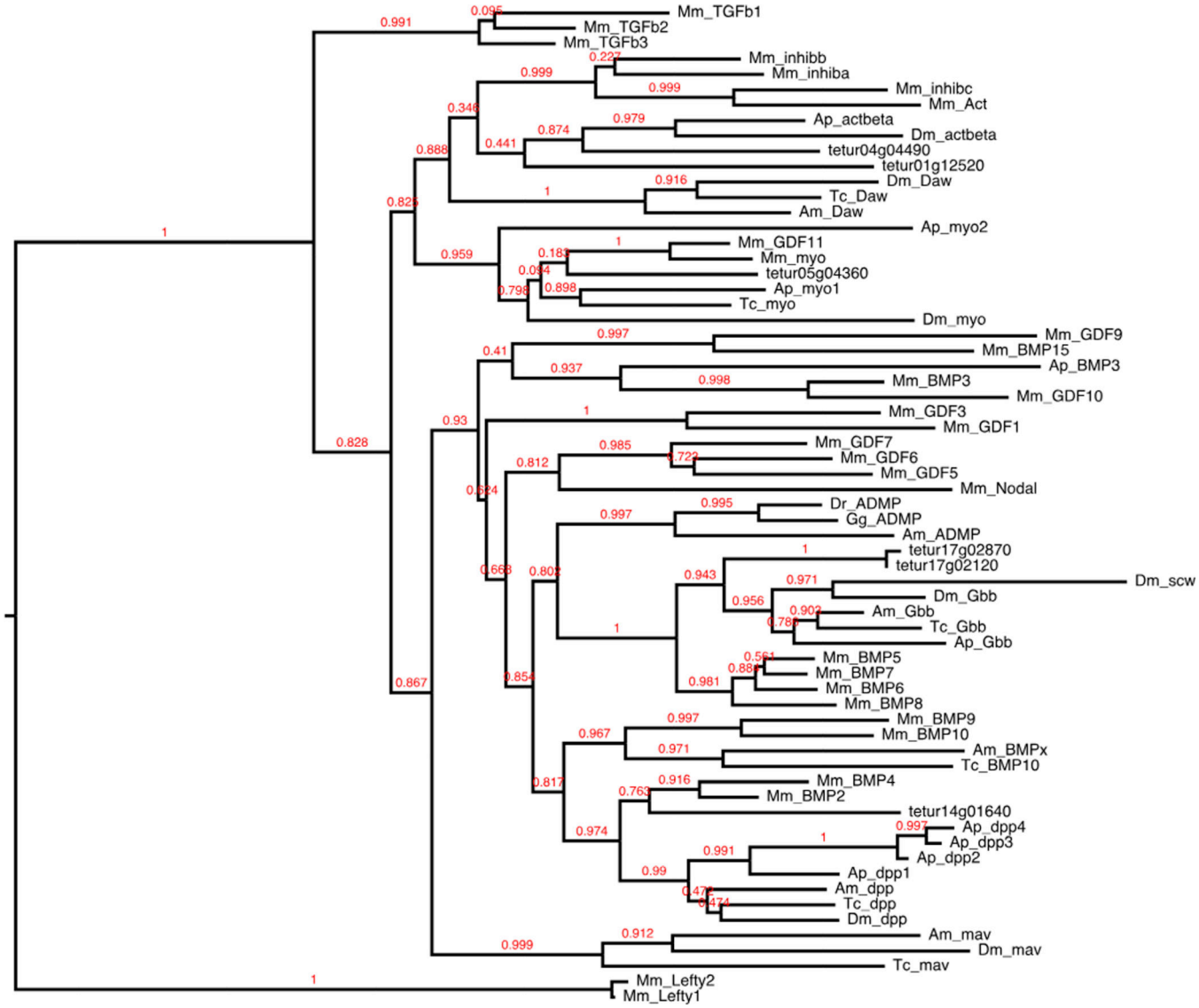

Fig. 1.

