

## Diversity and Functional Evolution of Terpene Synthases in Dictyostelid Social Amoebae

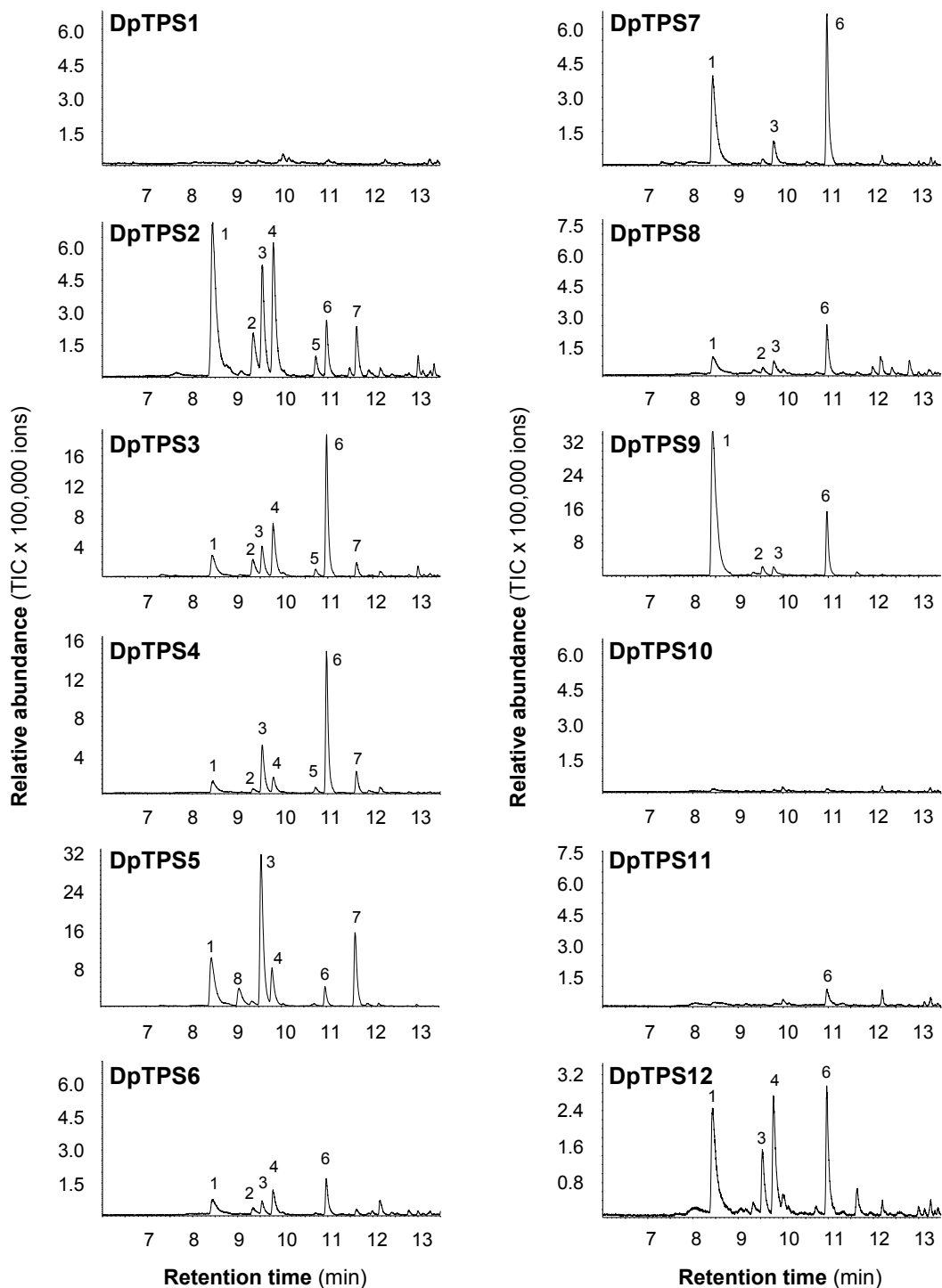
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**Table S1. List of terpene synthase genes from *Dictyostelium lacteum***

