中国农业科学院
CHINESE ACADEMY OF AGRICULTURAL SCIENCES

细胞色素P450s介导的烟粉虱烯啶虫胺抗药性分析

Study on cytochrome P450s causes whitefly nitenpyram resistance

“实用生物信息技术”课程期末总结交流报告会

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2024-01

目录

烟粉虱
Bemisia tabaci

别名：小白蛾、银叶粉虱

目：半翅目

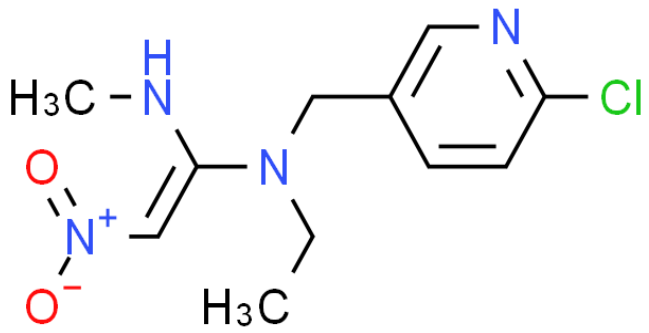
科：粉虱科

属：小粉虱属

烟粉虱是一种重大的外来入侵害虫，为害600多种寄主植物（烟粉虱的寄主包括烟草、番茄、番薯、木薯、棉花、十字花科、葫芦科、豆科、茄科、锦葵科等），传播300多种植物病毒

烟粉虱是世界粮农组织（FAO）认定的全球第二大农业害虫，它还是全球唯一被冠以“超级害虫”的昆虫

烯啶虫胺
nitenpyram



相较于其他蔬菜害虫，烟粉虱表现出更高的烯啶虫胺抗药性

烯啶虫胺是一种**新烟碱类杀虫剂**（分子式：$C_{11}H_{15}ClN_4O_2$），主要作用于**昆虫神经**，对害虫**突触受体具有神经阻断作用**，可用于水稻、蔬菜等作物，对各种蚜虫、叶蝉和蓟马显示了卓越活性

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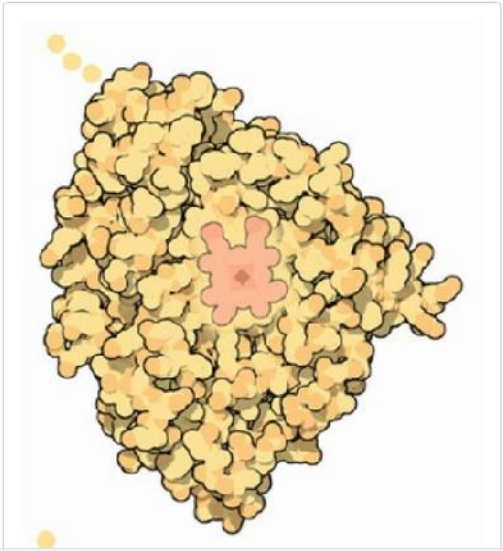
Molecule of the Month: Cytochrome p450

Cytochrome p450 detoxifies and solubilizes drugs and poisons by modifying them with oxygen

细胞色素P450s是一个解毒酶家族在烟粉虱对杀虫剂的解毒过程中扮演了重要的角色

If you have a headache and take a drug to block the pain, you'll notice that the effects of the drug wear off in a few hours. This happens because you have a powerful detoxification system that finds unusual chemicals, like drugs, and flushes them out of your body. This system fights all sorts of unpleasant chemicals that we eat and breathe, including drugs, poisonous compounds in plants, carcinogens formed during cooking, and environmental pollutants. The cytochrome p450 enzymes are our first line of defense in this chemical battle.



Adding Oxygen

The cytochrome p450 enzymes find unusual molecules and add oxygen atoms to them. In most cases, this has the effect of making the molecule more soluble in water, and thus, easier to flush out of the body. The added oxygen also provides a ready handle for other detoxifying enzymes to take hold and further modify, and destroy, these toxic molecules. This task of adding oxygen is chemically tricky, and cytochrome p450 enzymes use a powerful molecular tool to perform the reaction: an iron atom in a heme group (described in more detail later).

p450 Everywhere

Cytochrome p450 enzymes are found in all organisms. Each organism builds several different enzymes, each of which act on a different selection of molecules. Typically, bacteria make about 20 different forms of these enzymes and we produce about 60. Plants often make hundreds of different forms. This is because plants make unusual pigments and exotic toxins to protect themselves. Many of the reactions needed to make these molecules are performed by specialized cytochrome p450 enzymes. For more information on

细胞色素P450在生物体内是如何发挥作用的?