TGF-beta ligands. Full-length protein sequences were used to construct this maximum likelihood tree of TGF-beta ligands. Protein sequences for mouse (*Mm*), *Drosophila melanogaster* (*Dm*), *Tribolium castaneum* (*Tc*), and *Apis mellifera* (*Am*) were identified from the previous phylogenetic reconstruction of Van der Zee et al. (2008). *Acyrthosiphon pisum* (*Ap*) sequences were curated from Shigenobu et al. (2010) and from NCBI GenBank

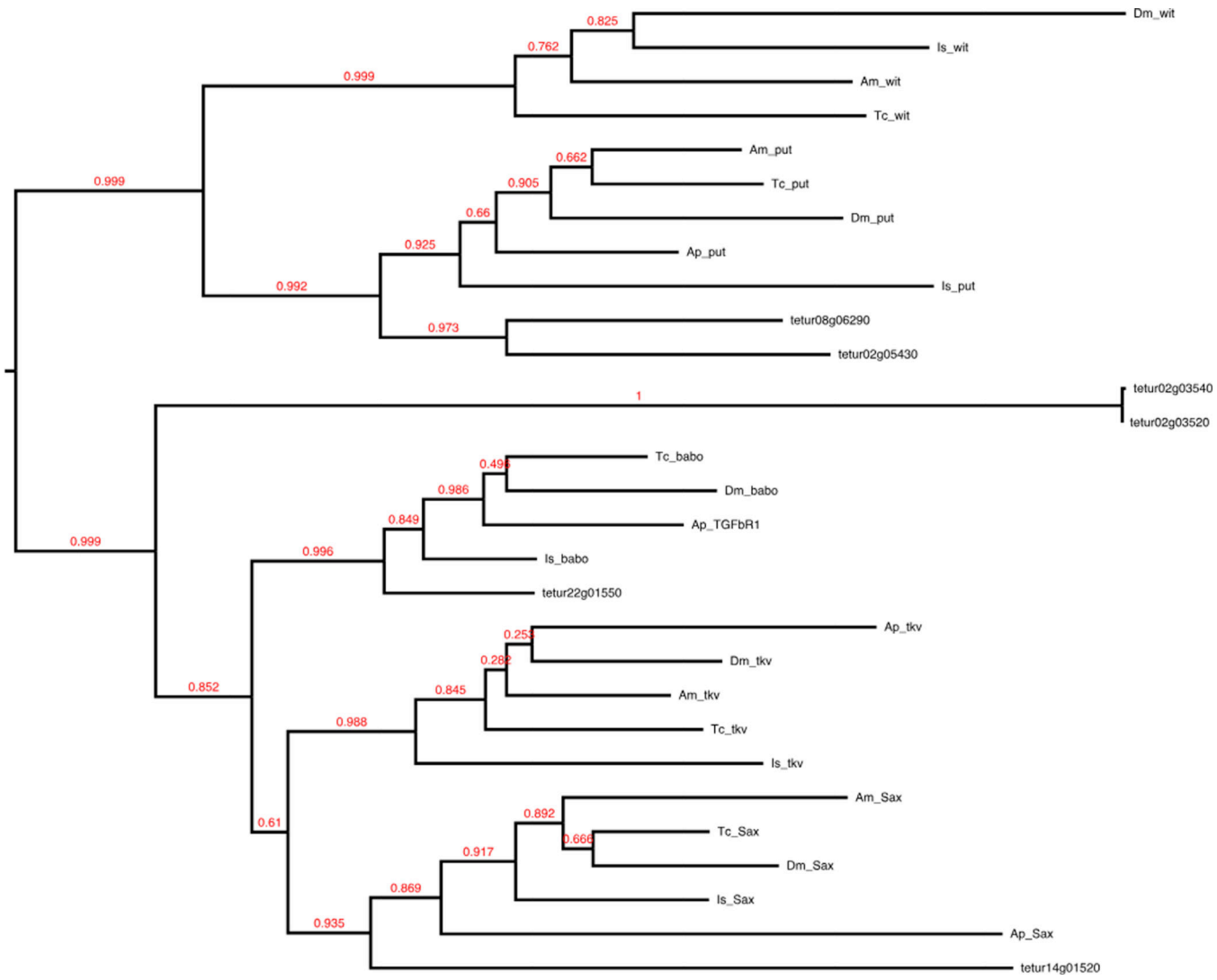

Fig. 2.

