Exploring the Taxonomic Domain of Applicability of an Adverse Outcome Pathway Network Using Predictive Bioinformatic Approaches

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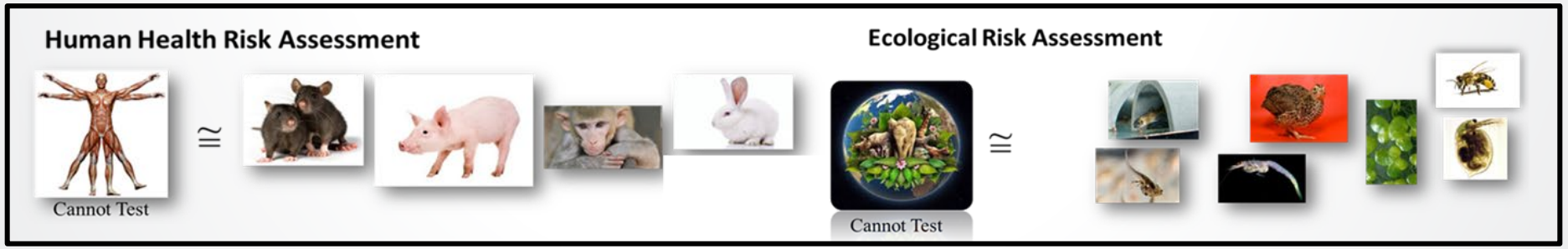

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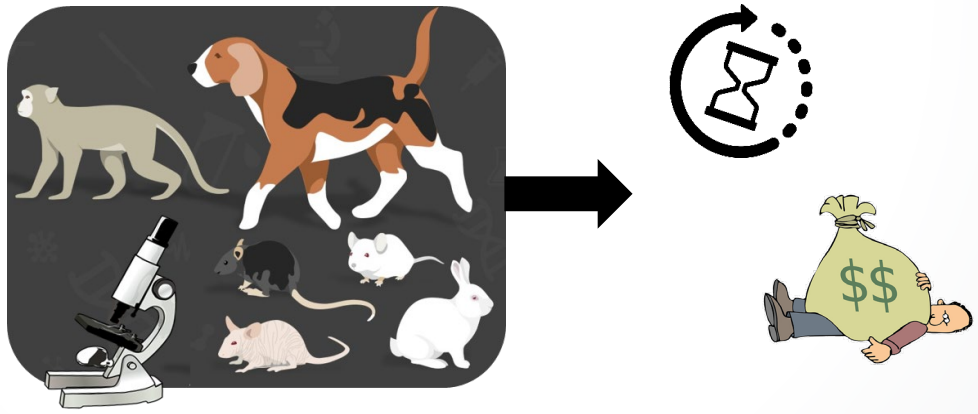
Chemical Safety Evaluations

- Collectively, we are concerned about chemical safety
- Challenge is that there are so many chemicals introduced
  - difficult to perform every test on every organism

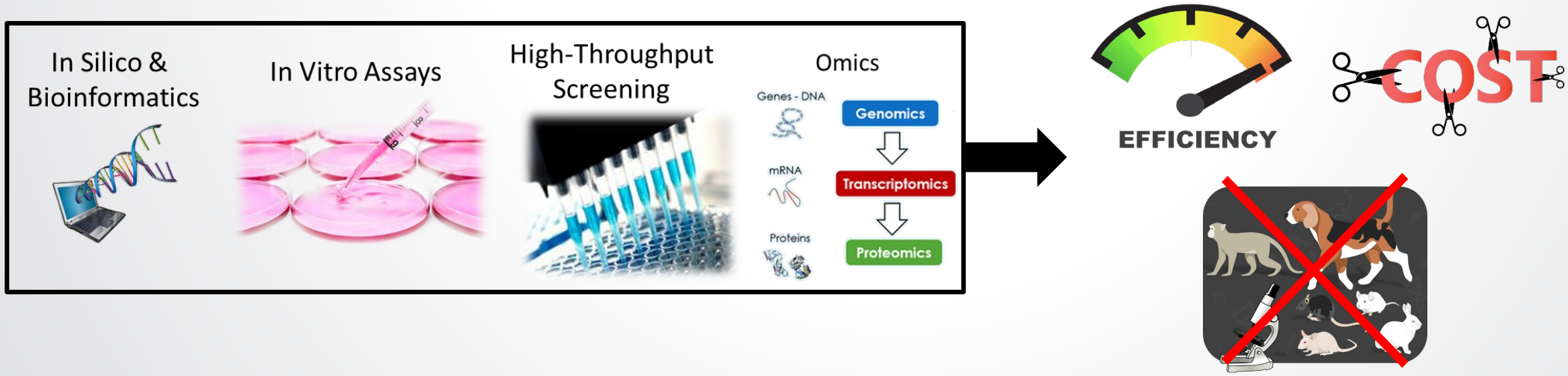
Whole Animal Testing Methods



- Researchers are working to evaluate chemical safety more efficiently
- Recognition that approaches have to change to meet needs

