

The genetic basis of species-specific sensitivities to 6PPD-Quinone in Salmonids

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Background

An ozonation product of an antioxidant used in car tire rubbers is threatening Coho salmon (*Oncorhynchus kisutch*) populations. 6PPD-Quinone is acutely lethal to a few Salmonids at environmentally relevant concentrations but causes no acute lethality in closely related fish species.

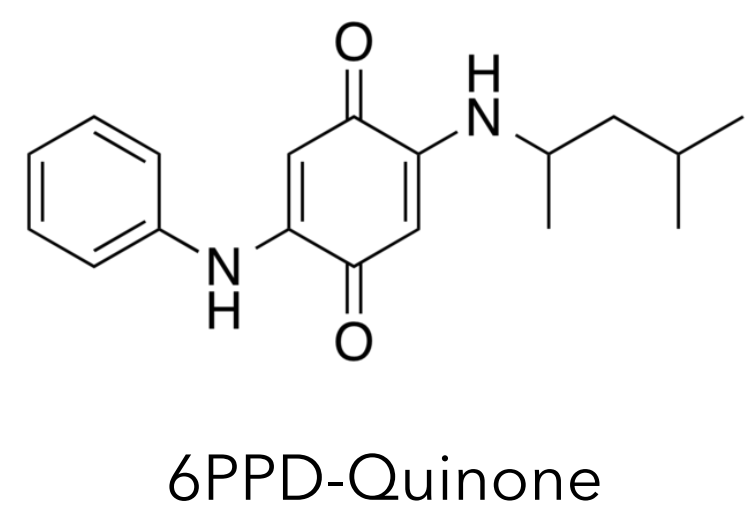
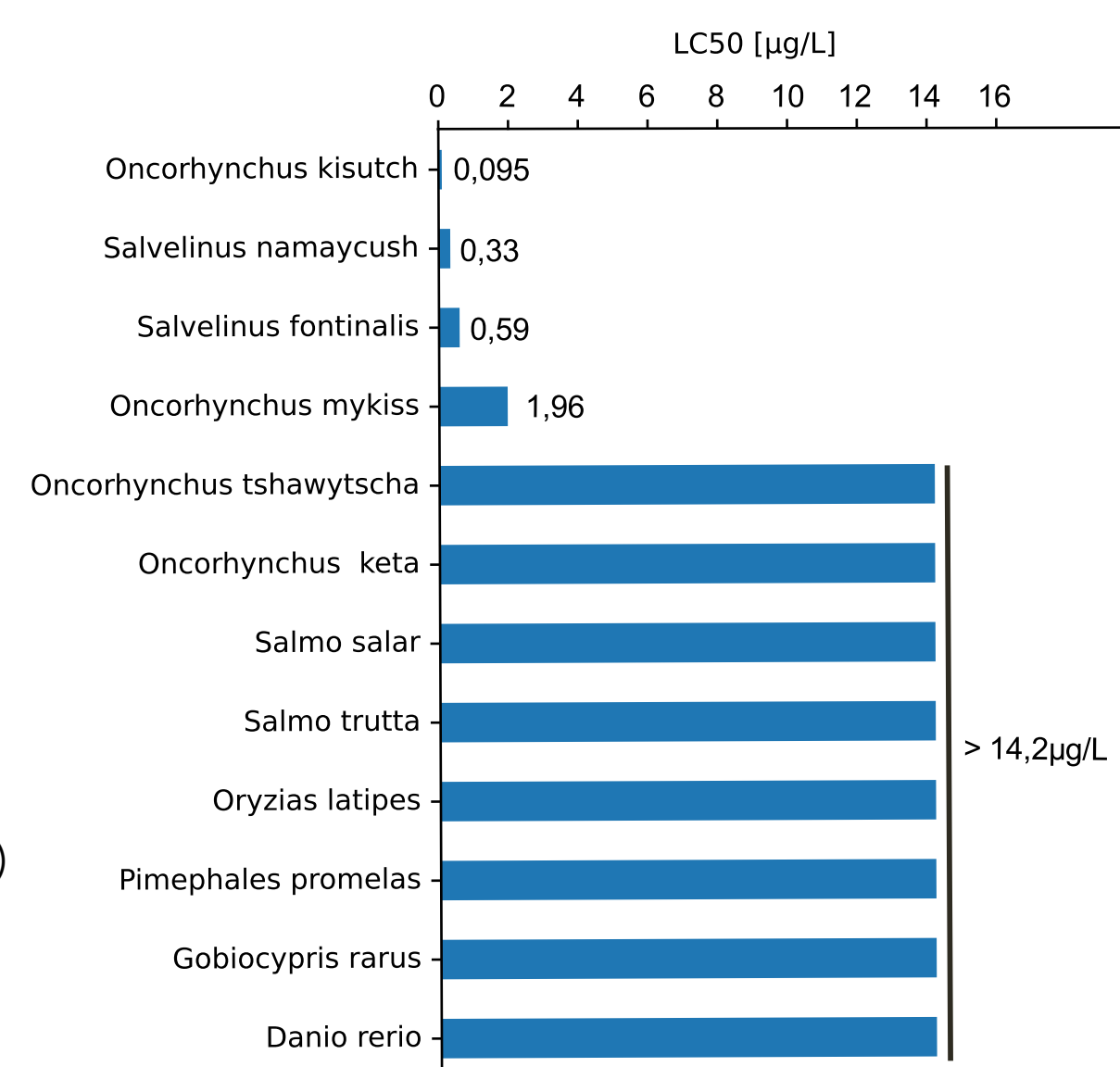


