

Identification and analysis of the transferrin protein family in the honey bee, *Apis mellifera*

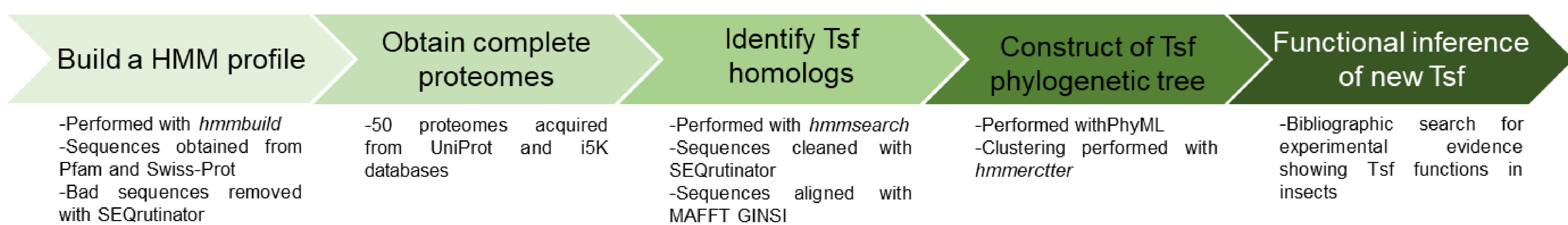
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ABSTRACT

The increasing availability of insect genomes information has formed the basis of new approaches to the study of the bee's health. The search for similarity in protein sequences has provided important insights into the molecular mechanisms that regulate diverse biological traits of organisms. Transferrins (Tsf) are proteins that play a central role in iron transport, growth, differentiation and immunity in vertebrates. Tsf consist of a 13-member evolutionarily related protein family among the putative Tsf of vertebrates, invertebrates, and plants. In the Insecta class, at least four Tsf family members (Tsf 1-4) have been identified. Recently, Tsf1 was shown to play an important role in the nutritional immunity of *Drosophila melanogaster* by sequestering iron from invading pathogens. Tsf2 from *D. melanogaster* also binds iron and it works in the formation of epithelial cell junctions. At the moment, there is no empirical evidence on the state of binding to iron and the function of Tsf3 and Tsf4. In the honey bee *Apis mellifera* an exhaustive search for Tsf has not yet been carried out. The goals of this project are: i) to identify all the Tsf members in a diverse set of insect proteomes, ii) to perform a comprehensive phylogenetic analysis, iii) to analyze the characteristic motif and iron-binding residues of *A. mellifera* Tsf, iv) to predict their functional annotation. We initially used *hmmbuild* to create an insect Tsf Hidden Markov model (HMM) profile using a set of insect Tsf sequences from Pfam and Swiss-Prot databases. Subsequently, the HMM profile was used to search (*hmmssearch*) against 50 proteomes of organisms belonging to the different orders of insects, including several representatives of the order Hymenoptera. Next, we removed pseudogenes and entries from incorrect gene models with a dedicated automated pipeline. Finally, the resulting hits were recognized as putative Tsf and such sequences were subjected to alignment and, finally we identify whether they have the Tsf domains. The phylogenetic reconstruction of insect Tsf was performed with a maximum likelihood approach (PhyML). In this work, we were able to identify in *A. mellifera* members of the Tsf family and the evolutionary relationships. Due to the accumulation of studies supporting the immunological role of insect Tsf against infections, our results allow to identify new candidates to study the specific roles of Tsf in honey bee health.