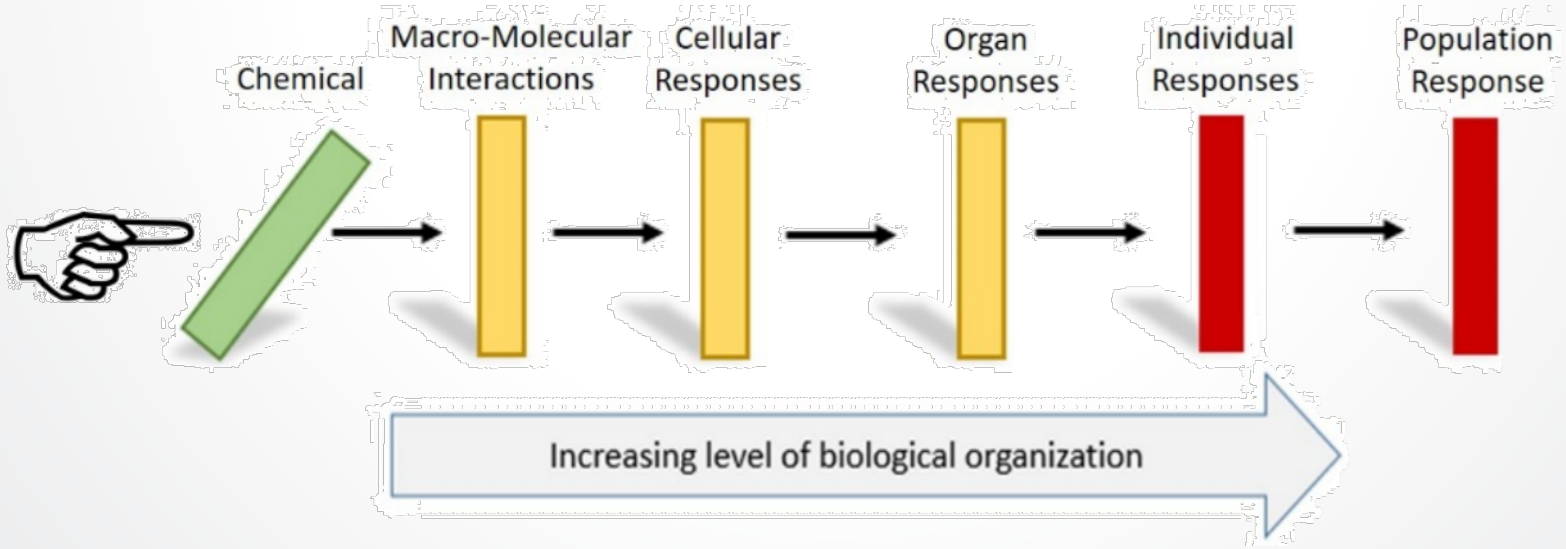
Movement from Whole Animal Testing to Pathway-Based Approaches

- Whole animal testing → New Approach Methods (NAMs)
  - Informatics, -omics, in vitro, in silico methods
- New tools developed because of the increase of new technology and availability of sequence data



Adverse Outcome Pathway (AOP)

- AOP framework organizes existing knowledge regarding the linkage between a molecular initiating event (MIE) and an adverse outcome (AO) at a biological level of organization relevant to risk assessment
- KE events (KEs) and key event relationships (KERs) connect the MIE and AO
- Lays out the biology that is known and unknown to direct research efforts



https://www.epa.gov/sites/production/files/2018-02/documents/aop_fact_sheet.pdf

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

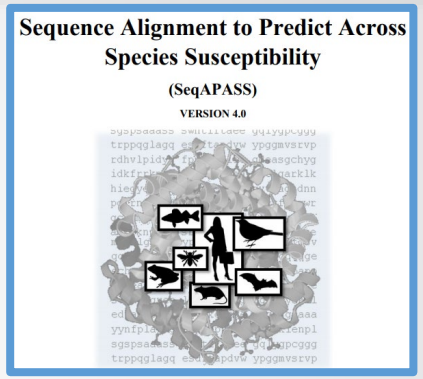
Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS): A Web-Based Tool for Addressing the Challenges of Cross-Species Extrapolation of Chemical Toxicity

