////Title: Identifying an Achilles' Heel in Insecticide Resistance

////Stand-first: Insecticide resistance is an ongoing challenge for agriculture and the control of insect-transmitted diseases. In a recent study at Purdue University, a team of scientists led by Dr Barry Pittendrigh [Pitt-uhn-dree] identified a potential chink in the armour of insecticide resistance in fruit flies. If this Achilles' heel can be exploited, it bodes well for the future control of destructive insects.

////Body text:

Insect pests can wreak havoc in crop fields, decimating yields and ultimately threatening our food security. Certain insect species can also cause serious health concerns for humans by transmitting infectious diseases. For example, mosquito-transmitted malaria continues to be a significant cause of ill health and death in many parts of the world.

Insecticides are used to control insect populations, but target insects can become resistant to these chemicals over time. By finding ways to inhibit resistance, the effectiveness of insecticide treatments could be restored. However, as recently as 2017, only one effective inhibitor of insecticide resistance was commercially available. To address this difficulty, Dr Barry Pittendrigh and his team at Purdue University are investigating potential new inhibitors of insecticide resistance.

…

The fruit fly *Drosophila melanogaster* [dro-so-fuh-lah muh-la-now-ga-stir], commonly referred to as *Drosophila*, has been used in investigations into insecticide resistance for decades. Many of these studies have focused on DDT – a well-known insecticide that many insect populations have now developed resistance to.

The so-called '91-R' [ninety-one R] strain of *Drosophila* is highly DDT-resistant, and these flies show reduced cell penetration and increased removal of DDT. These processes all require energy to power them. Previous comparisons between DDT-resistant strains and non-resistant strains of *Drosophila* have identified that energy metabolism pathways are critical to DDT resistance.

One such energy metabolism pathway is the insulin signalling pathway, which plays a significant role in energy regulation in animals, including mammals and insects. In *Drosophila*, the insulin signalling pathway is important for growth and development, regulation of lifespan, metabolism of foreign substances and stress resistance.

…

Together with his colleagues, Dr Pittendrigh used genomics tools to search for alterations in genes that code for proteins in the insulin signalling pathway, in both 91-R flies and flies of a strain called 91-C [ninety-one C], which are not resistant to DDT. The two strains are of common origin. The team also looked for differences between the males and females of both strains, in terms of their lifespans, resistance to starvation, glycogen levels in response to starvation and the impact of dietary sugar.

They found variations in insulin signalling pathway genes between the two strains of *Drosophila.* Specifically, a total of eight insulin signalling pathway genes with increased activity and seven with decreased activity were identified in the DDT-resistant 91-R flies compared to 91-C flies. The team noticed the largest differences in two genes that code for enzymes called PEPCK [pep-C-K] and GSK3β [G-S-K-three-bay-tah]. These two enzymes are associated with energy metabolism, and both were significantly reduced in the resistant flies.

PEPCK helps to metabolise proteins and fats to produce glucose for energy during times of fasting or starvation, while GSK3β inactivates an enzyme that converts glucose into glycogen, which can be stored for later use.

The team's results suggest that while fly populations were evolving to become resistant, they also evolved processes that allowed them to cope with the energy demands of multiple resistance mechanisms.

…

These results prompted Dr Pittendrigh and his colleagues to explore chemicals that can inhibit the action of PEPCK and GSK3β. The inhibitors they chose were hydrazine sulphate for the PEPCK enzyme, and lithium chloride for GSK3β.

After feeding the resistant 91-R flies with hydrazine sulphate mixed in a sugar solution, the team noticed that levels of DDT resistance were markedly reduced. In fact, this chemical increased the effectiveness of DDT in both resistant and non-resistant strains, causing up to a 218-fold decrease in resistance in the DDT resistant fly populations and up to a 5-fold increase in susceptibility in the non-resistant flies. Lithium chloride, which is known to inhibit the GSK3β enzyme, also reduced the resistance to DDT, but far less dramatically.

However, the effectiveness of the inhibitors was dependent on the nutritional status of the flies, with the greatest reductions occurring in well-fed 91-R flies. Previous studies have shown that DDT is more toxic to smaller individuals; thus, DDT toxicity and resistance appear to be directly linked to energy metabolism and fly weight.

In this study, the nutritional status of the flies did influence the toxicity of DDT and also the effectiveness of hydrazine sulphate and lithium chloride at reducing resistance levels. Interestingly, in the absence of DDT or inhibitors, resistant flies only outlived susceptible flies under optimal feeding conditions, with their lifespan generally being less than that of 91-C flies under starvation, and excess sucrose conditions.

…

Next, the team studied glycogen levels in both strains. Their results indicate that 91-R females store less energy in the form of glycogen than 91-C females, and consume a larger percentage of their stored glycogen during the early stages of starvation.

The use of lithium chloride to inhibit GSK3β would therefore be expected to make the 91-R flies more resistant to DDT, not less. However, the expression of PEPCK and GSK3β, which is already significantly reduced in 91-R, is further inhibited by hydrazine sulphate and lithium chloride, respectively. This additional inhibition likely reduced the function of the enzymes to levels where they were incapable of performing their physiological roles at all.

…

Through this work, Dr Pittendrigh and his colleagues have demonstrated the importance of the role of the insulin signalling pathway, and the enzymes PEPCK and GSK3β, in the maintenance of DDT resistance. They have also revealed important insights into the vulnerabilities of resistance mechanisms. They hope that these Achilles' Heel resistance traits can be exploited in future to develop compounds that can combat insecticide resistance in our most damaging and dangerous pest species.

This SciPod is a summary of the paper 'The insulin signalling pathway in *Drosophila melanogaster.* A nexus revealing an "Achilles' heel" in DDT resistance', published in *Pesticide Biochemistry and Physiology*. doi.org/10.1016/j.pestbp.2020.104727

For further information, you can connect with Barry Pittendrigh at pittendr@msu.edu