Figure 1. Chemical structure (top) and 24h median lethal concentrations (LC50) of 6PPD-Quinone on tested species.



Hypothesis

Independent gene loss events within the Salmonidae have made some species more susceptible to 6PPD-Quinone toxicity.

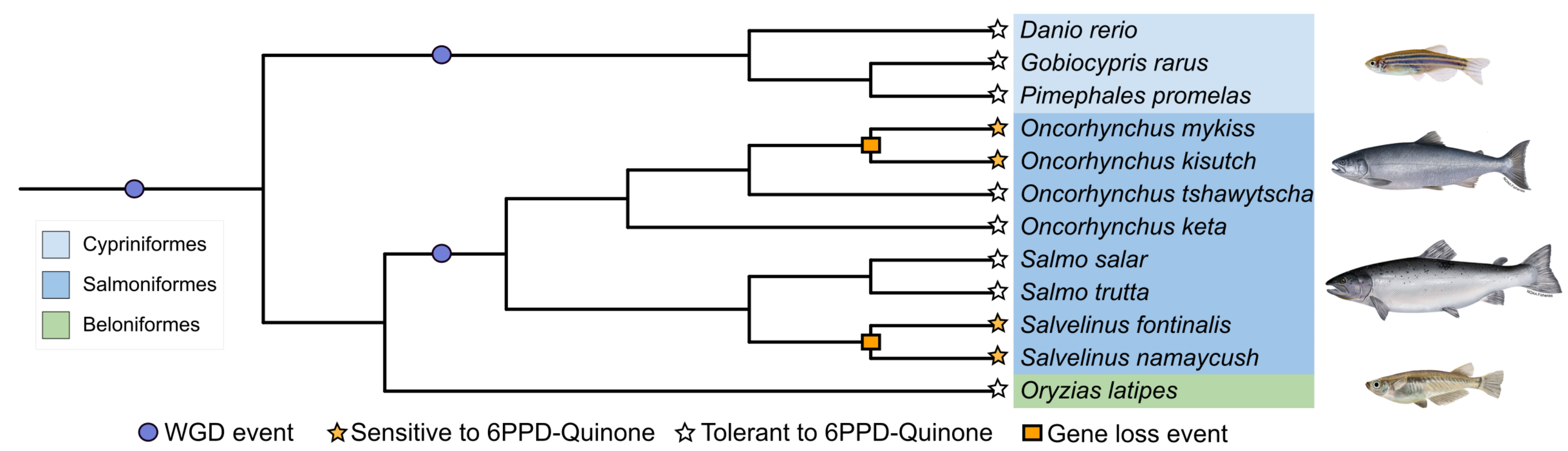


Figure 2. Phylogenetic tree of the study species (subtree from Langschieid et al. 2023). Blue and green areas represent fish orders. Orange rectangles highlight the hypothesis of independent gene loss events within Salmoniformes. WGD = whole genome duplication.

Identifying lost or modified genes in salmonids

An ortholog search found 24 Zebrafish genes that were present in 6 out of 8 6PPD-Q tolerant fish species and absent or modified in sensitive *Oncorhynchus* and *Salvelinus* species.

