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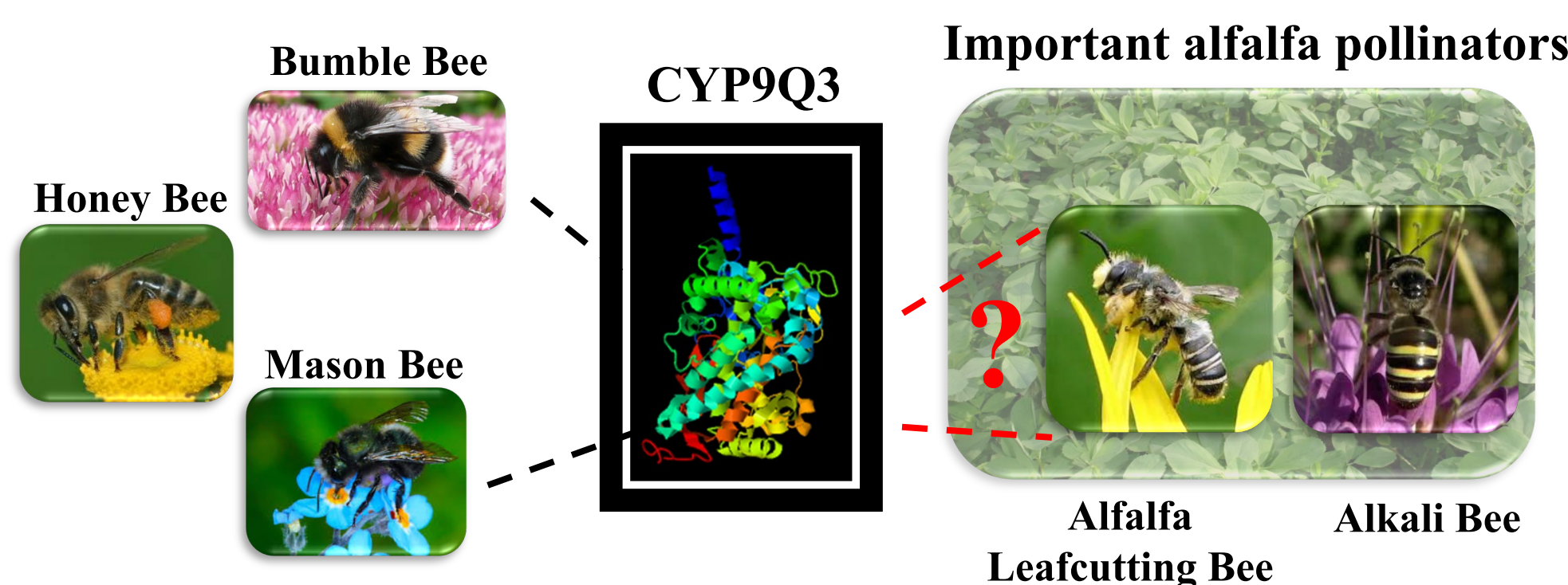
1: U.S. Environmental Protection Agency, Office of Research and Development, Great Lakes Toxicology and Ecology Division, Duluth, MN 55804
2: U.S. Environmental Protection Agency, Office of Pesticide Programs, Environmental Fate and Effects Division, Washington, D.C. 20460
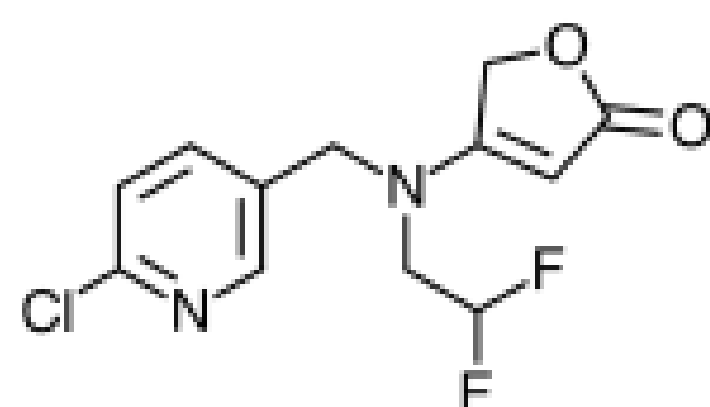
Donovan J. Blatz I Blatz.Donovan@epa.gov I 218-529-5245
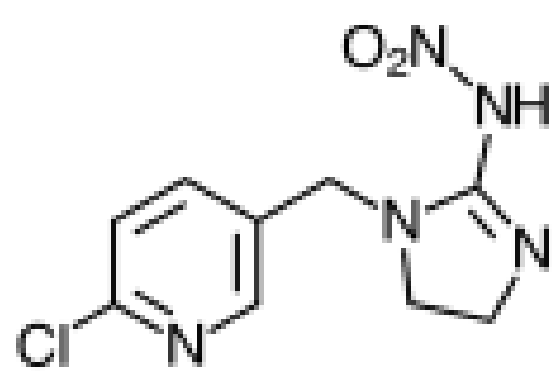
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Introduction

