

Supplementary Information

Selective pressure against horizontally acquired prokaryotic genes as a driving force of plastid evolution

Briardo Llorente^{1,2,*}, Flavio S. J. de Souza^{2,+}, Gabriela Soto^{2,+}, Cristian Meyer², Guillermo D. Alonso^{2,3}, Mirtha M. Flawiá^{2,3}, Fernando Bravo-Almonacid^{2,4}, Nicolás D. Ayub^{5,6} and Manuel Rodríguez-Concepción^{1,*}

¹ Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, 08193 Barcelona, Spain.

² Instituto de Investigaciones en Ingeniería Genética y Biología Molecular Dr. Héctor Torres, Consejo Nacional de Investigaciones Científicas y Técnicas (INGEBI-CONICET), C1428ADN Buenos Aires, Argentina.

³ Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina.

⁴ Departamento de Ciencia y Tecnología. Universidad Nacional de Quilmes, B1876BXD Bernal, Argentina.

⁵ Instituto de Genética Ewald A. Favret, Centro de Investigación en Ciencias Veterinarias y Agronómicas, Instituto Nacional de Tecnología Agropecuaria (CICVyA-INTA), B1712WAA Castelar, Argentina.

⁶ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), C1033AAJ Buenos Aires, Argentina.

* Corresponding authors: briardo.llorente@cragenomica.es or manuel.rodriguez@cragenomica.es

+ These authors contributed equally to this work.