烟粉虱对烯啶虫胺产生了抗药性

细胞色素P450可以帮助烟粉虱解毒

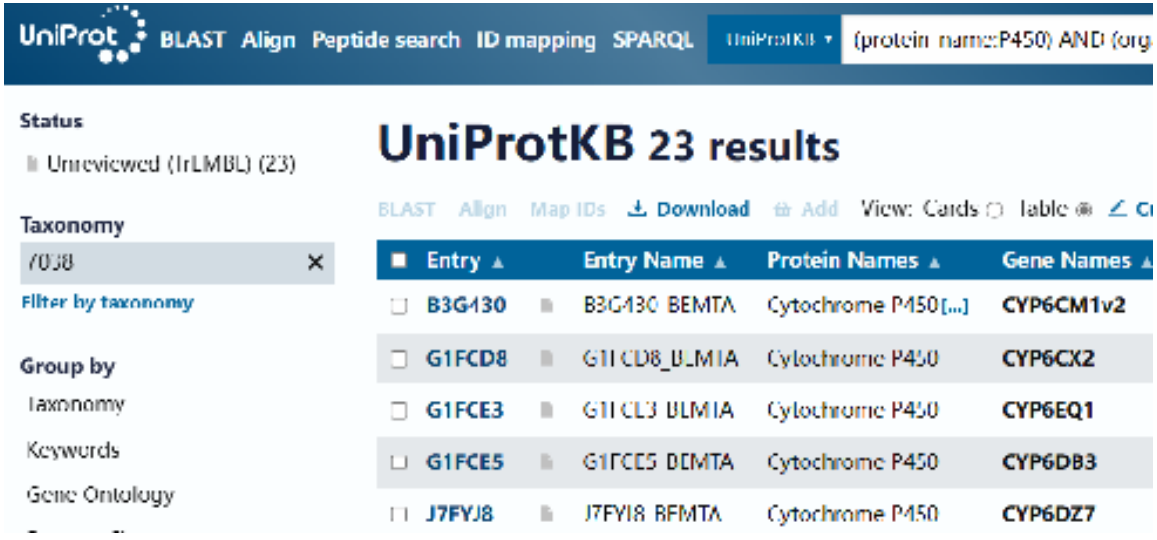
在uniprot中利用

高级搜索烟粉虱

P450s家族得到

23个结果及系统

发生树



代表性的P450s

3cpp

Phyre2 Investigator

Top model

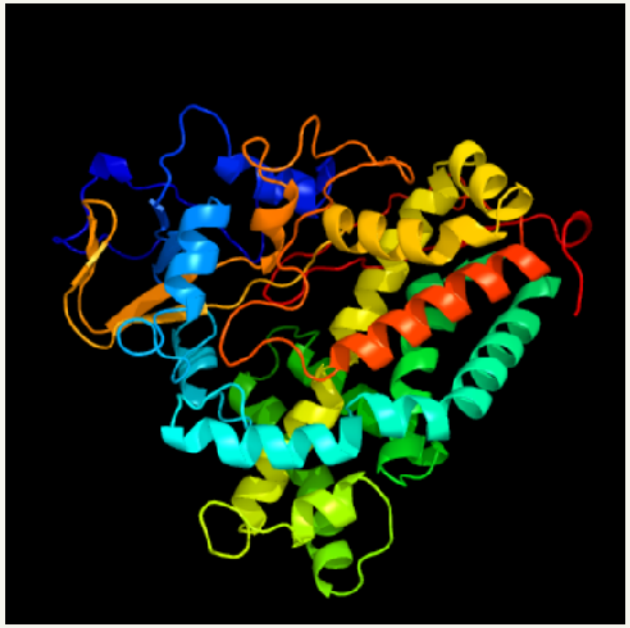


Image coloured by rainbow N → C terminus

Model dimensions (Å): **X**:67.081 **Y**:46.492 **Z**:59.660

Model (left) based on template d1re9a_

Top template information

Fold: Cytochrome P450
Superfamily: Cytochrome P450
Family: Cytochrome P450
PDB entry: PDBe RCSB PDBj

Confidence and coverage

Confidence: **100.0%** Coverage: **98%**

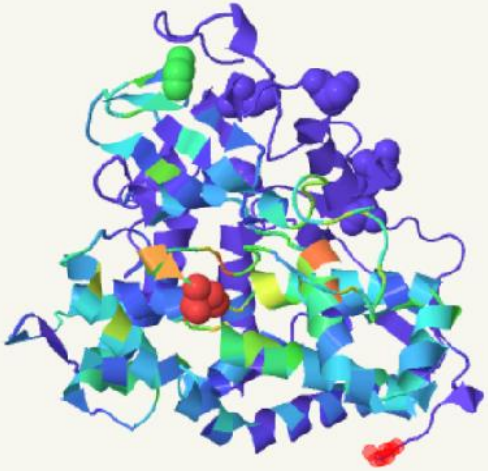
404 residues (98% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

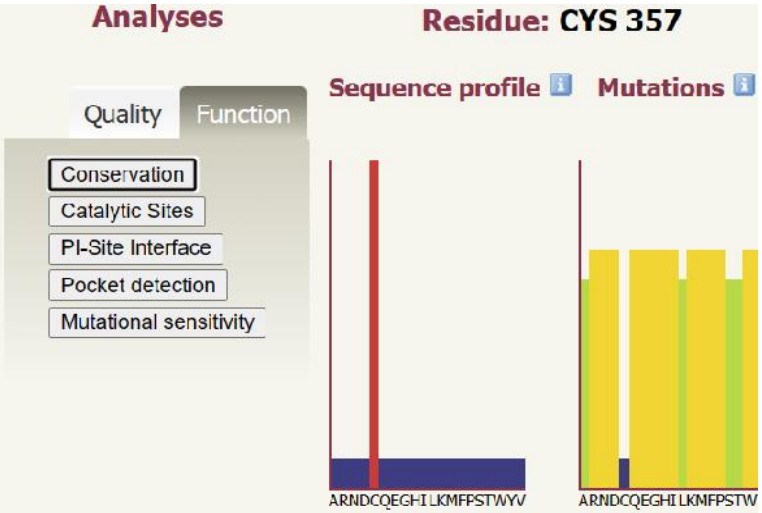
3D viewing

Interactive 3D view in JSmol

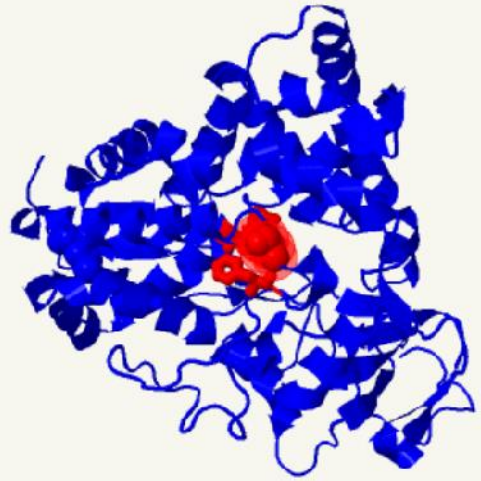
For other options to view your downloaded structure offline see the FAQ

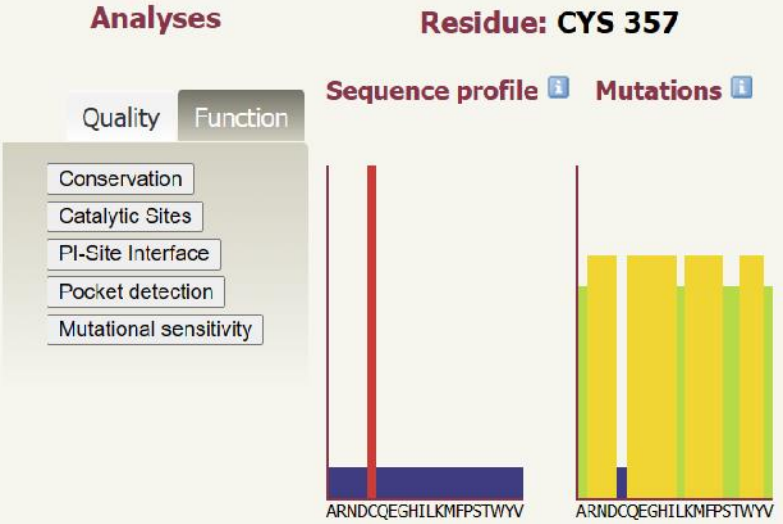
保守位点


Relative
Conservation
High
Low


Analyses
Residue: CYS 357
Quality
Function
Conservation
Catalytic Sites
PI-Site Interface
Pocket detection
Mutational sensitivity
Sequence profile
Mutations
ARNDCQEGHILKMFPSTWYV
ARNDCQEGHILKMFPSTW