TGF-beta receptors. Full-length protein sequences were used to construct this maximum likelihood tree of TGF-beta receptors. *Tetranychus* thickveins paralogs group away from the thickveins cluster but with the type I receptors. Mm, Dm, Tc, and Am protein sequences were identified from references in Fig. 1. *Ixodes scapularis* (*Is*) sequences were curated from NCBI GenBank

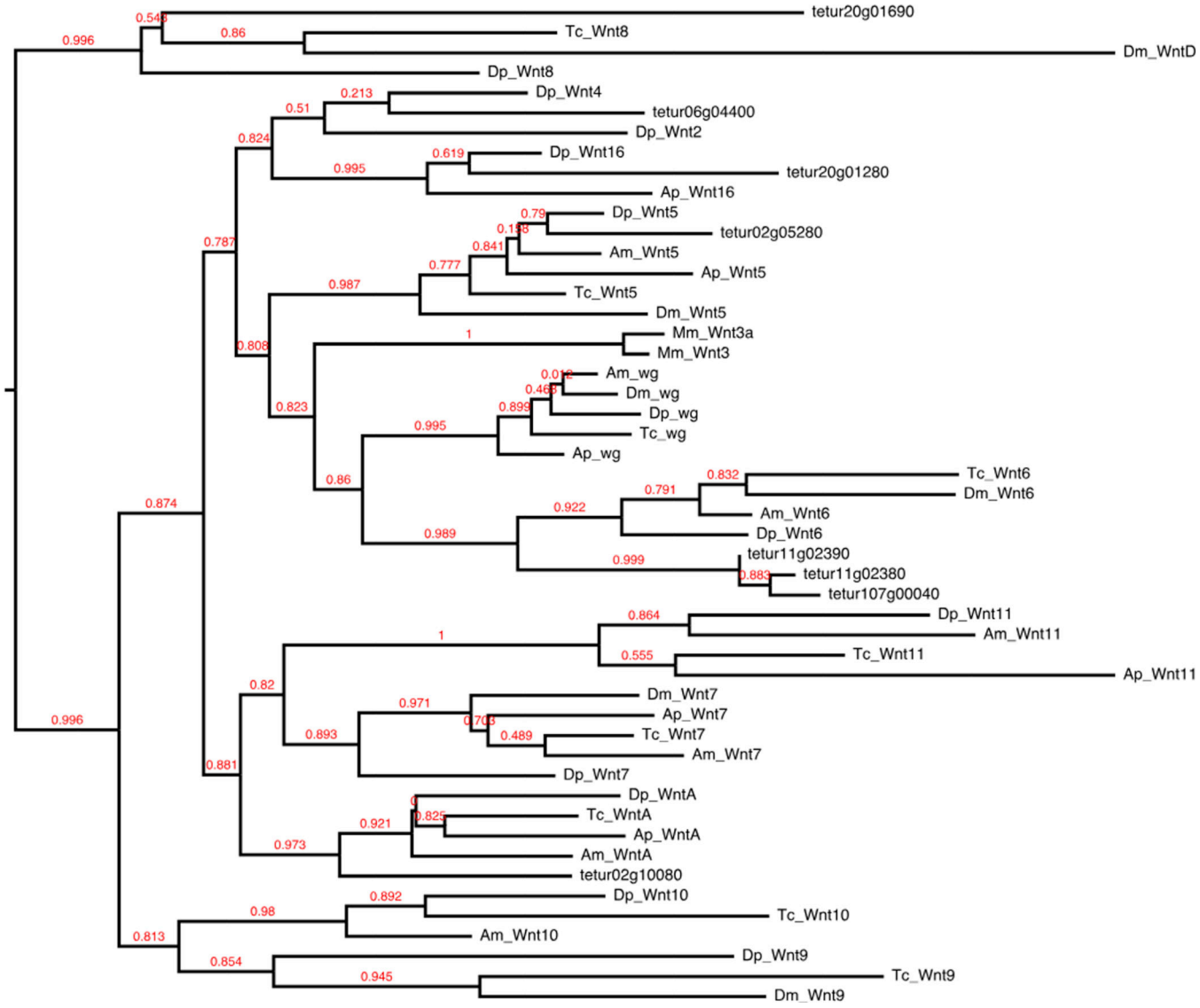

Fig. 3.