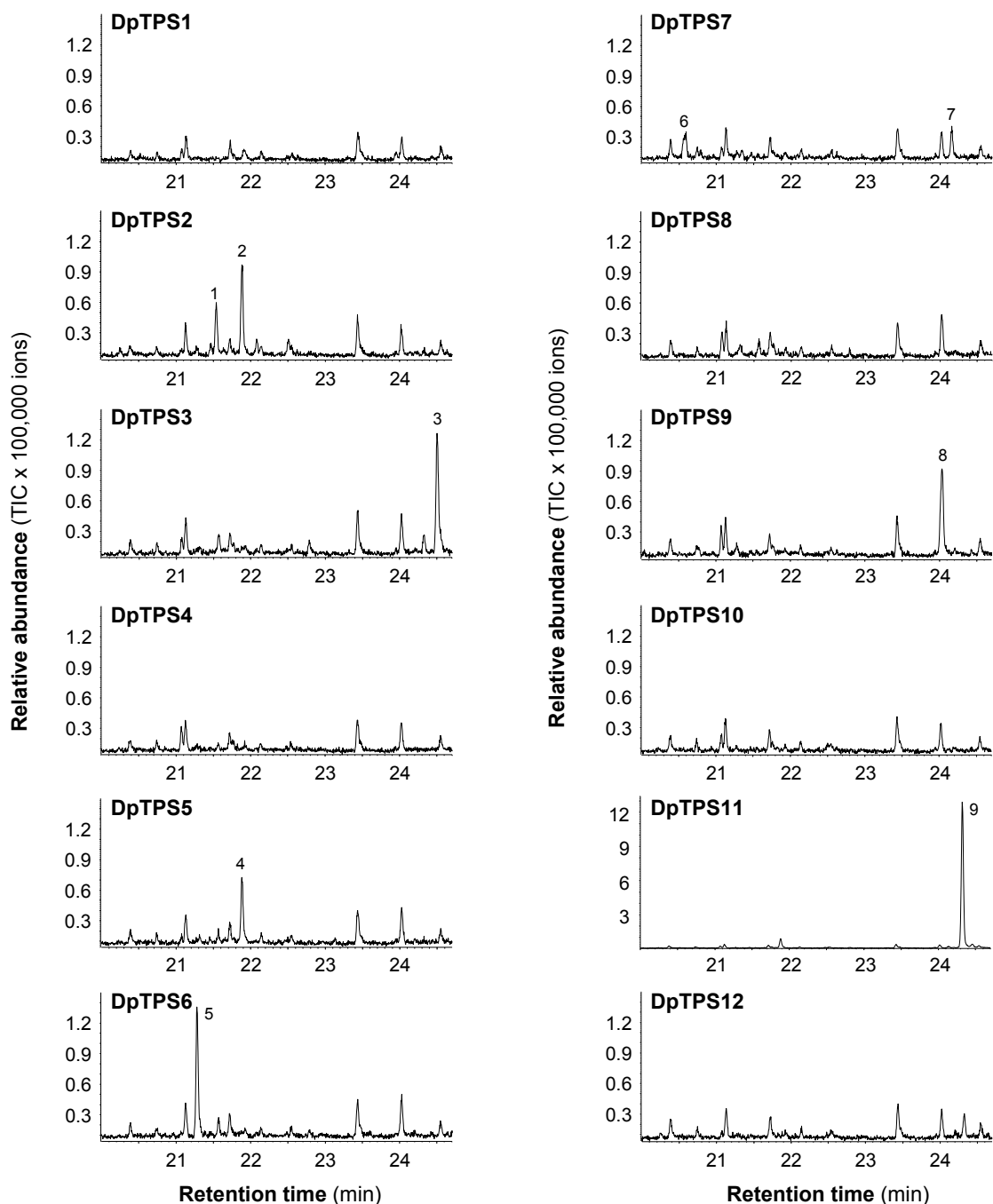
Gene name	Gene ID	location	Protein size	Number of introns
DITPS1	DLAC_07096	LODT01000031.1:625710-626848	354	1
DITPS2	DLAC_10965	LODT01000051.1:c207457-206420	345	0
DITPS3	DLAC_05221	LODT01000025.1:c629567-628551	338	0
DITPS4	DLAC_02702	LODT01000013.1:c532509-531535	324	0
DITPS5	DLAC_05826	LODT01000028.1:1030493-1031416	308	0
DITPS6		LODT01000028.1:1031498-1032448	317	0
DITPS7		LODT01000028.1:1032728-1033668	279	1