功能位点


Identity
Different
Non-site


Analyses
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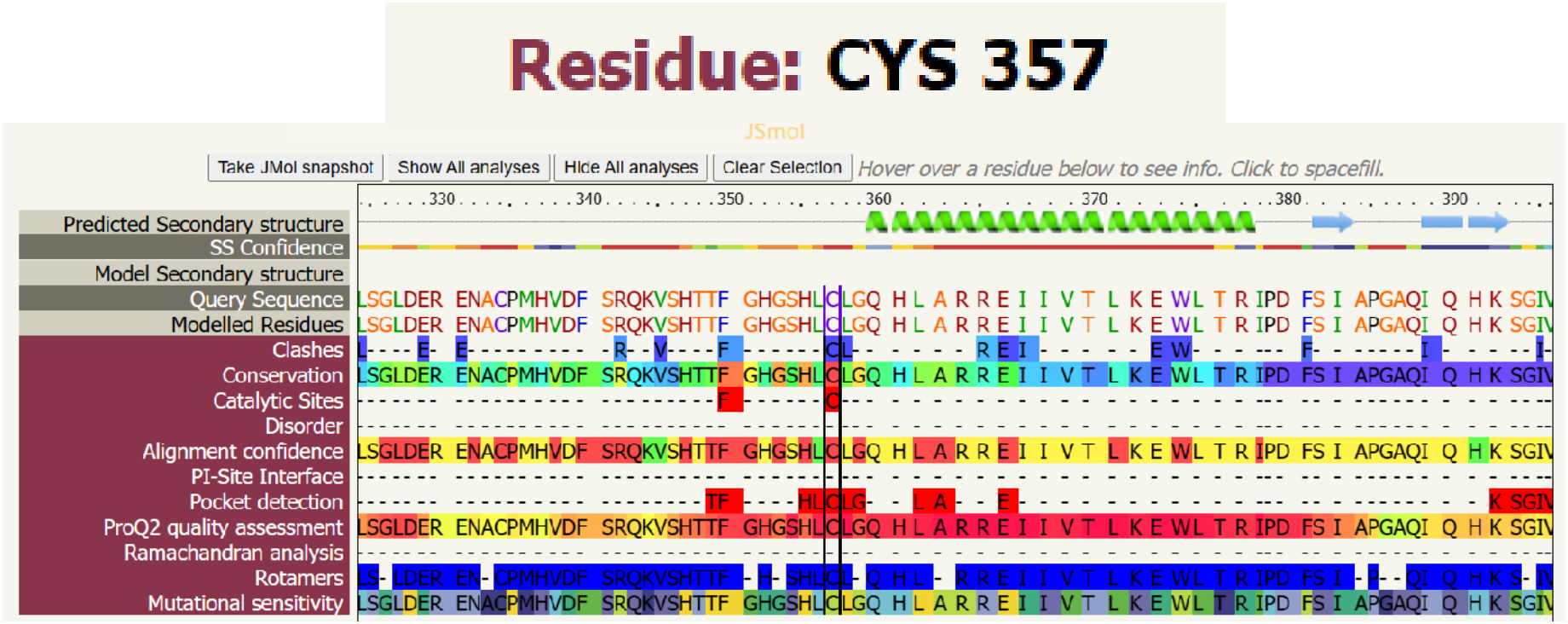
Mutational sensitivity
Sequence profile
Mutations
ARNDCQEGHILKMFPSTWYV
ARNDCQEGHILKMFPSTWYV