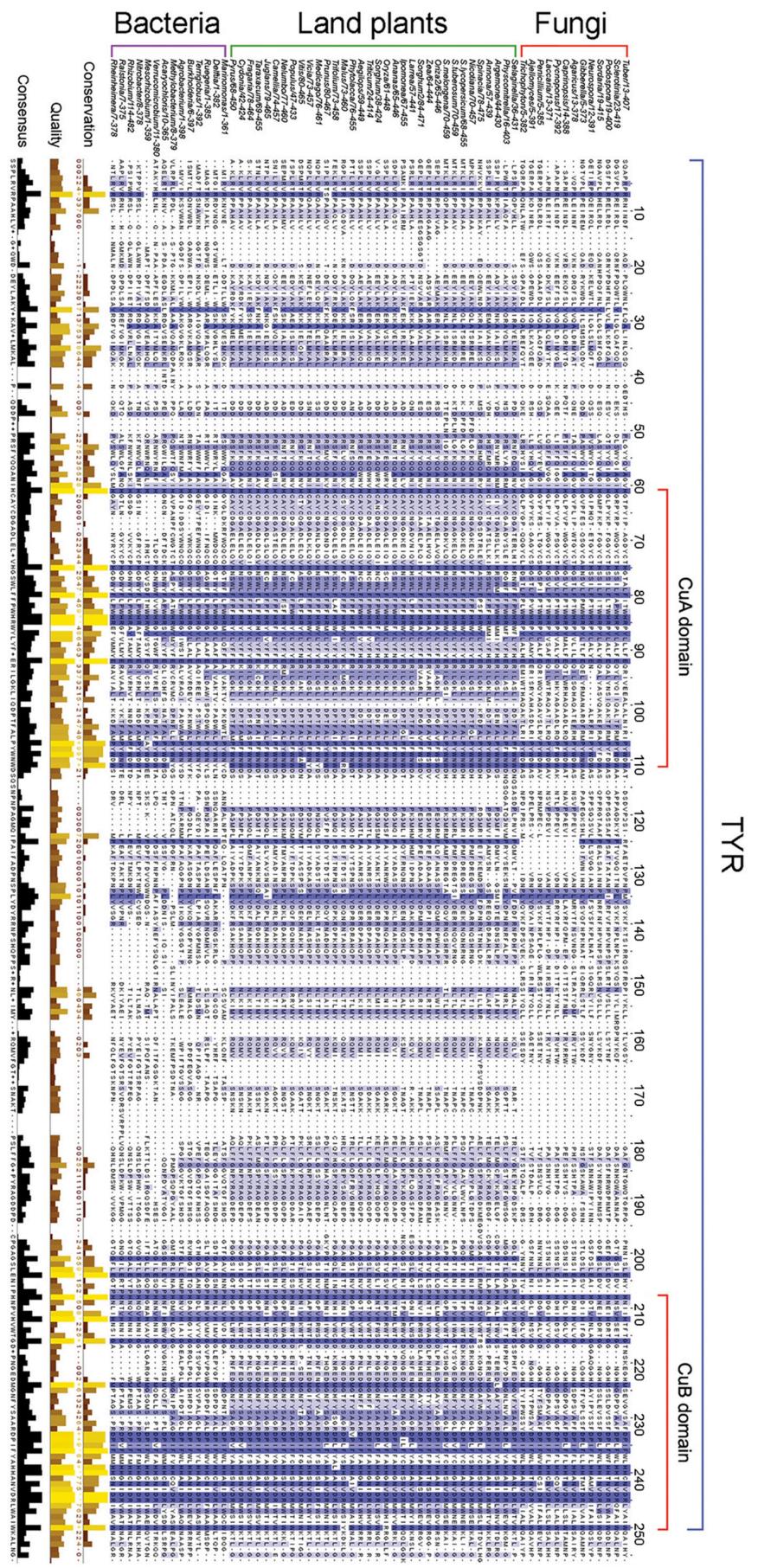


Figure S1 continues in next page

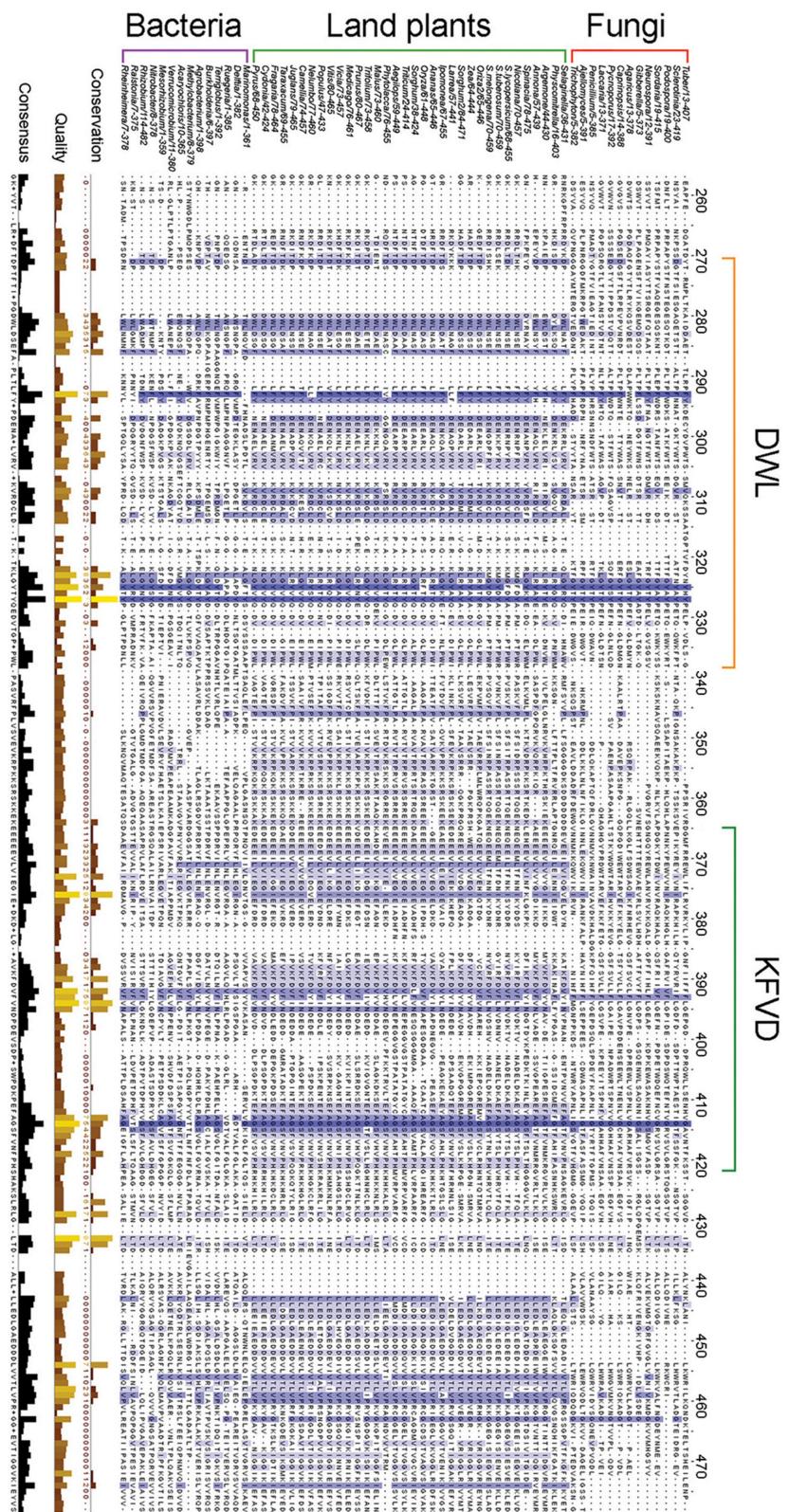


Figure S1 | Sequence alignments of PPO domains from bacteria, land plants and fungi.

Alignments include the TYR (PFAM00264), DWL (PFAM12142) and KFDV (PFAM12143) domains of PPO. The highly conserved copper-binding motifs A (CuA) and B (CuB) of the TYR domain are also indicated.

Table S1. GenBank accession IDs for protein sequences used in Figure 1b.