WORKFLOW



RESULTS

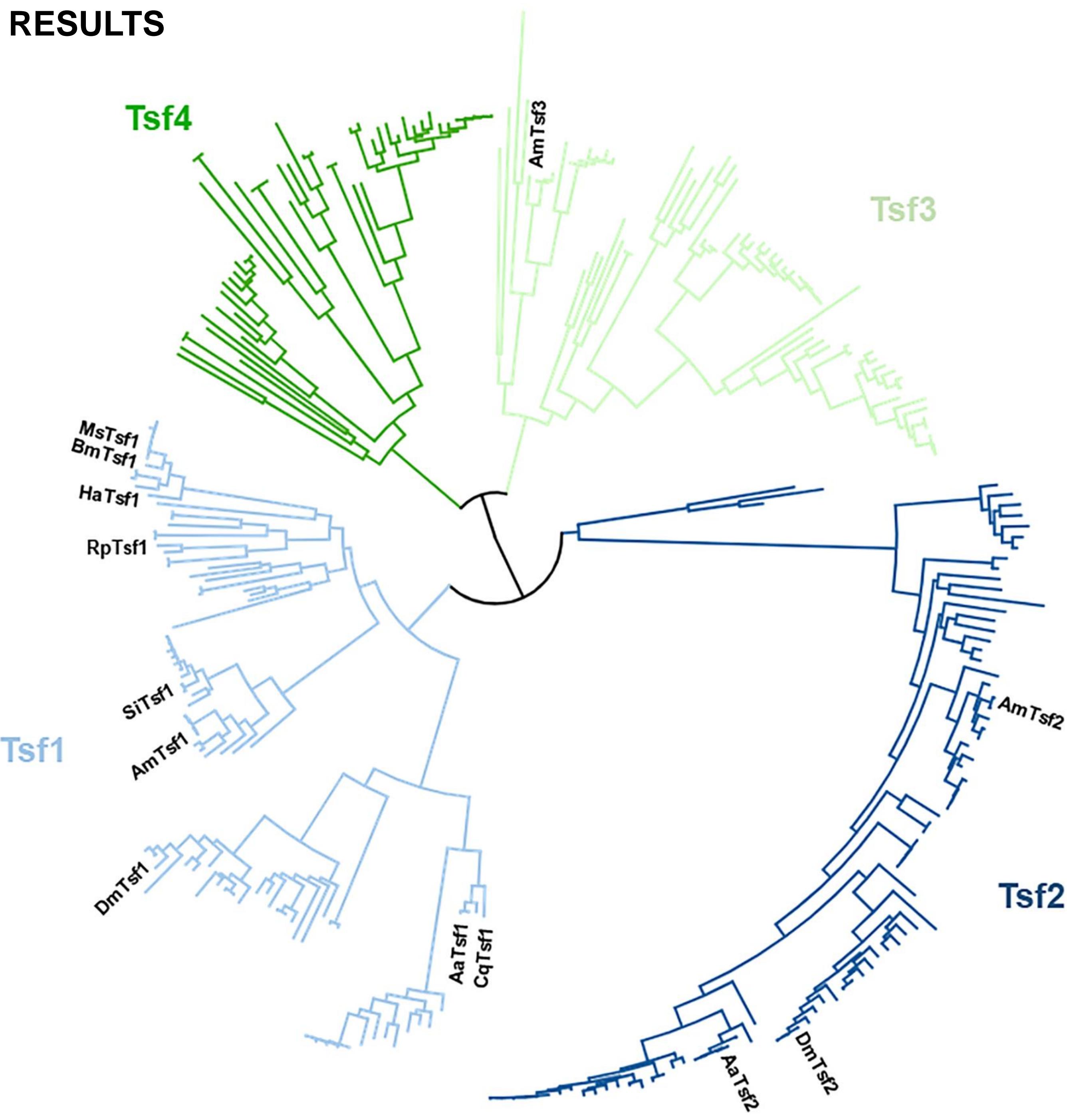


Figure 1. Phylogenetic tree of insect transferrins performed with the maximum likelihood method. Tsf1 orthologs are indicated in light blue, Tsf2 in blue, Tsf3 in light green and Tsf4 in green.

Table 1. Functional properties of empirically studied insect transferrins determined by molecular and/or biochemical analysis.

Order	Species	Abbreviation	Assigned function	Ref.
Diptera	<i>Aedes aegypti</i>	AaTsf1	Upregulation upon bacterial infection Upregulation after blood feeding	1, 2 3
		AaTsf2	Upregulation in larval and pupal stages	2
	<i>Culex quinquefasciatus</i>	CqTsf1	Upregulation upon parasite infection	4
	<i>Drosophila melanogaster</i>	DmTsf1	Nutritional immunity Upregulation upon fungal infection Iron trafficking Iron binding	5, 6 7 8 9
		DmTsf2	Epithelial septate junction assembly Iron binding Upregulation upon fungal infection	10 9 9
	<i>Glossina morsitans</i>	GmTsf1	Upregulation after blood feeding	11
Lepidoptera	<i>Sarcophaga peregrina</i>	SpTsf1	Iron trafficking	12
	<i>Bombyx mori</i>	BmTsf1	Upregulation upon bacterial, fungal and viral infection Iron binding Bactericidal activity	13 12
	<i>Helicoverpa armigera</i>	HaTsf1	Upregulation at late larval and pupal stages Xenobiotic tolerance	14 14
Hemiptera	<i>Manduca sexta</i>	MsTsf1	Iron binding Upregulation upon bacterial infection Bacteriostatic activity	15, 16, 9 17 17
	<i>Rhodinius prolixus</i>	RpTsf1	Upregulation upon bacterial and parasite infection	18
	<i>Apis mellifera</i>	AmTsf1	Upregulation at early pupal stages Upregulation upon bacterial infection Downregulation after juvenile hormone treatment Downregulation after hydroxyecdysone treatment Upregulation at late larval stages Upregulation upon parasite infection Suppression of immune response upon parasite infection	19 19 19 20 20 21 21
Hymenoptera	<i>Solenopsis invicta</i>	SiTsf1	Upregulation upon fungal infection	22