**Table S2 Primers used for full-length cDNA cloning**

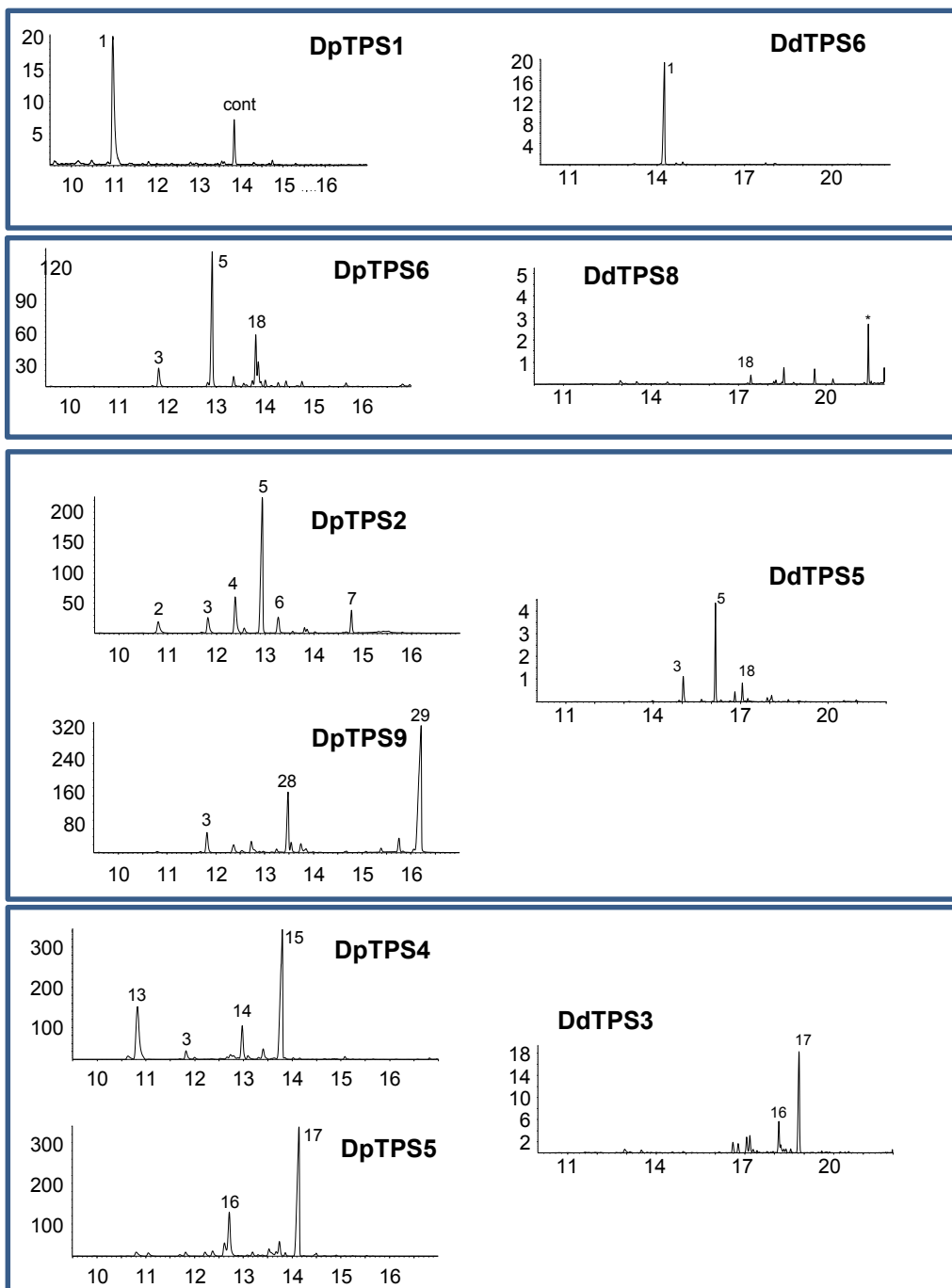
Gene Name	Primers	Sequences
<i>DpTPS1</i>	Forward	5'-ATGTCATTATCATTAAGCGATATTAATTC-3'
	Reverse	5'-TTAATTTATAATTTCTCTGGTGGTAAAATG-3'
<i>DpTPS2</i>	Forward	5'-ATGAATTTAACACTTAAAGATTTTCATG-3'
	Reverse	5'-TTATAGTTCATTATTGATTAATTTATTATC-3'
<i>DpTPS3</i>	Forward	5'-ATGCAAAAAGAAATTTATTATACATTTGAC-3'
	Reverse	5'-TTAATGATTTAAATTATAACGTGGGTAG-3'
<i>DpTPS4</i>	Forward	5'-ATGAATAGCTTAGCAAACAATGGC-3'
	Reverse	5'-TTATATCCAAAATATATCAGGTGTTAAAATTG-3'
<i>DpTPS5</i>	Forward	5'-ATGCAAGAAAACCAAATCTTTTAAG-3'
	Reverse	5'-TTAATAAAGATTTGAATTTAAGATCTTTTC-3'
<i>DpTPS6</i>	Forward	5'-ATGAAACAACATATACTTTGGGATTTAAATAAC-3'
	Reverse	5'-TTATTTATAACGGTTACAGATTGAAGAGAC-3'
<i>DpTPS7</i>	Forward	5'-ATGCAAGAAAATCAAGAGTGTCAAATG-3'
	Reverse	5'-TTATTTATAATCAGCTTCACAAACAGTG-3'
<i>DpTPS8</i>	Forward	5'-ATGCAAGAAGAAATATTATACAAATGGAATTATG-3'
	Reverse	5'-TTATTCATTAATAATCTTTAATTCAGCTTC-3'
<i>DpTPS9</i>	Forward	5'-ATGTCTCTATCTTTTAAAAATATAGTTTTTC-3'
	Reverse	5'-TTATAAACTGGAGGCATCTATTCATTATC-3'
<i>DpTPS10</i>	Forward	5'-ATGGAAGCTATAAAACAAAAAGAAG-3'
	Reverse	5'-TCATCGAAAAAATGTTTTATGTTGGAC-3'
<i>DpTPS11</i>	Forward	5'-ATGGATATAAATAAACAAAAACCAAATG-3'
	Reverse	5'-TTAATTATTTTTATTGATATAGTTTTTGTTG-3'
<i>DpTPS12</i>	Forward	5'-ATGTATTCTTTATTTAAAAATATAAAATTC-3'
	Reverse	5'-CTAACCAAATAATGATTTAGTATTTAAATAAG-3'
<i>AsTPS1</i>	Forward	5'-ATGTCACTATCTTTAAATGATATCAAATTC-3'
	Reverse	5'-TTAAGCCTCACGGAACCTTCTCATTC-3'
<i>DfTPS1</i>	Forward	5'-ATGTTATCATTAAACGAAATCAAGTTTC-3'
	Reverse	5'-CTAAAGTTTGGACATCAAGTTCCTTG-3'
<i>DITPS1</i>	Forward	5'-ATGTCACTGTCACTTAATGATATTAATTC-3'
	Reverse	5'-TTATACTCTTTTCTTTCTTTAAGTCTC-3'
<i>PpTPS18</i>	Reverse	5'-ATGTTGCCTTAAACGAAATCAAATTC-3'
	Reverse	5'-TTATAGATAGCTTTTCGAGGGAGGTC-3'