- Examining potential interactions between a new pesticide, Flupyradifurone, and cytochrome p450 CYP3 family of enzymes across bee species; Honey Bee *(Apis Mellifera)*, Bumble Bee *(Bombus Terrestris)*, Mason Bees *(Osmia Bicornis)*, Alfalfa Leafcutting Bees *(Megachile Rotundata)*, and Alkali Bees *(Nomia Melanderi)*
- The family of cytochrome p450 CYP3 family of enzymes are known to detoxify some pesticides
- The US EPA Sequence Alignment to Predict Across Species Susceptibility Tool (SeqAPASS v4.0; https://seqapass.epa.gov/seqapass/) can be used to determine chemical susceptibility across species by evaluating conservation of key amino acids
- The CYP9 family of metabolizing enzymes are present in three bee species which have similar sensitivity to the pesticide, Flupyradifurone

CYP9Q3
Bumble Bee
Honey Bee
Mason Bee
Important alfalfa pollinators
Alfalfa Leafcutting Bee
Alkali Bee

Flupyradifurone

Flupyradifurone

Imidacloprid

- In the same mode of action as neonicotinoids and sulfoxaflors[3]
- Acts on sucking pest species targeting the nicotinic acetylcholine receptors (nAChR)[3]
- Honey bee, Bumble bee and Mason bee all have a similar sensitivity to Flupyradifurone while the pesticide is **170x more toxic** to Alfalfa leafcutting bees[3]

