# Evidence for activation of nitenpyram by a mitochondrial cytochrome P450 in *Drosophila melanogaster*

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## Abstract

## Graphical abstract

## Highlights

## Keywords

## Introduction

Cytochrome P450s (P450) genes are found in all forms of life and are involved in diverse physiological processes. Some insect P450s have essential developmental roles in hormone synthesis and degradation, while others act as drug metabolizing enzymes (DMEs) capable of modifying the chemical structure of insecticides from different classes and affecting resistance. Increased expression of P450s is often positively associated with resistance, particularly to the neonicotinoid class of insecticides (**Bass et. al 2015**). Individual P450s have been associated with neonicotinoid resistance by comparing transcript levels between resistant and susceptible strains or by genetic mapping (Kalajdzic et. al 2013, Yang et. al 2013), and some candidates have been confirmed by transgenic overexpression in model organisms (**check: is this only Drosophila?**) (Daborn et. al 2002, Zhu et. al 2010, Bass et. al 2013). (**P450?**) metabolites of the neonicotinoid imidacloprid have been identified and characterised *in vitro* and *in vivo* (Joussen et. al 2008, Hoi et. al 2014), and these metabolites are not always less toxic than the original insecticide. For example, the olefin metabolite of imidacloprid is more toxic (**has a lower LD50?**) than imidacloprid and has higher affinity for the neonicotinoid target protein, but was more **rapidly?** excreted from *Drosophila melanogaster* larvae (Nauen et. al 1998, Fusetto et. al 2017). These studies have challenged the earlier paradigm that P450-mediated metabolism always leads to detoxification.

**Sentence or two about nitenpyram**

P450 genes form four phylogenetic clades, three encoding proteins localised to the endoplasmic reticulum and one encoding enzymes localised to mitochondria (**Feyereisen 1999, Ranson et. al 2002**). Although mammalian mitochondrial P450s are associated predominantly with developmental functions (**Guengerich et. al 2005**), insect P450s from mitochondrial and non-mitochondrial clades function in insecticide metabolism and development (**Gilbert et. al 2004, Daborn et. al 2007**). Here, we report evidence that *Cyp12a5*, a mitochondrial P450, is involved in the metabolism of nitenpyram in *D. melanogaster*, possibly forming a metabolite with higher toxicity than nitenpyram.

## Material and methods

We used the method.

## Results

### RNAi of the cytochrome P450 redox partner, *dare*, results in decreased nitenpyram mortality

To detect phenotypes arising from disruption of cytochrome P450 functions in specific tissues, we generated an RNAi system to interrupt the functions of the P450 redox partners, *Cpr* and *dare*, under control of the GAL4-UAS system. For each redox partner gene, two non-overlapping UAS-RNAi constructs were transformed into the *w*1118 strain of D. melanogaster (supporting figure 1). Both ubiquitous RNAi with the 5′tubulin driver and specific RNAi in the ring gland using the 5′phantom driver resulted in lethality, and the latter was partially rescued by addition of 20-hydroxyecdysone (supporting figure 2), suggesting that RNAi of the redox partners targets inhibits P450 functions.

The system was then used to specifically examine To detect phenotypes related to P450-based metabolism of insecticides in the insect digestive system., RNAi of the P450 redox partners was performed using the 5′Cyp6g1HR-3a driver, which produces GAL4 in the midgut, Malpighian tubules and fat body. Quantitative real-time RT-PCR in midguts from first instar larvae, third instar larvae and adults indicated that RNAi removed 60–80% of Cpr mRNA and 70–90% of dare mRNA (Ttable 1). In crosses between heterozygous flies carrying the driver transgene and a balancer chromosome (genotype 5′Cyp6g1HR-3a / TM6B, Tb1) and the Cpr and dare UAS-RNAi line s, there was no reduction significant difference inof emergence of progeny carrying the driver chromosome (Ffigure 1A), suggesting that RNAi of the P450 redox partners in these tissues does not cause lethality.

To assess the effect of Cpr and dare knockdown in the D. melanogaster metabolic tissues, flies expressing the UAS-RNAi constructs under control of the 5′Cyp6g1HR-3a driver were reared on nitenpyram or dicyclanil or exposed to DDT as adults. Mortality of first instar larvaeSurvival of flies expressing either dare RNAi construct raised on two different concentrations of nitenpyram was increased compared to control crosses (Supporting Figure 2). This suggests that metabolism of nitenpyram by one or more mitochondrial P450s expressed in the midgut, Malpighian tubules or fat body leads to the formation of a more toxic product.

## Discussion

a combination of RNAi and GAL4-UAS overexpression is used to characterize the roles of a subset of P450s and their redox partners in vivo using D. melanogaster. RNAi knockdown of the P450 reductases Cpr and dare was lethal if performed ubiquitously or in the ring gland. Partial rescue with ecdysone suggested that this was due to the essential role Cpr and dare play in facilitating essential P450 function. Conversely, Cpr and dare knockdown in the digestive tissues (malpighian tubules, midgut and fat body) had no fitness cost but, inhibition of dare led to an increase in nitenpyram resistance. Investigation of a subset of mitochondrial P450s showed that RNAi knockdown of Cyp12a5 phenocopied the dare RNAi (increased resistance) and that GAL4/UAS overexpression of the same gene lead to a decrease in nitenpyram resistance. These results suggest that Cyp12a5 may play a role in increasing the toxicity of nitenpyram.

Test citation (Daborn et al., [2002](#ref-daborn_single_2002)).

## Acknowledgements

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## References

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