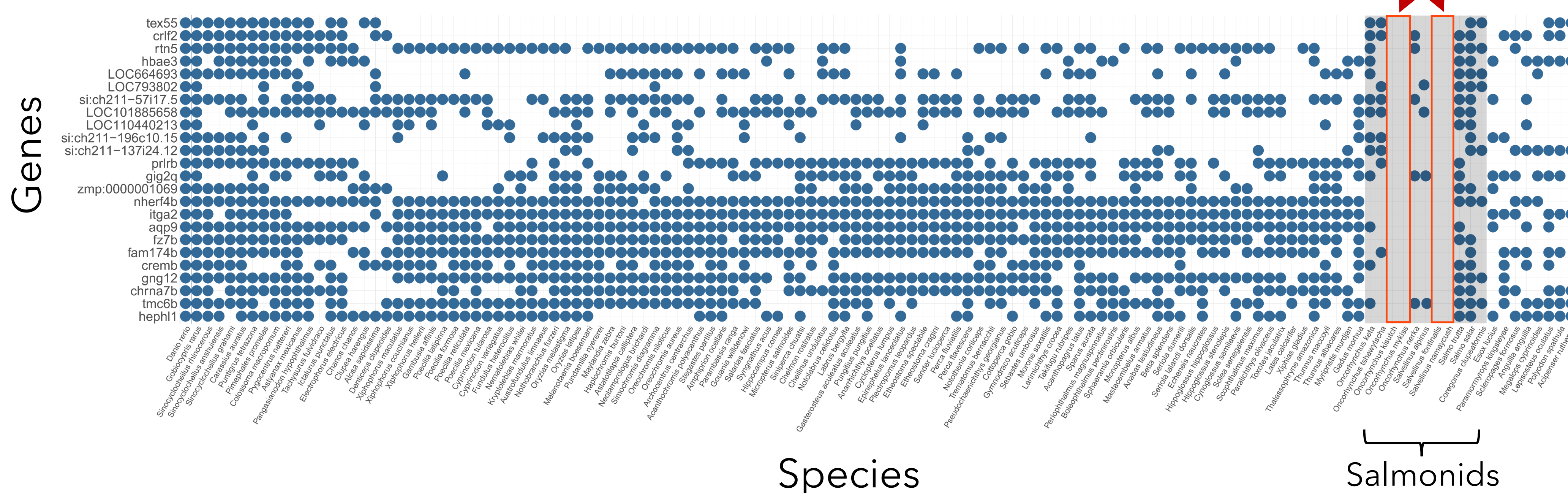


Figure 3. Phylogenetic profiles with gene presence/absence patterns of 24 *Danio rerio* genes (vertical axis) across 120 fish species (horizontal axis). Blue dots represent orthologs (genes of common ancestry) with the corresponding *D. rerio* gene; empty spaces represent genes that were not found in search taxa.

Biological pathways affected by these genes might be related to 6PPD-Q's toxic mode of action.