CYP3 Detoxifying Enzymes

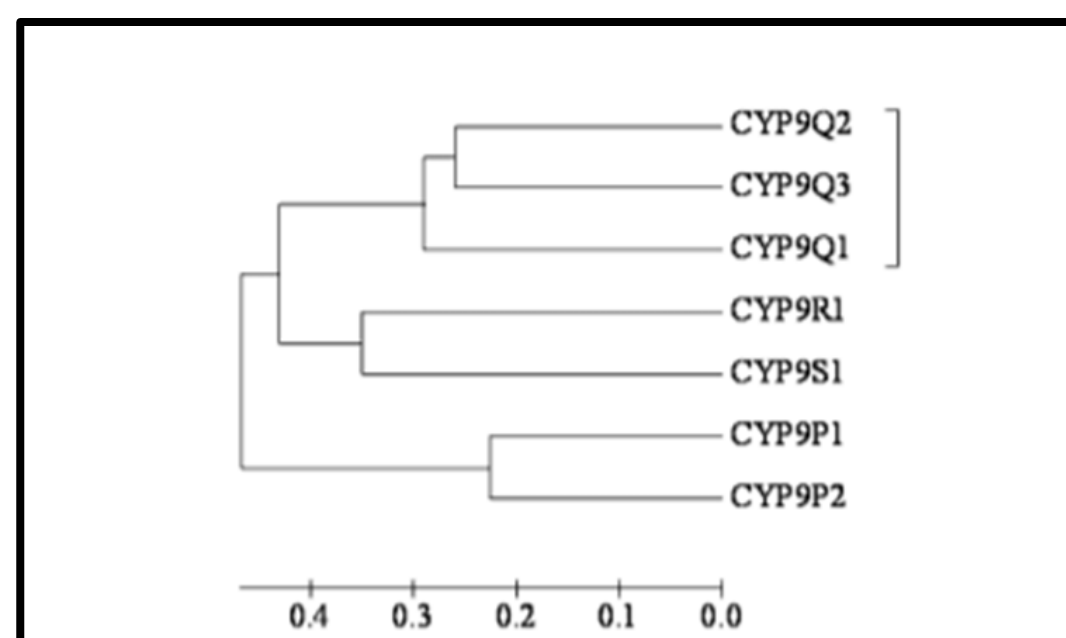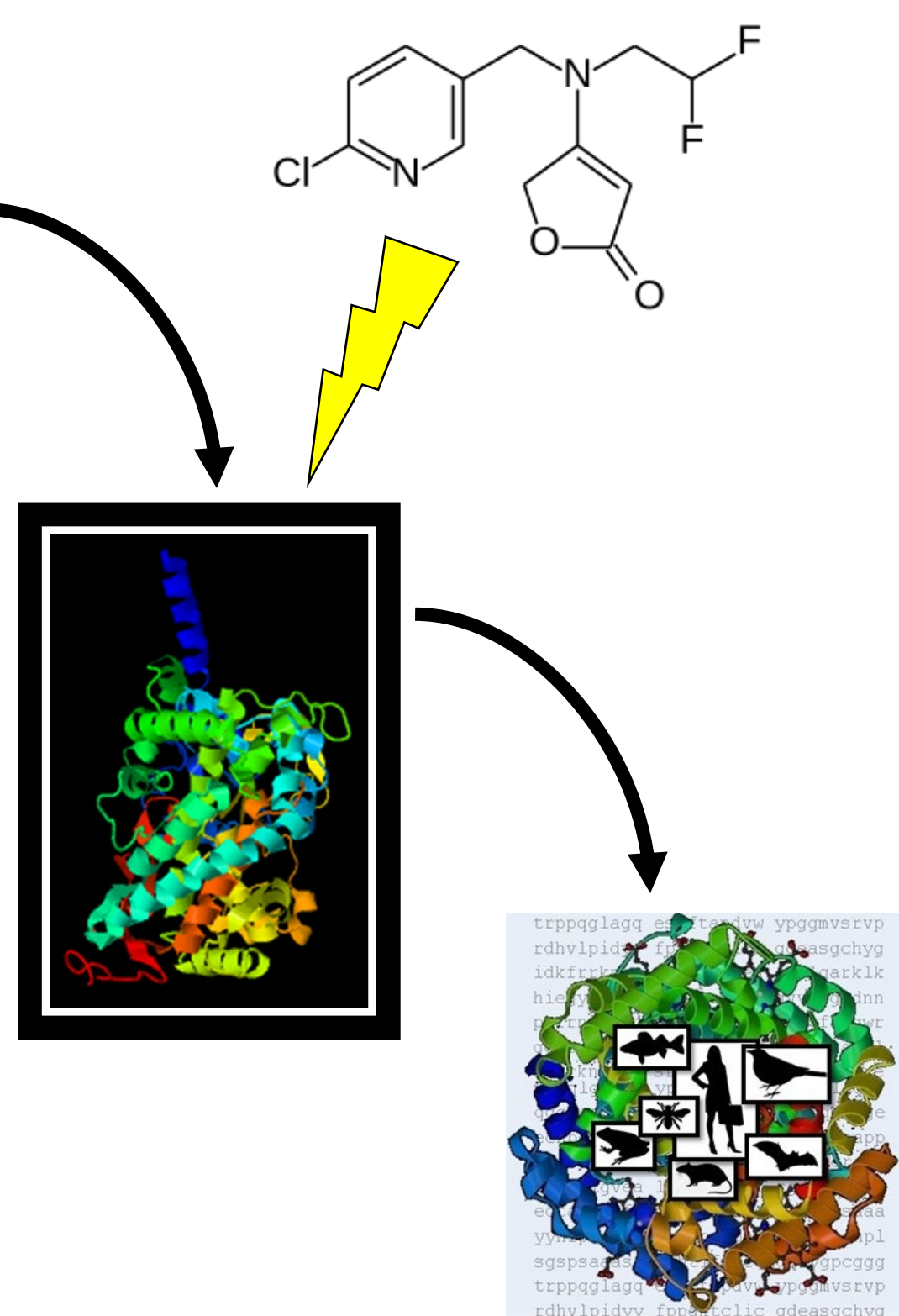CYP9Q2
CYP9Q3
CYP9Q1
CYP9R1
CYP9S1
CYP9P1
CYP9P2