Carlie A. LaLone,[*,1] Daniel L. Villeneuve,[*] David Lyons,[†] Henry W. Helgen,[‡] Serina L. Robinson,[§,2] Joseph A. Swintek,[¶] Travis W. Saari,[*] and Gerald T. Ankley[*]

https://seqapass.epa.gov/seqapass/

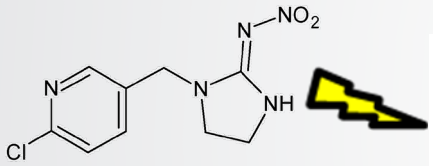
- Developed to understand target conservation across taxonomic groups to be able to predict chemical susceptibility
- Assesses protein sequence and structure similarity
- Need knowledge of a chemical-protein interaction in a species

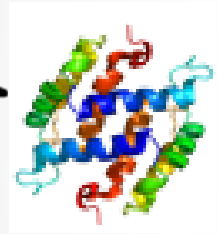
SeqAPASS: The Basics



Level 1: Primary Amino Acid Sequence Alignments

Chemical





Query Protein

+

Query Species

Level 1 Analyses

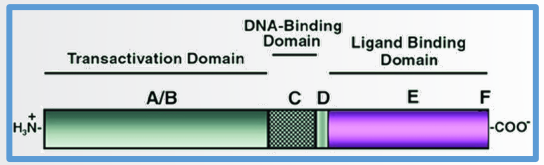
Susceptibility Prediction

Adapted from LaLone et al. (2016)

SeqAPASS: The Basics

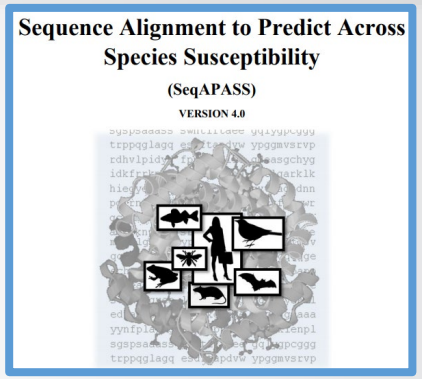
Level 2: Conserved Functional Domain Alignments

Literature Review
- Identify domains of interest



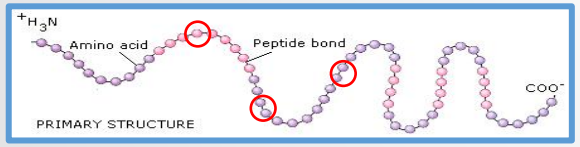
Level 2 Analyses

Susceptibility Prediction



Level 3: Critical Amino Acid Residue Conservation

Literature Review
- Identify key amino acid residues



Level 3 Analyses

Are the amino acids a total match or partial match?

Final Susceptibility Prediction

Adapted from LaLone et al. (2016)

Hypothesis and Objectives

Hypothesis

I hypothesize that SeqAPASS is a sufficient screening approach to understand pesticide susceptibility across species and can be used as an initial line of evidence for extrapolation of toxicity knowledge from one species to others