Wnt ligands. Mm, Dm, Tc, Am, Ap, and *Daphnia pulex* (*Dp*) protein sequences were identified from Janssen et al. (2010)

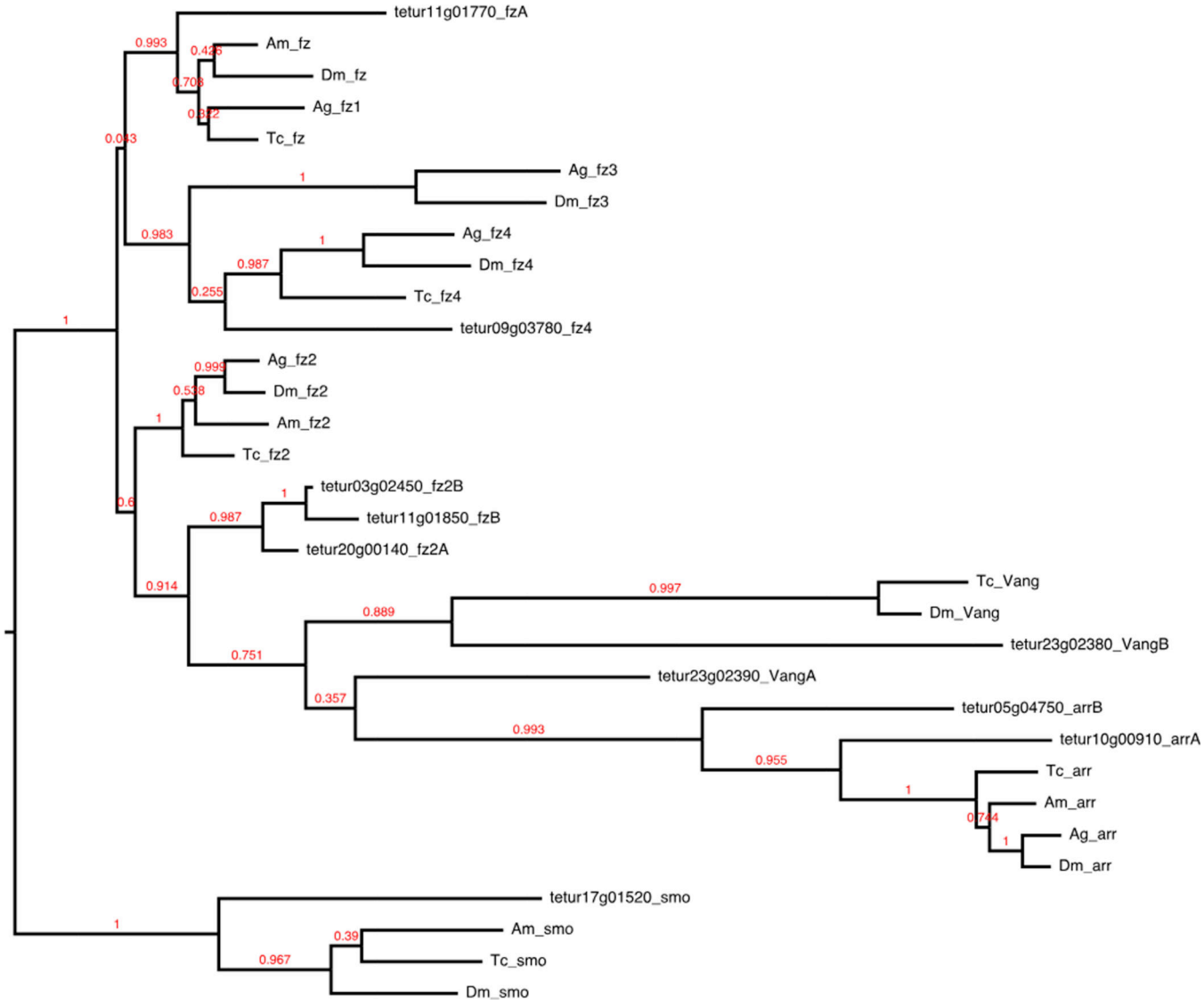

Fig. 4.