Figure 1: A phylogenetic tree of the CYP9 family of enzymes for the honey bee [Modified from Moa et al., 2011][2].

- There are 28 P450 genes that are associated with the CYP3 family of detoxifying enzymes[2]
  - CYP6 and CYP9
- The subfamily CYP9Q enzymes found in insects, including the honey bee, are known for their detoxification properties[2]
- The CYP9Q enzymes contain a fairly conserved catalytic pocket where the pesticide will bind[2]
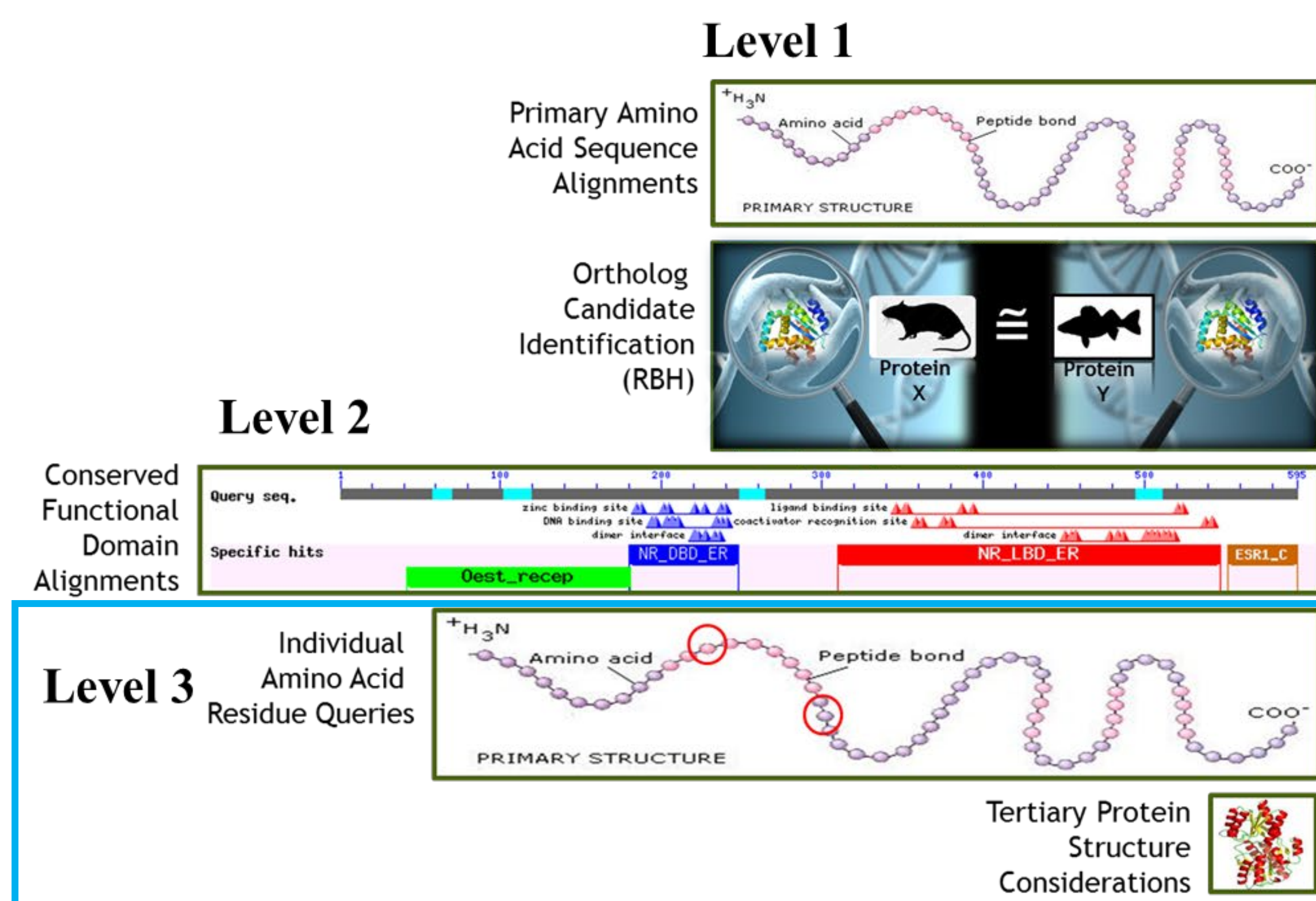
Case Study: Examine CYP9Q Conservation Using SeqAPASS to Predict Pesticide Susceptibility