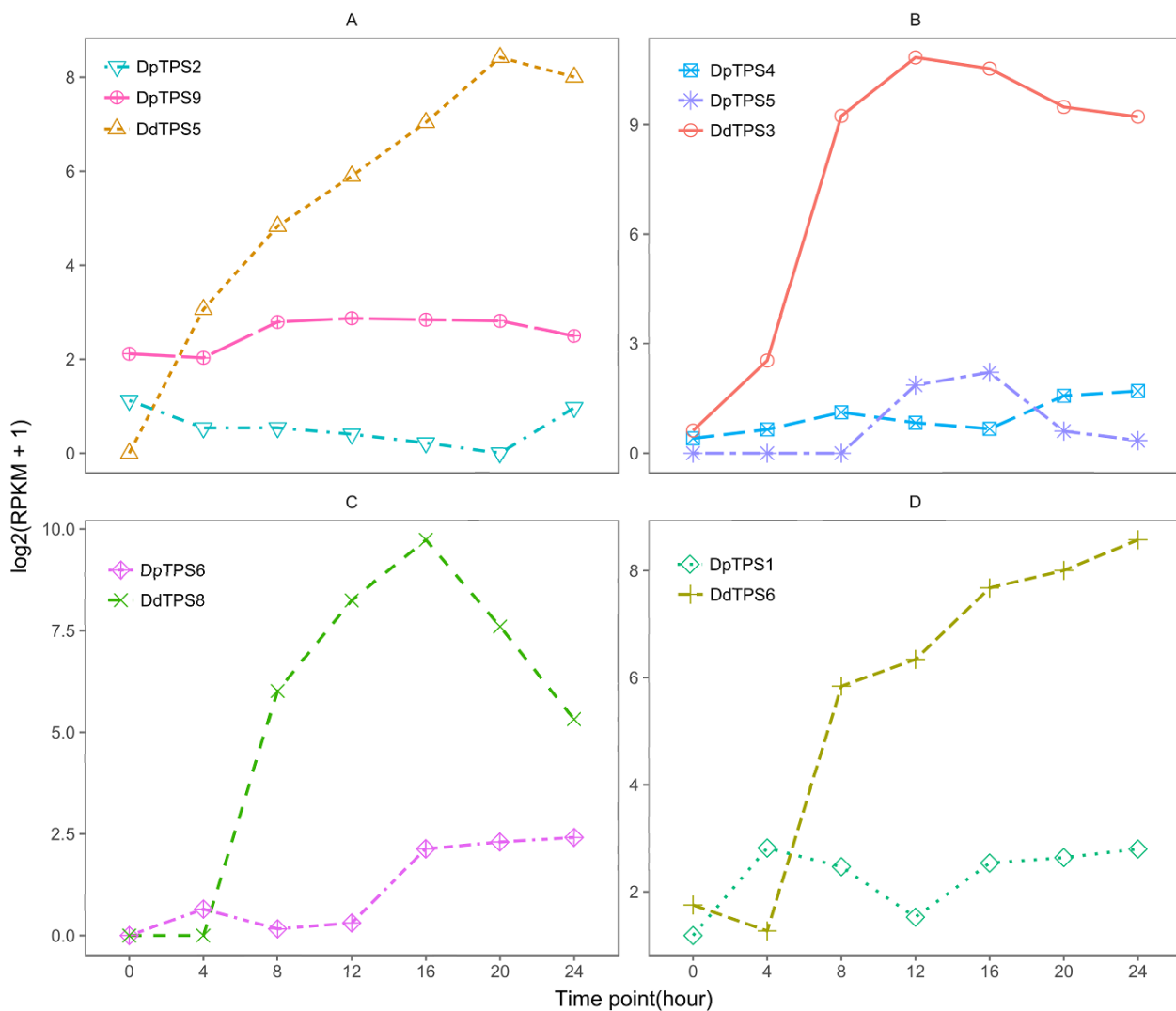
**Figure S1. Monoterpene products of *Dictyostelium purpureum* terpene synthases.** The *TPS* genes were heterologously expressed in *Escherichia coli* and crude protein extracts were incubated with the substrate GPP in the presence of 10 mM Mg<sub>2</sub>Cl as cofactor. The produced monoterpenes were collected from the headspace of the assays using solid-phase microextraction and were analyzed with gas chromatography/mass spectrometry. 1, β-myrcene\*; 2, limonene\*; 3, (*Z*)-β-ocimene; 4, (*E*)-β-ocimene; 5, terpinolene\*; 6, linalool\*; 7, neo-allo-ocimene; 8, α-terpinene\*.