Wnt receptors. Dm, Tc, Am, and *Anopheles gambiae* (*Ag*) protein sequences were curated from NCBI GenBank

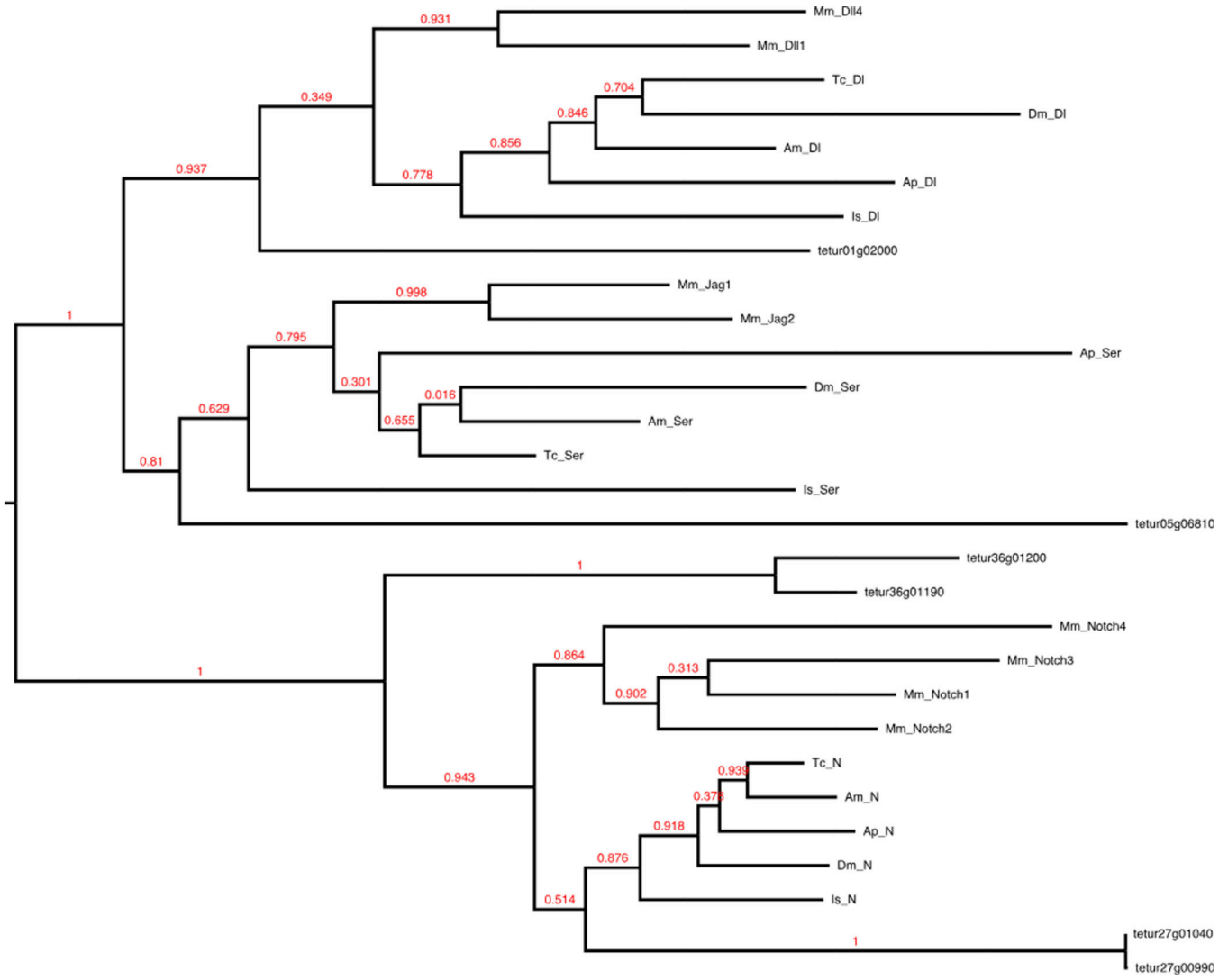

Fig. 5.

Notch receptors and ligands. Full-length protein sequences were used to construct this maximum likelihood tree of Notch receptors and ligands