Case Study Objective: To determine whether existing data describing the interaction between CYP9Q in one bee species and a pesticide can inform why there are differences in sensitivity to Flupyradifurone across bee species using the SeqAPASS tool

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

Level 1
Primary Amino Acid Sequence Alignments
PRIMARY STRUCTURE
Ortholog Candidate Identification (RBH)
Protein X
Protein Y

Low Level of Complexity

Level 2
Conserved Functional Domain Alignments

Level 3
Individual Amino Acid Residue Queries
PRIMARY STRUCTURE
Tertiary Protein Structure Considerations

High level of Complexity

- SeqAPASS was developed to create a strategic and automated approach for assessing protein similarity. We utilize available information, from publicly accessible databases, for our SeqAPASS evaluations. This allows us to be flexible in the approach for each SeqAPASS run

Understanding Protein Conservation

1. Must know the molecular target (e.g. pesticides)
2. Must identify a sensitive species

Apis mellifera
Apis cerena cerena
CYP9Q3
Best matched sequence

Chemical Molecular Target in Target Species ≅ Compare to **Millions** of Proteins From **Thousands** of Species

Greater similarity = Greater likelihood that chemical can act on the protein
Line of Evidence: Predict Potential Chemical Susceptibility Across Species

- Level 1 allows for comparison of the full length protein sequence from a known sensitive species to all other species with sequence information available
- Level 2 evaluates conservation of functional domains, such as a ligand binding domain, across species
- Level 3 requires previous knowledge of critical amino acid residues that are important for the chemical-protein interaction for comparisons across species
- Each Level of the SeqAPASS evaluation provides an additional line of evidence toward conservation of a protein and can be used to predict chemical susceptibility

SeqAPASS Level 3 Results Identify Difference in Key Amino Acid

SRS2

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CYP9Q1   ELKSVLTRYTNDVIARCVYGVSVDSVNEPENIFYRYGQVASQLSTFFQNLMIFVHRNSPR   229
CYP9Q2   EMKALLTRYTNDVIAACIYGVNVDSIKEPRNVFYMYGRVGATLIGLFKNLKIMVHRNMPW   234
CYP9Q3   EMKALLSRYANDVIASCVYGVSVDSLDPKNIFYVYGRRGTNVVGLFKSMFVLIHRNMPW   232
CYP9P1   EGKNLFTRYSNDVIATVAFGISVNSIEHPDNEFYRRGIDVSTFSGTFRFIKFMLFRLNPR   240
CYP9R1   ELKDAFTRYTNDVIATCAFGVEVNSMKDRKNKFYVYGREGTTTFGSWAS-IKFFVTRVLFV   232
          *  *   ::**;:*****  *:.*.*:*::.   * **   *     **        *
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Figure 2: Sequence alignment of five CYP9 proteins highlighting the conservation of the lysine residue at position 219 (CYP9Q3) that is important for detoxification [Modified from Moa et al,. 2011][2].

