

LABORATORY-DIRECTED EVOLUTION AS A TOOL FOR ANTICIPATING INSECTICIDE RESISTANCE

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The evolution of insecticide resistance provides a eukaryotic model system for studying enzyme evolution. Understanding the molecular basis of insecticide resistance can assist both the development of new methods to combat resistance and the anticipation of future resistance. Three insect species have independently evolved catalytic organophosphate (OP) insecticide resistance through a single active-site mutation (Gly>Asp) in the α E7 enzyme¹⁻³. To explore the evolutionary potential of α E7, we subjected α E7 from the blowfly *Lucilia cuprina* to nine rounds of mutation and selection, resulting in a >1000-fold increase in OP-hydrolase activity and a $k_{cat}/K_M > 10^6 \text{ M}^{-1} \text{ min}^{-1}$. Kinetic and structural analysis of the evolutionary trajectory revealed the molecular basis for the increase in catalytic efficiency. Mutations occurring in the early stages of the trajectory enrich the productive side chain conformation of the key aspartic acid residue, while mutations in later stages remodel the binding pocket. Remarkably, mutations appearing in the later rounds yielded larger improvements in catalytic efficiency compared to initial mutations, indicating that the initial Gly>Asp mutation represents only a fraction of the α E7 evolutionary potential. Worryingly, this suggests that the Gly>Asp could be the first of many steps toward efficient OP-insecticide detoxification.

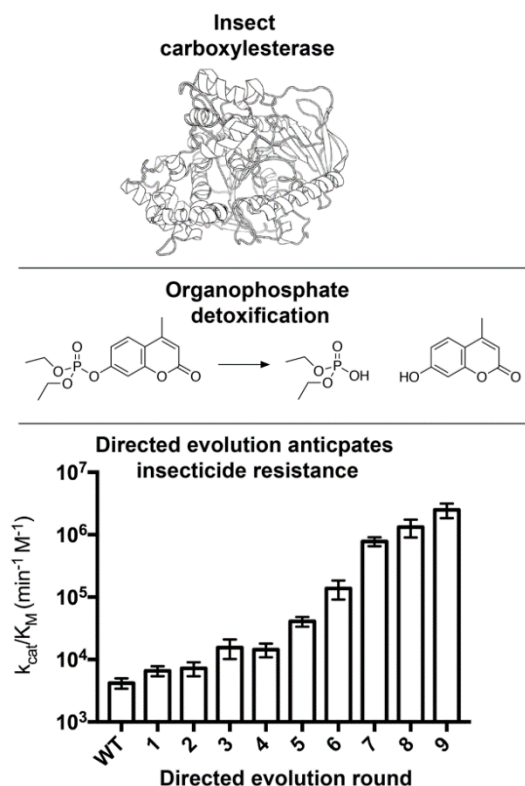


Figure 1 – Laboratory-directed evolution as a tool for anticipating insecticide resistance

References

1. Jackson, C. J. *et al.* Structure and function of an insect α -carboxylesterase (α Esterase7) associated with insecticide resistance. *Proc. Natl. Acad. Sci. U. S. A.* 110, 10177-82 (2013).
2. Correy, G. J. *et al.* Mapping the accessible conformational landscape of an insect carboxylesterase using conformational ensemble analysis and kinetic crystallography. *Structure.* 24, 1-11 (2016).
3. Mabbitt, P. D.* , Correy, G. J.* *et al.* Conformational disorganization within the active site of a recently evolved organophosphate hydrolase limits its catalytic efficiency. *Biochemistry.* 55, 1408-17 (2016).