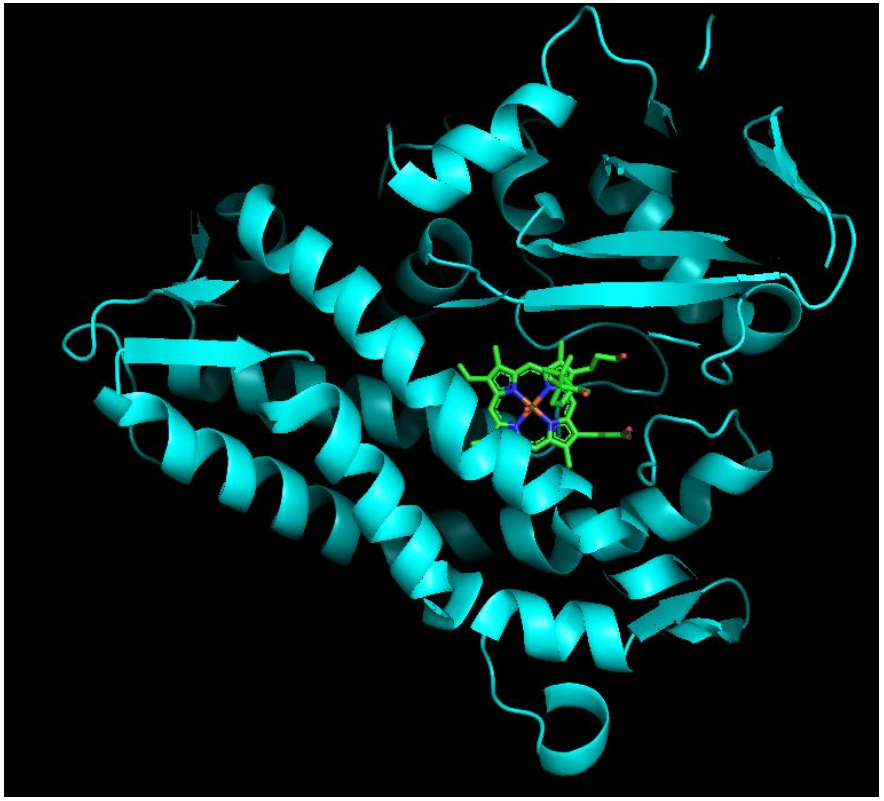
核心功能区，催化位点，高度保守，参与形成口袋

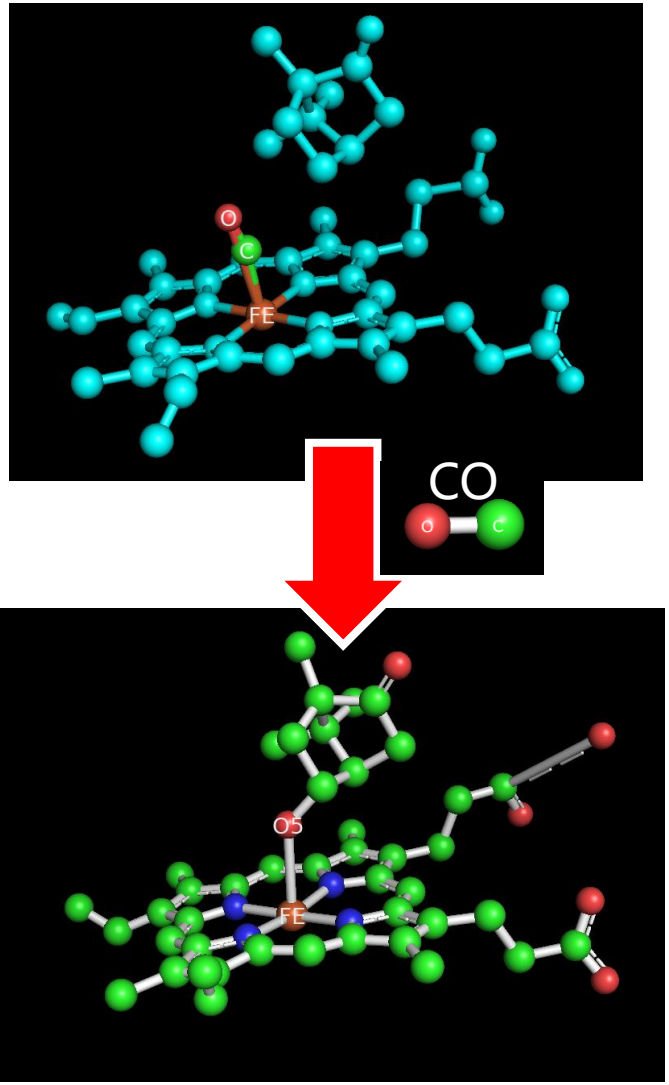


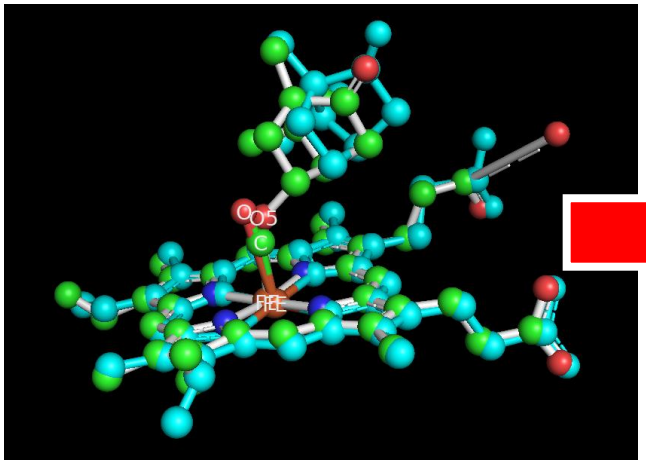
357位的半胱氨酸

是如何协助P450行使解毒功能的?