- A critical amino acid residue, Lysine at position 219 (K219), was identified in CYP9 enzymes to bind and metabolize a pyrethroid, Tau-flauvalinate[2]
- A catalytic pocket for CYP9Q3 containing 3 phenylalanines and one isoleucine also aids in binding of the pesticide[2]
- CYP9Q3 has been shown to bind bulkier molecules and demonstrate a broader substrate specificity suggesting a more accessible/non-selective active site[2]

Protein Name	Common Name	Similar Susceptibility	Amino Acid and Position
cytochrome P450 9Q3	Asiatic honeybee	Y	219K
cytochrome P450 9e2	Honey bee	Y	219K
PREDICTED: cytochrome P450 9e2-like isoform X1	Giant honeybee	Y	219K
Cytochrome P450 9e2	Stingless bees	Y	454K
cytochrome P450 9e2	Buff-tailed bumblebee	Y	220K
PREDICTED: cytochrome P450 9e2-like	Orchid bees	Y	219K
uncharacterized protein LOC100740972	Common eastern bumble bee	Y	220K
PREDICTED: cytochrome P450 9e2-like	Little honeybee	Y	216K
cytochrome P450 9e2-like	Carpenter bees	Y	218K
cytochrome P450 mono-oxygenase	Red mason bee	Y	219T
PREDICTED: cytochrome P450 9e2-like	Digger bees	Y	217L
PREDICTED: cytochrome P450 9e2-like	Bees	Y	219V
PREDICTED: cytochrome P450 9e2-like	Alfalfa leafcutting bee	N	215W

Figure 3: A level 3 SeqAPASS (v4.0) output table showing the species of interest (highlighted) along with other bee species[1].

Y = Yes
N = No
Total Match
Partial Match
Not a Match

- Using SeqAPASS Level 3, comparisons were made across species to honey bee lysine at position 219. A meaningful difference in the amino acid was observed in the predicted sequence for Alfalfa leafcutting bee yielding a Similar Susceptibility prediction of "No" compared to the template species

Side Chain Classification: acidic, basic, aromatic, etc.
MW as surrogate for size: > 30g/mol different size
Susceptibility different than template = Both Class and Size Differ

Summary & Conclusions

- Limited sequence information available for bee species
  - Advocate for genome sequencing and annotation of key species
  - No sequence data for alkali bee *(Nomia melanderi)*, a key alfalfa pollinator
    - Only 16 protein sequences for *Nomia sp. (Nomia westwoodi; Nomia amboinensis, Nomia ridleyi, Nomia iridescens, Nomia yunnanensis, Nomia tetrazonata)*
    - Predictive approaches require sequence data
- Limited data on species-pesticide interactions that highlight key amino acids, which can be utilized by SeqAPASS
  - SeqAPASS requires previous knowledge/literature of critical residues to help inform a level 3 amino acid comparisons between species
- With available information conservation of CYP9Q across bee species
  - Level 3 critical individual amino acid comparisons
    - Species specific differences between *Apis/Osmia/Bombus* and *Megachile rotundata* @ K219, indicating that the pesticide-CYP9Q interaction may differ
      - Amino acid sequence, in part, may contribute to differences in sensitivity to Flupyradifurone if CYP9Q is important for detoxification

References / Acknowledgements

1. LaLone et al. 2016, SeqAPASS: A Web-Based Tool for Addressing the Challenges of Cross-Species Extrapolation of Chemical Toxicity. *Toxicol Sci*, 153 (2): 228-245 (2016)
2. Moa et al. 2011, CYP9Q-mediated detoxification of acaricides in the honey bee (Apis mellifera). *PNAS*, 108 (31): 12657-12662 (2011)
3. Hayward et al. 2019, The leafcutter bee, *Megachile rotundata*, is more sensitive to *N*-cyanoamidine neonicotinoid and butenolide insecticides than other managed bees. *Nature Ecology & Evolution*, 3: 1521-1524 (2019)
4. Roy et al. 2010, I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocols*, 5: 725-738 (2010)