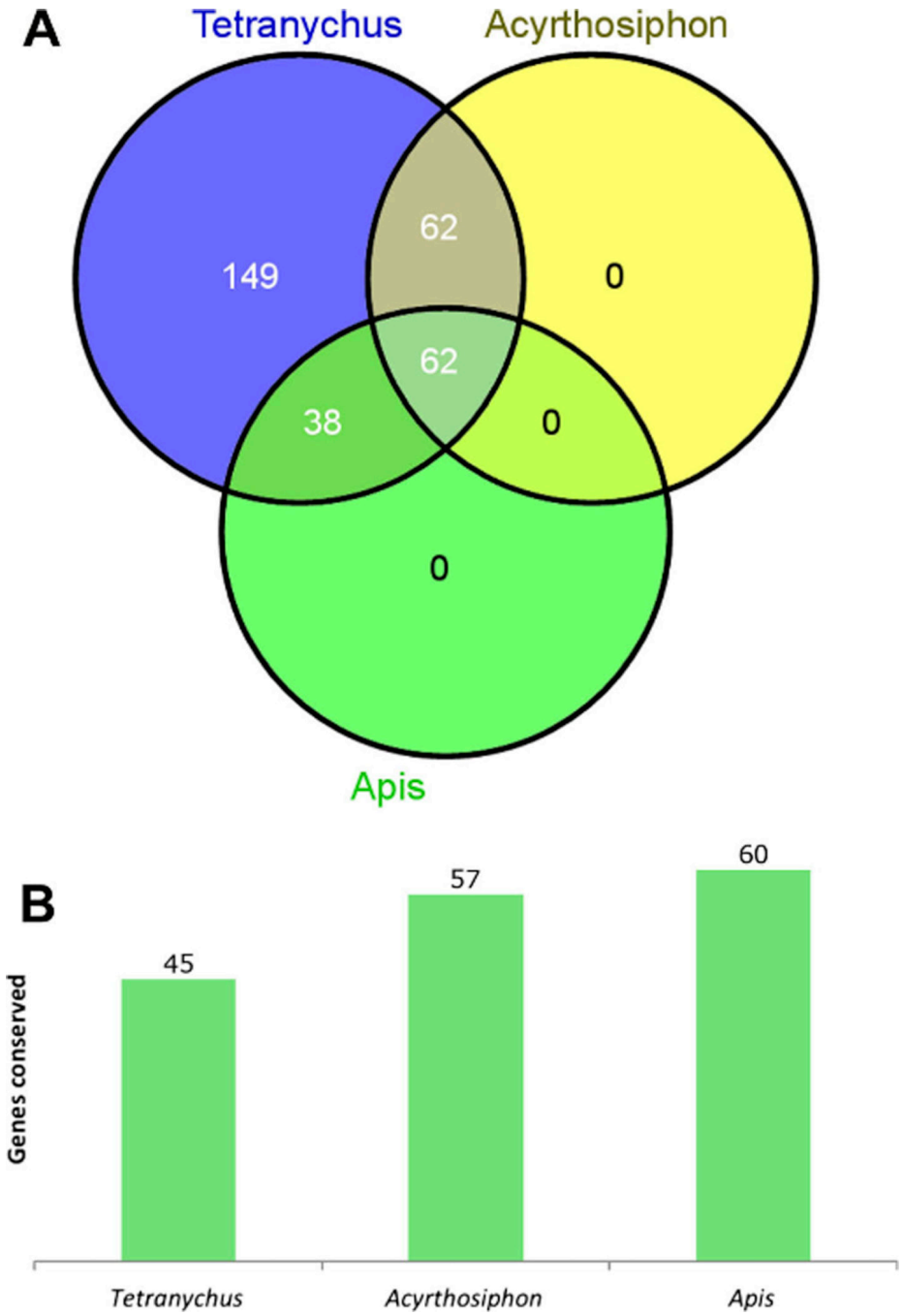

Fig. 6.

Number of genes conserved among genes shared in arthropod surveys. **a** Venn diagram of *Drosophila* genes surveyed for in *Tetranychus*, *Acyrthosiphon*, and *Apis*. **b** Number of shared *Drosophila* orthologs conserved among 62 genes surveyed for in the three arthropod surveys. *Tetranychus* (44/62), *Acyrthosiphon* (57/62), and *Apis* (60/62)

Table 1

Inventory list of key components from signal transduction pathways in *Tetranychus*