Organism	Accession number	Phylum
<i>Acaryochloris marina</i>	WP_012167220	Cyanobacteria
<i>Agaricus bisporum</i>	Q00024	Basidiomycota
<i>Agrobacterium vitis</i>	WP_015916152	Proteobacteria
<i>Ajellomyces dermatitidis</i> ER-3	EEQ88565	Ascomycota
<i>Aphanizomenon flos-aquae</i>	WP_027401937	Cyanobacteria
<i>Arthrobotrys oligospora</i> ATCC 24927	EGX43307	Ascomycota
<i>Burkholderia thailandensis</i> 1	WP_015604107	Proteobacteria
<i>Burkholderia thailandensis</i> 2	WP_011400974	Proteobacteria
<i>Candidatus Nitrospira defluvii</i>	WP_013249721	Nitrospirae
<i>Chitinimonas koreensis</i>	WP_028446804	Proteobacteria
<i>Chlamydomonas reinhardtii</i>	XP_001701364	Chlorophyta
<i>Chromobacterium violaceum</i> ATCC 12472	AAQ60926	Proteobacteria
<i>Chroococcidiopsis thermalis</i>	WP_015153379	Cyanobacteria
<i>Coprinopsis cinerea</i> okayama7#130	XP_002911630	Basidiomycota
<i>Delftia acidovorans</i>	WP_016447903	Proteobacteria
<i>Gallaecimonas xiamenensis</i>	WP_008484930	Proteobacteria
<i>Fusarium graminearum</i>	CEF86307	Ascomycota
<i>Gloeocapsa</i> sp. PCC 7428	WP_015187306	Cyanobacteria
<i>Hahella ganghwensis</i>	WP_020405703	Proteobacteria
<i>Haliscoenenobacter hydrossis</i>	WP_013766505	Bacteroidetes
<i>Marinomonas mediterranea</i>	AAV49996	Proteobacteria
<i>Mesorhizobium</i>	WP_013530378	Proteobacteria
<i>Methylobacterium</i> sp. 4-46	WP_012335039	Proteobacteria
<i>Nelumbo nucifera</i>	ADC92563	Streptophyta
<i>Neofusicoccum parvum</i> UCRNP2	XP_007587508	Ascomycota
<i>Neurospora crassa</i> OR74A	XP_964823	Ascomycota
<i>Nisaea denitrificans</i>	WP_028466395	Proteobacteria
<i>Nitrobacter hamburgensis</i>	WP_011511370	Proteobacteria
<i>Nocardiopsis halotolerans</i>	WP_040684307	Actinobacteria
<i>Oceanospirillum beijerinckii</i>	WP_028299139	Proteobacteria
<i>Oryza sativa</i>	ACS15320	Streptophyta
<i>Physcomitrella patens</i>	AAX69084	Streptophyta
<i>Podospora anserina</i> S matt+	XP_001912783	Ascomycota
<i>Pseudanabaena</i> sp. PCC 6802	WP_019501743	Cyanobacteria
<i>Ralstonia solanacearum</i>	ACM68954	Proteobacteria
<i>Rheinheimera</i> sp. A13L	WP_008899933	Proteobacteria
<i>Rhizobium etli</i> CFN 42	AAM54973	Proteobacteria
<i>Ruegeria pomeroyi</i>	WP_011047142	Proteobacteria
<i>Sagittula stellata</i>	WP_005858917	Proteobacteria
<i>Sclerotinia sclerotiorum</i> 1980 UF-70	XP_001584564	Ascomycota
<i>Selaginella moellendorffii</i> 1	XP_002969567	Streptophyta
<i>Selaginella moellendorffii</i> 2	XP_002990879	Streptophyta
<i>Sinorhizobium meliloti</i>	WP_017267834	Proteobacteria
<i>Solanum tuberosum</i>	AAA85122	Streptophyta
<i>Sorangium cellulosum</i>	WP_020736163	Proteobacteria
<i>Sorghum bicolor</i>	XP_002446945	Streptophyta
<i>Talaromyces marneffei</i> ATCC 18224	XP_002149601	Ascomycota
<i>Terriglobus saanensis</i>	WP_013568839	Acidobacteria
<i>Triticum aestivum</i>	AAS00454	Streptophyta
<i>Tuber magnatum</i>	CAT00093	Ascomycota
<i>Verrucomicrobium spinosum</i>	WP_009958178	Verrucomicrobia
<i>Vicia faba</i>	Q06215	Streptophyta
<i>Vitis vinifera</i>	NP_001268045	Streptophyta
<i>Volvox carteri</i>	XP_002956492	Chlorophyta

Table S2. Primers used in this study.

Primer name	Sequence
StPPO _M F (C)	GGGGACAAGTTGTACAAAAAAGCAGGCTATGGCAAGCTTGCAATAGTAGTAG
StPPO _A F (C)	GGGGACAAGTTGTACAAAAAAGCAGGCTATGGACCAAAACCAACGTTGAAAC
StPPO R (C)	GGGGACCACTTGTACAAGAAAGCTGGGTTAACATCTGCAAGACTGATCGTCG
StPPO C-tag R (C)	GGGGACCACTTGTACAAGAAAGCTGGGTTACAATCTGCAAGACTGATCGTCG
StPPO _M N-tag F (C)	GGGGACAAGTTGTACAAAAAAGCAGGCTTACATGGCAAGCTTGCAATAGTAGTAG
StPPO _A N-tag F (C)	GGGGACAAGTTGTACAAAAAAGCAGGCTTACATGGACCAAAACCAACGTTGAAAC
StPPO qPCR F (Q)	TGAATGCGAATGAGCTTGAC
StPPO qPCR R (Q)	CAGCTGGAAATCACAGTCG
AtUBC qPCR F (Q)	TCAAATGGACCGCTCTTATC
AtUBC qPCR R (Q)	CACAGACTGAAGCGTCCAAG

The names are labelled with (C) or (Q) to indicate whether they were used for cloning or qPCR, respectively.