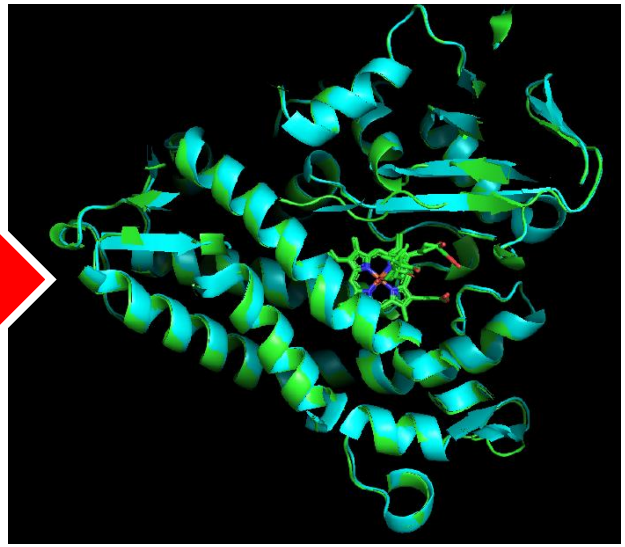
3cpp

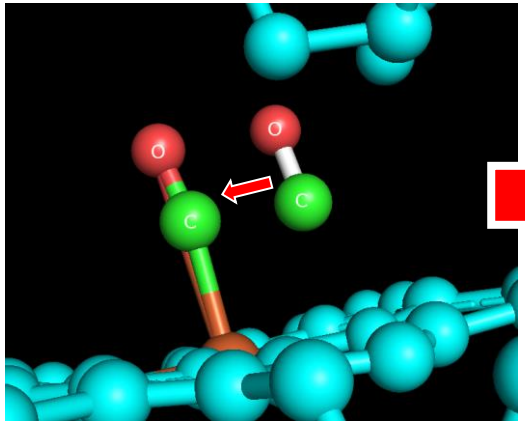


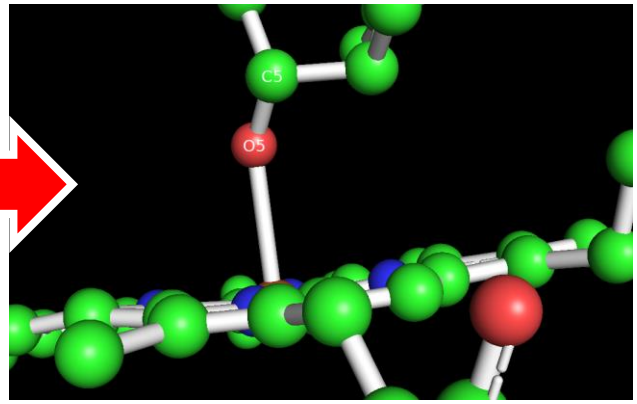




微小的构象变化



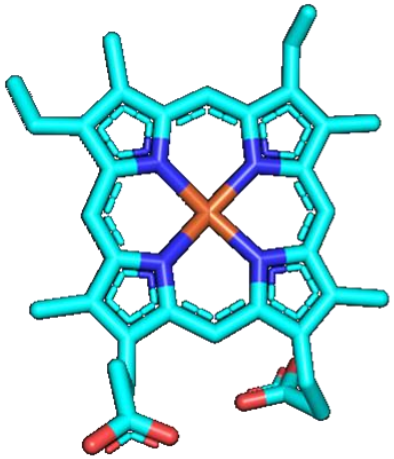


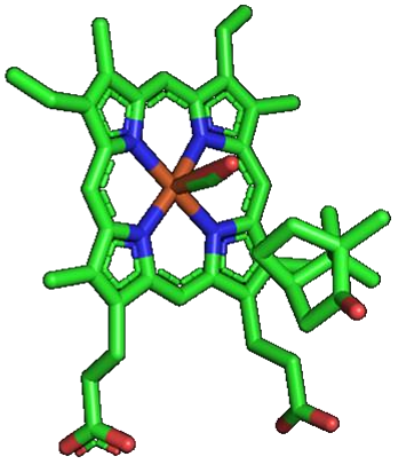


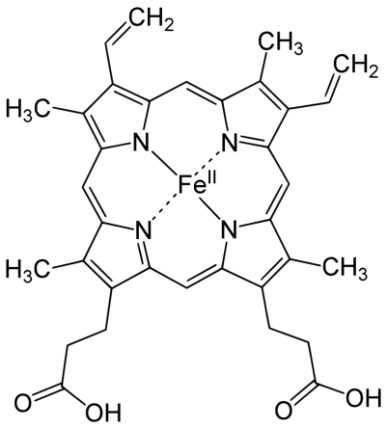
当P450s和有毒物质（CO、杀虫剂）结合后，会向其中加入一个氧原子，而另一个氧原子在反应过程中作为水分子释放出来