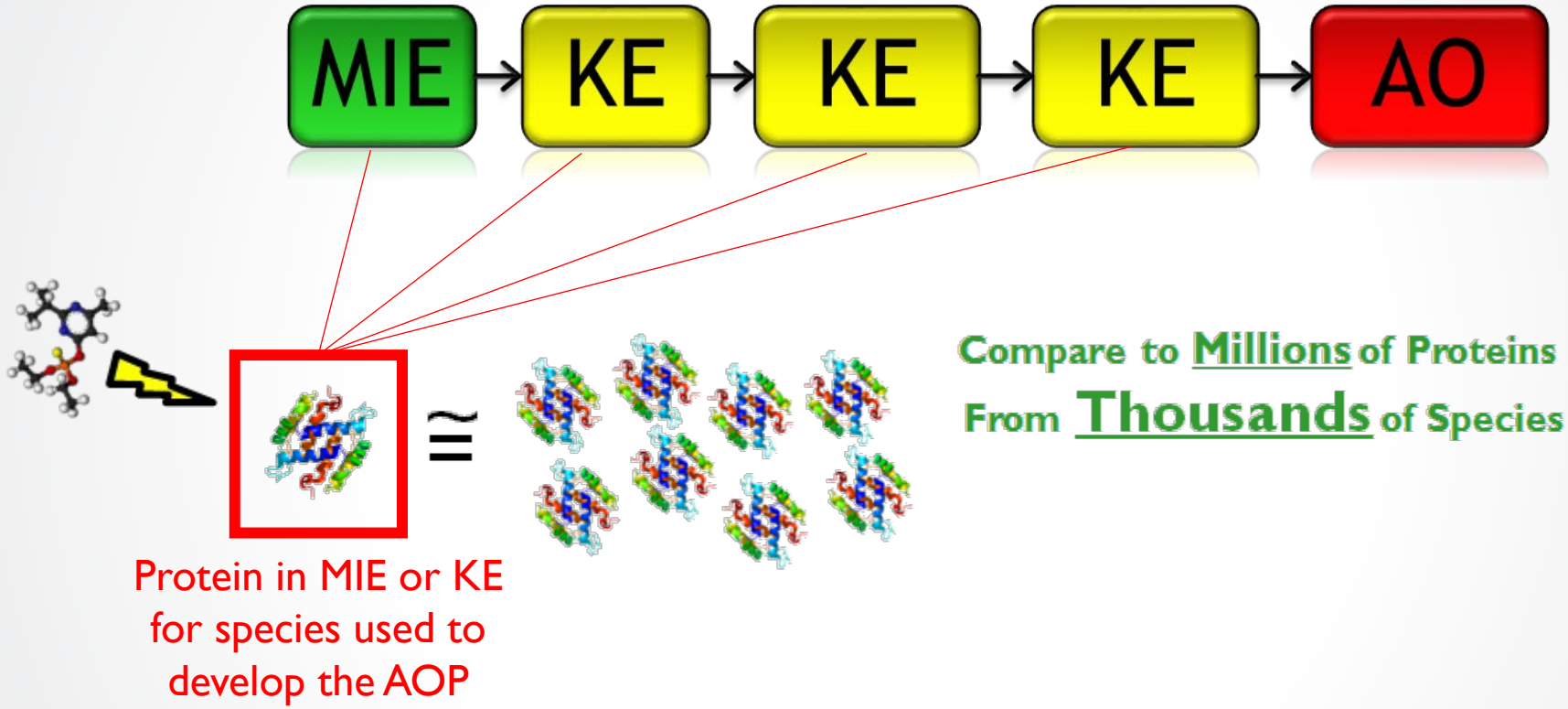
Objectives

→ Identify protein targets of AOP network involving honey bees (*Apis mellifera)* and neonicotinoids

→ Find domain of applicability across pollinators, specifically *Apis* and non-*Apis* bees

→ Evaluate cross-species effects of neonicotinoid pesticides for chemical safety evaluation