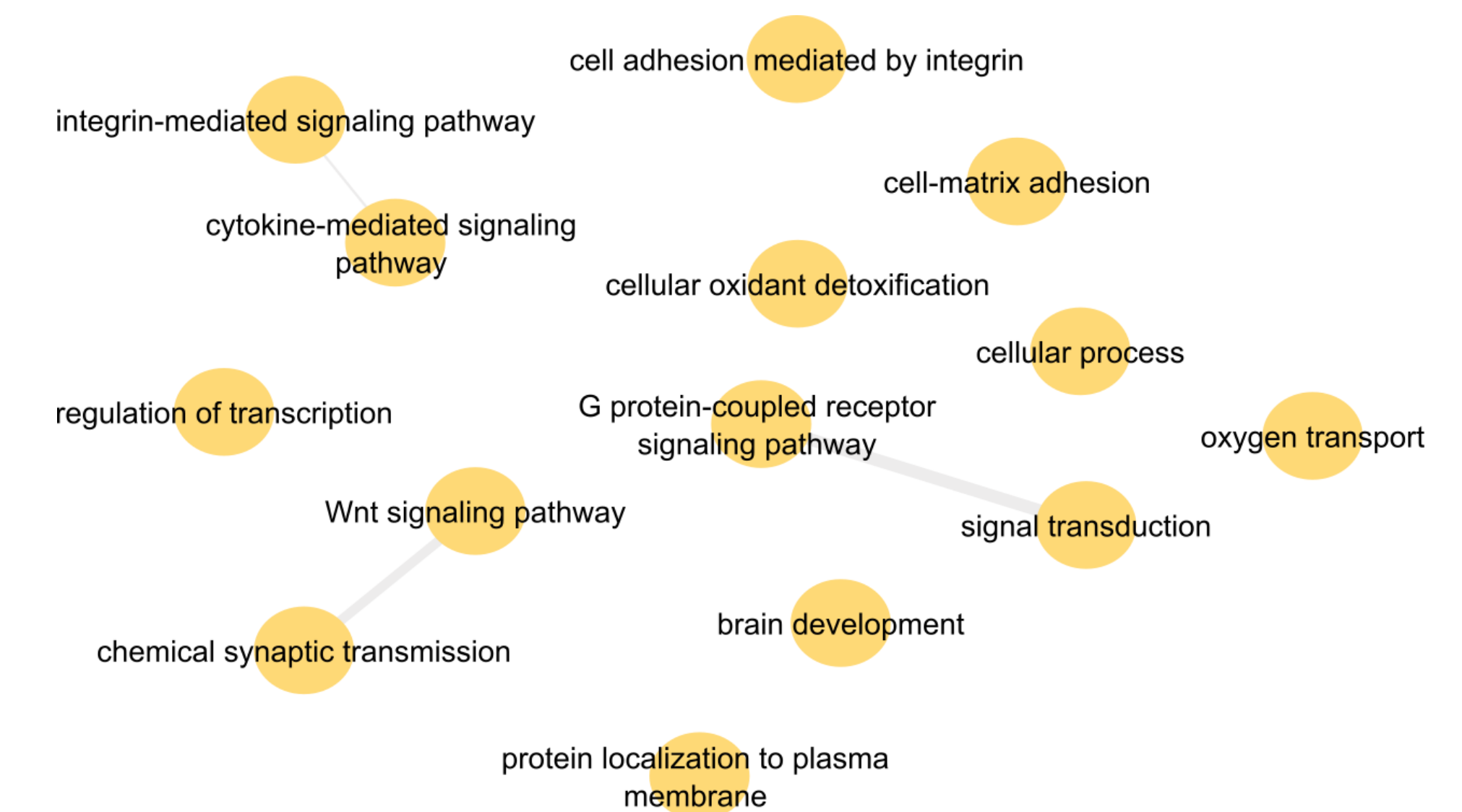
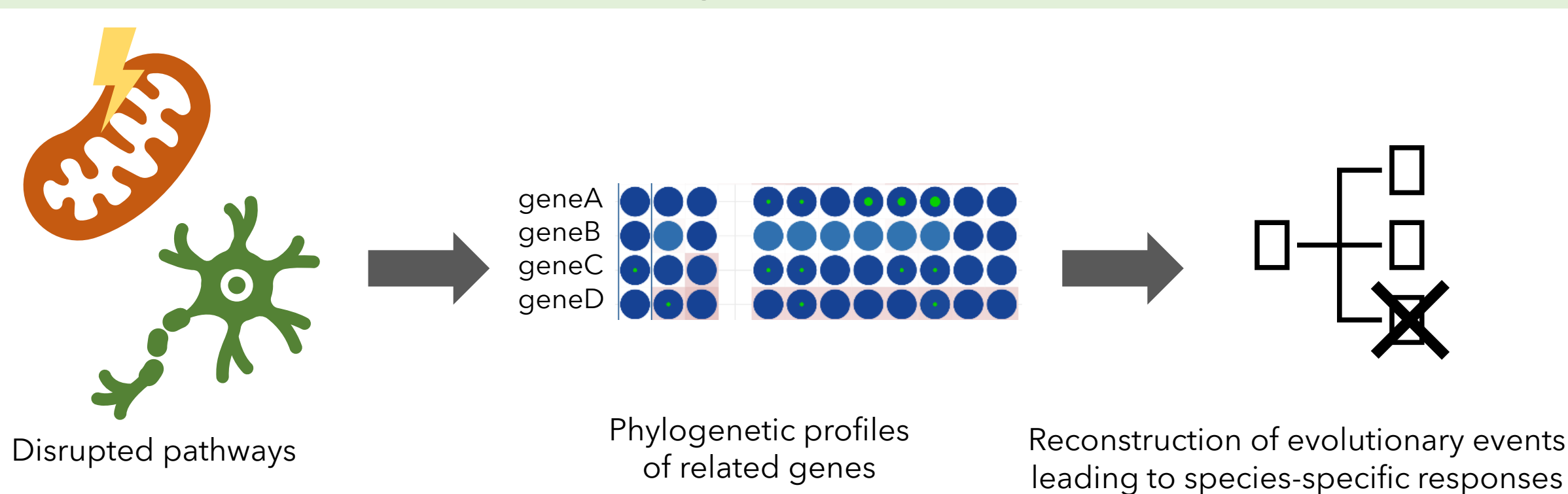


Figure 4. Non-redundant biological pathway GO terms associated with candidate genes. Edges connect highly similar terms (REVIGO).