References

- Yoshiga et al., (1997) Proc Natl Acad Sci USA. DOI: 10.1073/pnas.94.23.12337.
- Zhou et al., (2009) Insect Biochem Mol Biol. DOI: 10.1016/j.ibmb.2008.12.004.
- Harizanova et al., (2005) Insect Mol Biol. DOI: 10.1111/j.1365-2583.2004.00533.x.
- Paily et al., (2007) Parasitol Res. DOI: 10.1007/s00436-007-0474-2.
- Iatsenko et al., (2020) Proc Natl Acad Sci USA. DOI: <https://doi.org/10.1073/pnas.1914830117>.
- Yoshiga et al., (1999) Eur J Biochem. DOI: 10.1046/j.1432-1327.1999.00173.x.
- Levy et al., (2004) Mol Cell Proteomics. DOI: 10.1074/mcp.M300114MCP200.
- Xiao et al., (2019) Cell Rep. DOI: <https://doi.org/10.1016/j.celrep.2018.12.053>.
- Weber et al., (2020) Insect Biochem Mol Biol. DOI: <https://doi.org/10.1016/j.ibmb.2020.103438>.
- Tiklova et al., (2010) Nat Cell Biol. DOI: 10.1038/ncb2111.
- Strickler-Dinglasan et al., (2006) Insect Biochem Mol Biol. DOI: <https://doi.org/10.1016/j.ibmb.2006.09.003>.
- Kurama et al., (1995) Eur J Biochem. DOI: 10.1046/j.1423-1327.1995.00173.x.
- Yun et al., (2009) Dev Comp Immunol. DOI: 10.1016/j.dci.2009.05.008.
- Zhang et al., (2015) Insect Mol Biol. DOI: 10.1111/imb.12129.
- Huebers et al., (1988) J Comp Physiol B. DOI: 10.1007/BF00695327.
- Barfield et al., (1990) J Biol Chem. 17.
- Brumet et al., (2017) Insect Biochem Mol Biol. DOI: 10.1016/j.ibmb.2016.12.006.
- Ursic-Bedoya et al., (2007) Dev Comp Immunol. DOI: 10.1016/j.dci.2006.05.008.
- Kucharski et al., (2003) J Insect Sci. DOI: 10.1093/jis/3.1.27.
- Rodríguez-García et al., (2021) PLoS Pathog. DOI: 10.1371/journal.ppat.1009270.
- Valess et al., (2005) Gene. DOI: 10.1016/j.gene.2005.05.017.

- The selected insect proteomes in this work represented 12 orders and 50 insect species. We identified a total of 290 Tsf sequences which were represented in a phylogenetic tree (Fig 1).
- Tsf sequences were distributed into four main clades corresponding to the four orthologous groups found previously in Insect taxa.
- Experimental evidences show that Tsf1 from *D. melanogaster* and *Manduca sexta* (DmTsf1 and MsTsf1) and Tsf2 from *D. melanogaster* (DmTsf2) bind iron (Table 1). Furthermore, among the experimental evidences found for tsf1 in different species of insects, a function associated with the immune response predominated.
- Little experimental evidence exists in the literature regarding the functions of Tsf2 and null for Tsf3 and Tsf4. However, very conclusive results have shown that Tsf2 from *D. melanogaster* acts in epithelial septate assembly (Table 1).
- Protein sequences from each orthologous group were used to search for distinctive motifs using the MEME program. We are currently processing and analysing this data to reach more robust conclusions and infer possible functions for the members of the Tsf family less studied so far in insects.
- Orthologous sequences were found in *Apis mellifera* in clades 1, 2 and 3 of the phylogenetic tree. Previous works have suggested that AmTsf1 participates in the immune response and, also, a role in development has been suggested.
- Much of the studies carried out in insects have focused on the possible functions of Tsf associated with its ability to bind iron. Given that previous studies have suggested that Tsf3, Tsf4 and in many cases Tsf2 do not bind iron, and because these proteins are conserved in most of the insects evaluated in this work, it would be important that future studies aim to elucidate the role of these members of the Tsf family.



Lab website