定向加氧是一个复杂的化学反应

一个具有铁元素核心的血红素发挥了重要功能







P450s核心　血红蛋白核心　铁-卟啉复合体

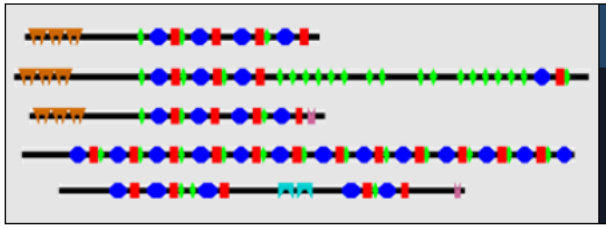
底部的**半胱氨酸**可激活铁原子，为有毒物质加上一个氧，并释放一个水分子

$$CO + O_2 + 2H^+ + 2e^- \rightarrow CO_2 + H_2O$$

筛选基因

MEGA11

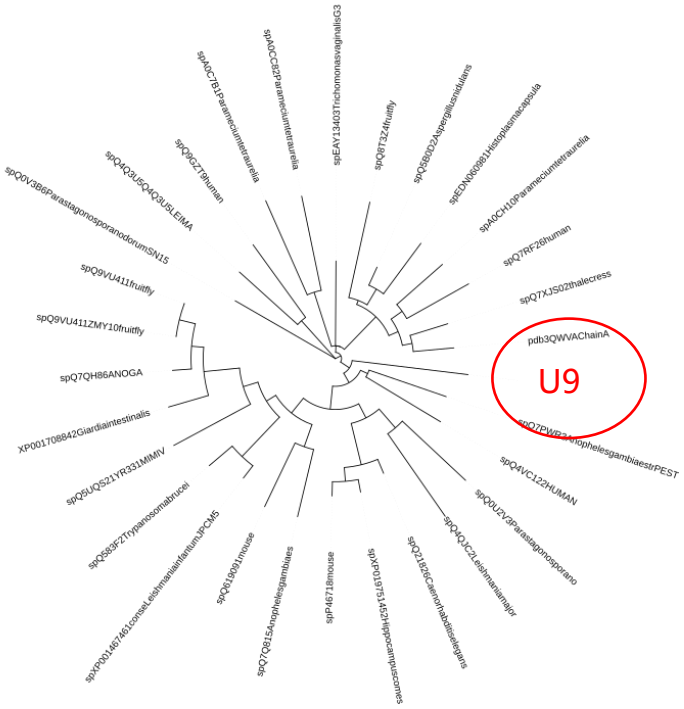
iTOL INTERACTIVE TREE OF LIFE

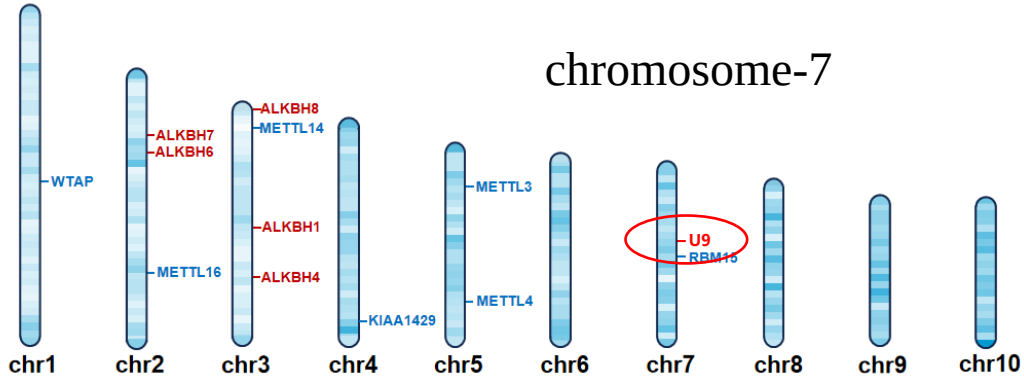


CDD

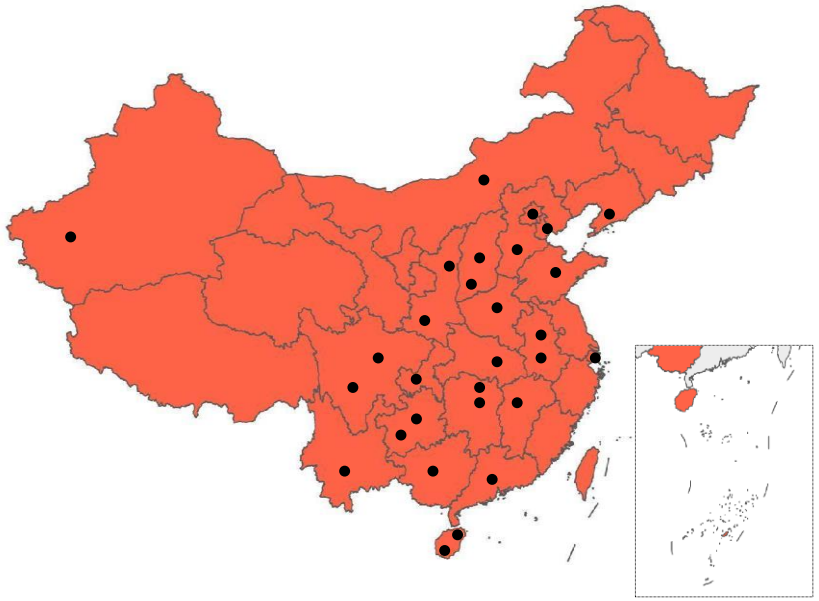
The Conserved Domain Database is a resource for the annotation of functional units in proteins. Its collection of domain models includes a set curated by NCBI, which utilizes 3D structure to provide insights into sequence/structure/function relationships.