Summary & Outlook



- A phylogenetic profile database has been established for over 20,000 fish genes across 12 species with differing tolerance levels to 6PPD-Q.
- New hypotheses regarding mechanisms of species-specific sensitivities can be easily tested for gene loss.
- Some candidate genes are widely conserved. Their modification might have resulted in loss of tolerance against 6PPD-Quinone.
- Three candidate genes play crucial roles in pathways negatively affected upon 6PPD-Q exposure (e.g. brain vascular permeability). These will be investigated further:
 - Frizzled-7 (*fz7b*)
 - Integrin alpha-2 (*itga2*)
 - Nicotinic cholinergic receptor alpha-7 (*chna7*)

A database to investigate 6PPD-Q toxicity

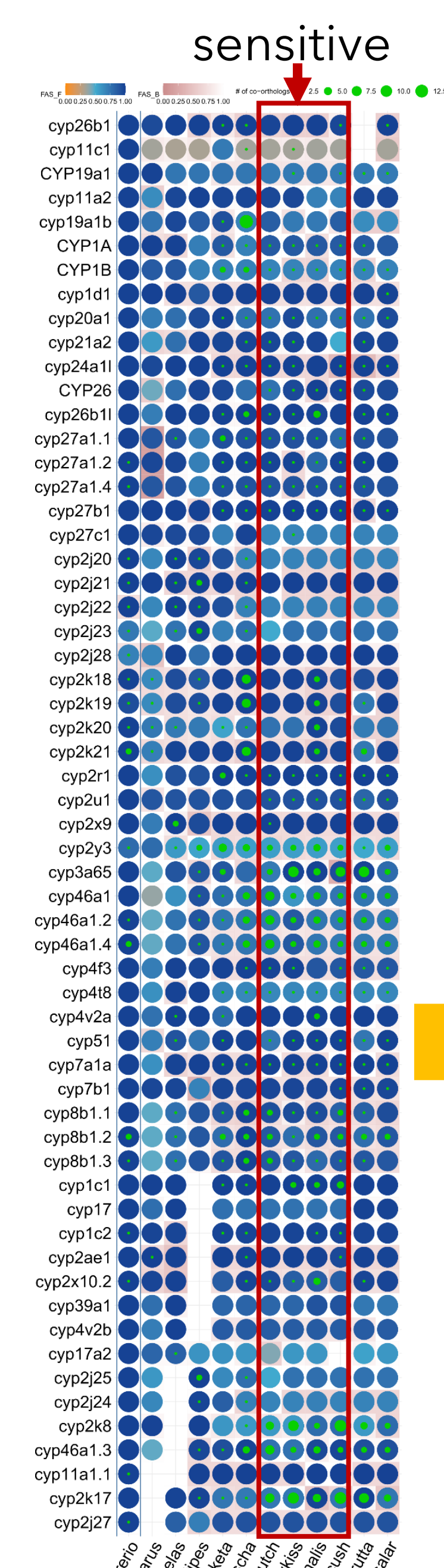


Figure 5. Phylogenetic profile of 60 genes encoding cytochrome P450 enzymes (*cyp* genes) across 12 fish species.

- 6PPD-Quinone's mode of action is not fully understood. Hypotheses have been proposed based on *in vivo* and *in vitro* studies.
- Ortholog search and annotation of genes involved in its mode of action can identify genetic patterns unique to sensitive species.

Biotransformation capacity?

- A proposed origin of 6PPD-Q tolerance relates to the ability of species to metabolize it (Montgomery et al. 2023). Cytochrome p450 (*cyp*) genes are mainly responsible for the detoxification of similar compounds.
- Phylogenetic profiles do not suggest *cyp* genes are absent or highly modified in sensitive Salmonids.
- Differences in species' biotransformation capacities of 6PPD-Q can still be at the gene expression level of *cyps*.

References

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