**Figure S2. Diterpene products of *Dictyostelium purpureum* terpene synthases.** The *TPS* genes were heterologously expressed in *Escherichia coli* and crude protein extracts were incubated with the substrate GGPP in the presence of 10 mM  $Mg_2Cl$  as cofactor. The produced diterpenes were extracted from the assays with hexane and were analyzed using gas chromatography/mass spectrometry. 1+2, unidentified DTs; 3+4, unidentified oxygenated DTs; 5+6, unidentified DTs; 7-9, unidentified oxygenated DTs; DT, diterpene hydrocarbon. Non-labeled peaks are contaminations.



**Figure S3. Comparison of sesquiterpene products of four putative orthologous groups of terpene synthases from *D. purpureum* (DpTPS) and *D. discoideum* (DdTPS).** The compound numbers follow the same scheme as presented in Figure 2. "\*" represents an unidentified sesquiterpene.



**Figure S4. Comparison of expression patterns of four putative orthologous pairs/groups of terpene synthase genes between *D. purpureum* and *D. discoideum* during 24 hour development.** Expression of TPS genes was measured by RPKM (reads per kilobase per million sequenced reads) based on RNA-Seq data produced from web-based interface program (<http://dictyexpress.biolab.si/>) and then displayed on a  $\log_2(\text{RPKM}+1)$  scale in this line plot.