锁定了一个P450s相关基因U9



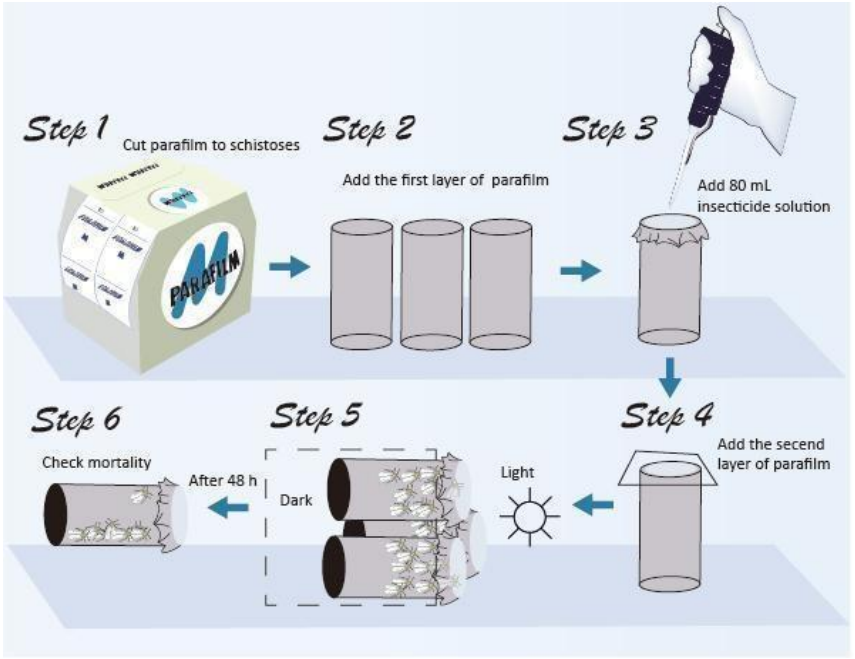


采集种群



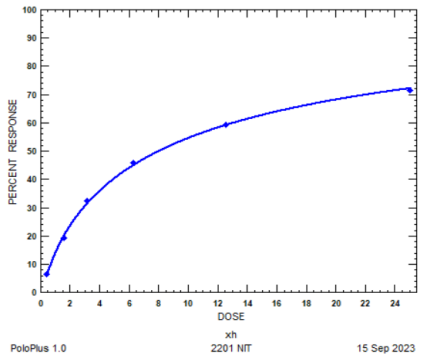
来自全国33个不同地区的三年的烟粉虱种群

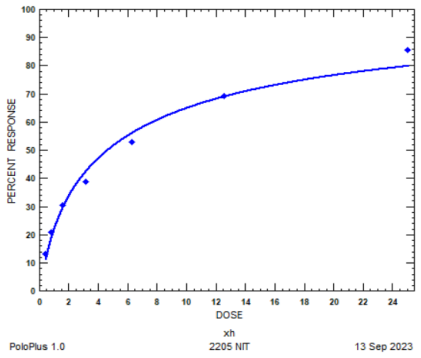
鉴定种群

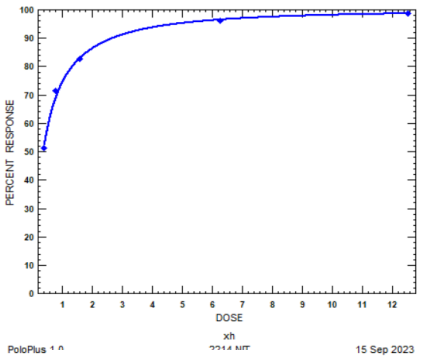


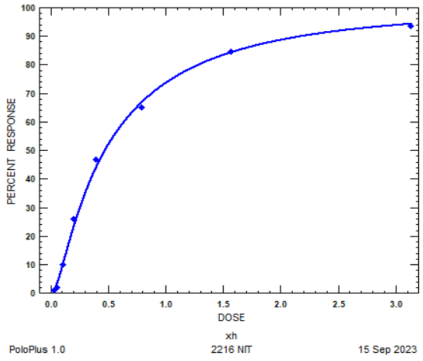
采用饲喂法，鉴定烟粉虱对烯啶虫胺抗性程度

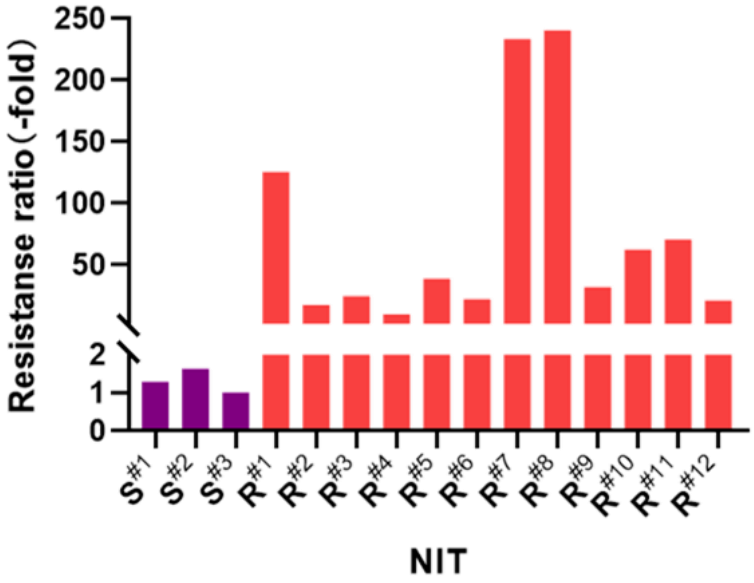
分析种群











采用Polo Plus 进行抗药性倍数分析

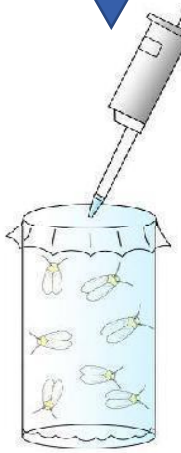
锁定了3个敏感种群，12个抗性种群

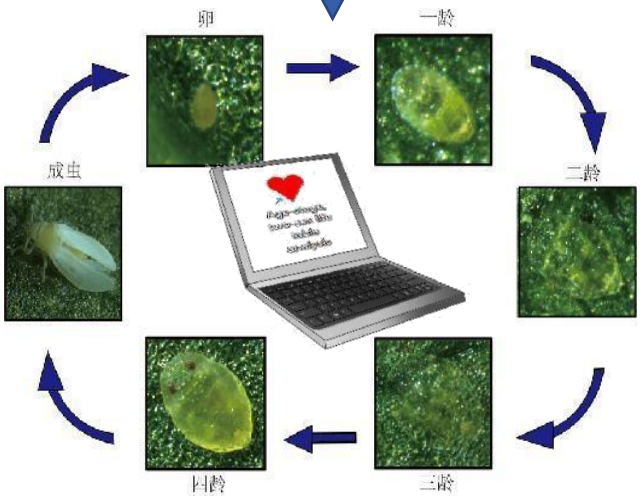
RNAi
（RNA干扰）

抗/敏

生命表

通过dsRNA
干扰U9的
正常表达



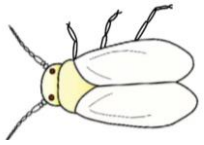


“齐心”生命表
分析发育参数

死亡率
显著变化

RT-qPCR
荧光定量PCR

证明U9
在抗药性方面
发挥作用

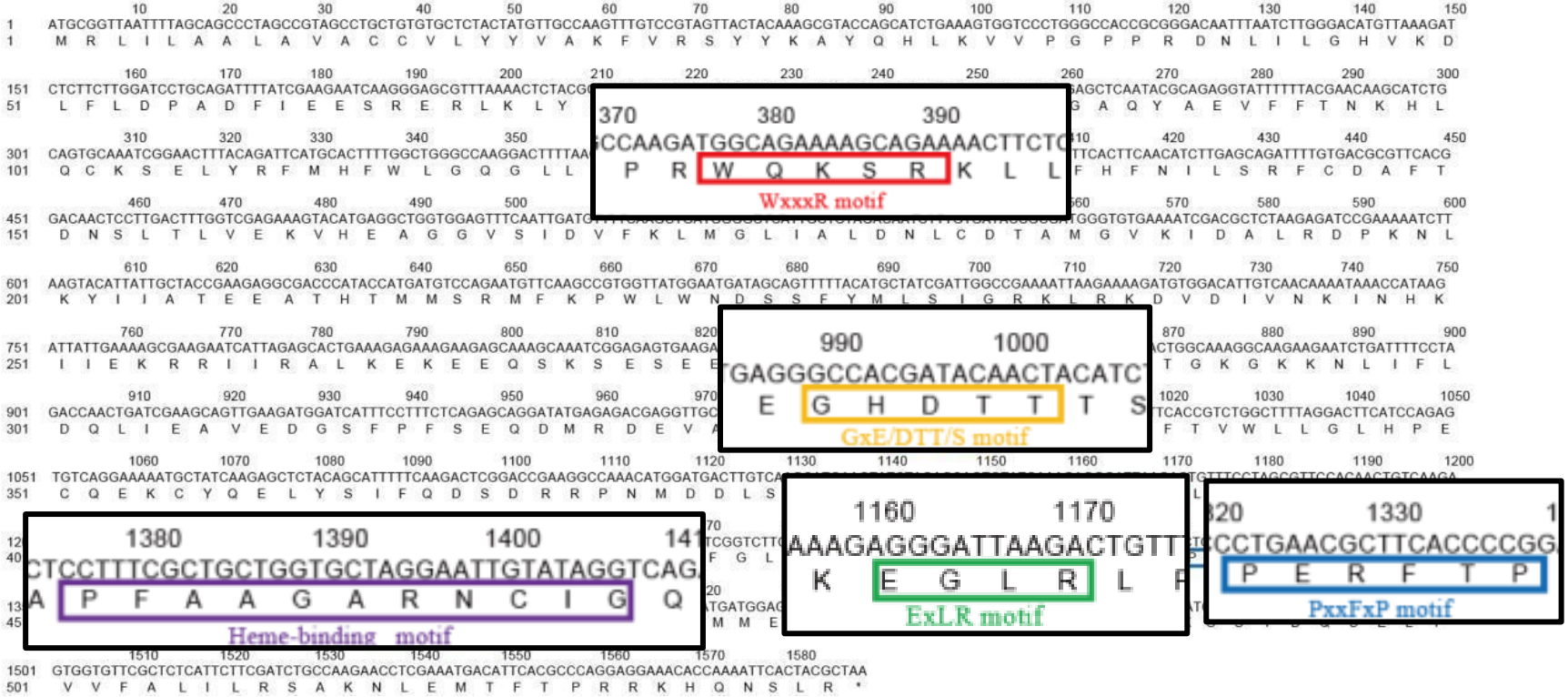


QuantStud...
Design & ...