Class/function	Name	Duplication
TGF-beta		
Ligand	*dpp*	
	actbeta	Duplication (2)
	Gbb *myo*	Duplication (2)
Extracellular modifiers	*tlk*	Duplication (4)
	sog *fs*	Duplication (2)
Type I receptor	*babo*	
	tkv *Sax*	Duplication (2)
Type II receptor	*put*	Duplication (2)
R-SMAD	*Mad*	
R-SMAD	*Smox*	
Co-SMAD (common)	*Med*	
Transcription factor	*Rbf* *nej* *shn* *snoN*	
Smad binding	*fuss (CORL)* *Rocla* *cul/linl9* *Sara*	Duplication (2)
	lack	Duplication (2)
(absences)	*DAW,* *ADMP, scw, mav, BMP-10, wit, tld*	
Wnt		
Ligand	*Wnt4* *Wnt5*	
	Wnt6 *Wnt8* *Wnt 16* *WntA*	Duplication (3)
Transmcmbranc receptor	*Vang*	Duplication (2)
	fz	Duplication (2)
	fz2 *fz4* *arr*	Duplication (2)
Activated by frizzled	*dsh*	Duplication (2)
Arm destruction complex	*APC* *sgg*	Duplication (2)
Downstream effector	*arm*	Duplication (3)
(β-Catenin binding	*pont*	
Antagonist of β-catenin	*Cby*	
	pan	Duplication (3)
Transcription factor	*CtBP*	

Class/function	Name	Duplication
Scaffolding protein	*ebi*	Duplication (2)
Ligand modifier	*wls*	
	por	
	shf	
Wnt-protein binding	*dlp*	
(absences)	*wg, wnt2, wnt3, wnt7, wnt9, wnt 10, wnt11, Axn, pygo, Igs, nkd*	
Notch		
Ligand	*Dl* *Ser*	
Transmcmbranc receptor	*N*	Duplication (4)
Receptor modifier	*O-fut* *fng*	
Protease	*Fur1*	Duplication (2)
Mctalloprotcasc	*kuz*	
γ-Secrctasc complex	*Psn* *pen-2* *nct*	
	Aph01	Duplication (2)
Transcription factor	*Su(H)*	Duplication (3)
	gro	Duplication (2)
	nub	Duplication (2)
(absences)	*Tace, dx, H, mam, E(spl)*	
RTK		
EGF		
Ligand	*spi* *vn*	
Transmcmbranc receptor	*EGFR*	
	kek-1	
	kek-3	Duplication (2)
EGFR inhibitor	*aos*	Duplication (2)
(absences)	*grk, Km, Yan*	
FGF		
Transmcmbranc receptor	*htl*	Duplication (2)
	sgl *sfl*	
ECM component	*trol*	
(absences)	*bnl, btl, pyr, ths, sty*	
Scvenless/MAPK		
Transmcmbranc receptor	*sev*	
	drk *Sos*	
Rasl	*Ras85D*	
MAPKKK (RAF)	*phi*	

Class/function	Name	Duplication
MAPKK (MEK)	*Dsor1* *hep* *lic*	
MAPK	*rl* *p38b*	
(absences)	*boss*	
JAK-STAT		
Receptor	*Dome*	
JAK	*hop*	
STAT	*Stat92E (mrl)*	Duplication (2)
(absences)	*os, upd2, upd3*	
Hh		
Ligand	*Hh*	
Transmembrane receptor	*ptc* *smo* *mgl*	
Transcription factor	*ci*	
Repressor	*Su(fu)*	Duplication (2)
(absences)	*cos, fu*	

TGF-beta transforming growth factor-β, *RTK* receptor tyrosine kinase, *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor, *FGF* fibroblast growth factor, *ECM* extracellular matrix, *MAPK* mitogen-activated protein kinase, *JAKSTAT* Janus kinase/signal transducers and activators of transcription, *Hh* hedgehog

Table 2

Lineage-specific and shared gene duplications found in the 124 *Drosophila* orthologs surveyed for in the *Tetranychus* and *Acyrthosiphon* genomes

	Tetranychus	Acyrthosiphon
activin-beta	2	
anterior pharynx defective 1	2	
APC-like	2	
canoe	2	
dishevelled	2	
frizzled	2	
frizzled 3	3	
glass bottom boat	2	
groucho	2	
heartless	3	
kekkon-3	2	
Notch	4	
nubbin	2	
pangolin/TCF-LEF1	3	
punt	2	
short gastrulation	2	
supernumerary limbs	2	
Suppressor of fused	2	
thickveins	2	
Wnt6	3	
argos	2	4
armadillo/β-catenin	2	2
Stat	2	2
Suppressor of Hairless	3	2
decapentaplegic		4
Domeless		5
kekkon-1		2
kekkon-2		2
Medea		5
Mothers against dpp		2
myoglianin		2
Rhomboid-4		2
shaggy/GSK-3		2
Number of duplicated orthologs	24	13
Total number of paralogs	55	36