检测不同龄期U9表达量
绘制时空表达谱

DNAMAN8

通过U9的ORF序列
预测蛋白质的保守结构域

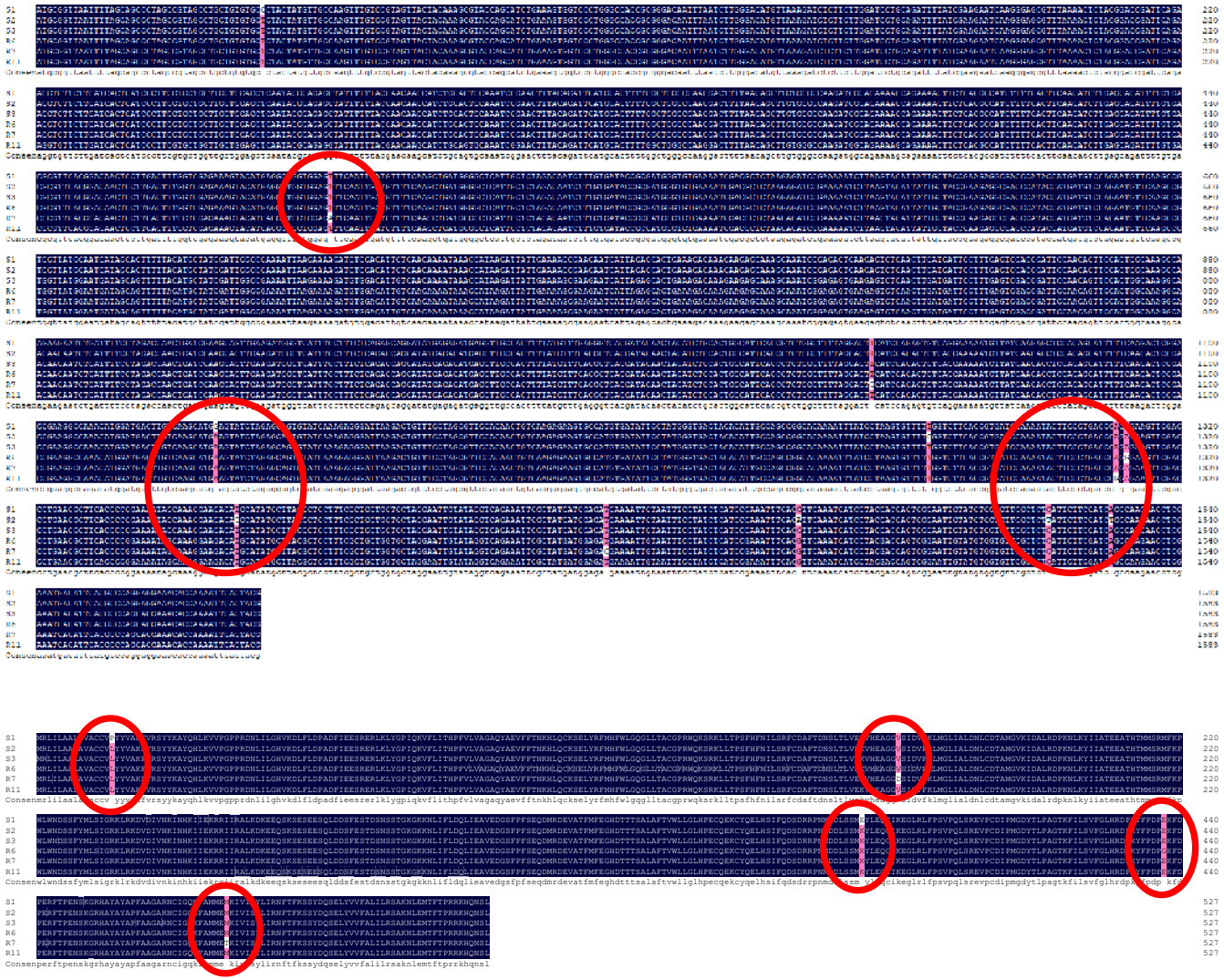


包含5个motif

该基因中的五个保守结构域是细胞色素P450家族共有的

对抗/敏种群的5个motif进行核酸/氨基酸对比

核酸序列



氨基酸序列

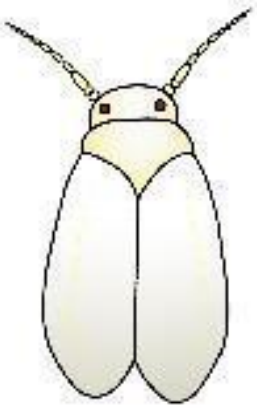
结论

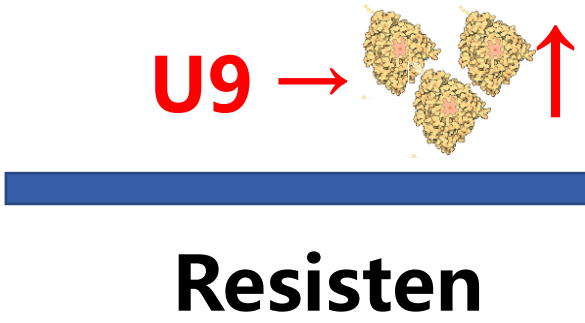
敏感/抗性种群在外显子区域没有一致的单核苷酸多态性

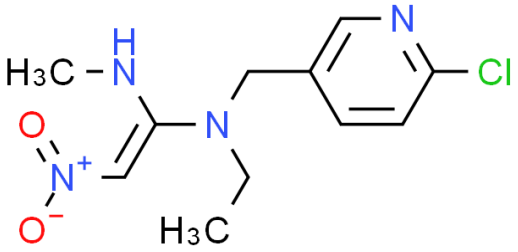
烟粉虱对烯啶虫胺的抗性

主要还是来自**U9表达量的改变**，而不是基因突变

结论：U9作为一个上游基因对P450s起到调控作用从而影响了烟粉虱对烯啶虫胺的抗性







后续会从蛋白质层面继续探究

U9、P450s与烯啶虫胺抗性之间的关系

Western Blot(WB)

Immunoprecipitation（IP)

pull-down

electrophoretic mobility shift assay（EMSA)

Yeast two-hybrid system……

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12. Cross-resistance and Fitness Cost Analysis of Resistance to Thiamethoxam in Melon and Cotton Aphid (Hemiptera: Aphididae)
13. Monitoring, cross-resistance, inheritance, and fitness costs of brown planthoppers, Nilaparvata lugens, resistance to pymetrozine in China
14. Dinotefuran resistance in Nilaparvata lugens: resistance monitoring, inheritance, resistance mechanism and fitness costs

Web

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THANKS