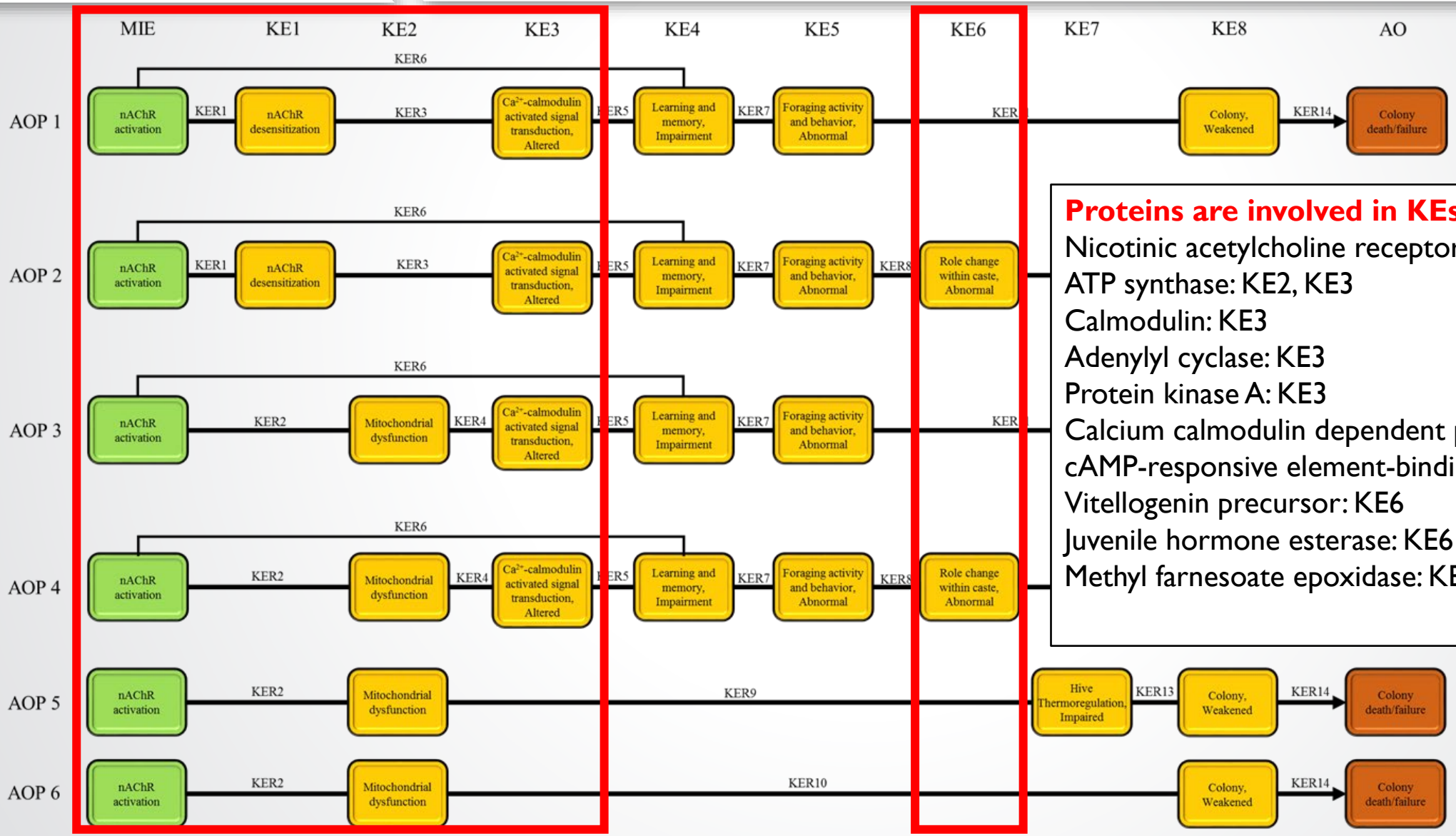
Evaluation of Protein Conservation Across Species



Conservation of MIE or KE across species = Extrapolate MIE or KE across species/taxa

Extrapolating biological pathway knowledge across species

AOP Descriptions: Proteins Are Involved in KEs



Proteins are involved in KEs:

Nicotinic acetylcholine receptor: MIE, KE1
ATP synthase: KE2, KE3
Calmodulin: KE3
Adenylyl cyclase: KE3
Protein kinase A: KE3
Calcium calmodulin dependent protein kinase II: KE3
cAMP-responsive element-binding protein: KE3
Vitellogenin precursor: KE6
Juvenile hormone esterase: KE6
Methyl farnesoate epoxidase: KE6

LaLone et al. (2017)

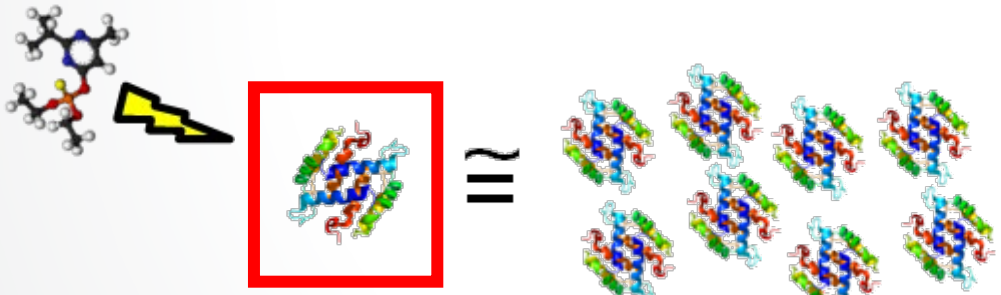
Defining the Taxonomic Domain of Applicability

?

How broadly can we extrapolate this AOP across species?

Integrating Principles

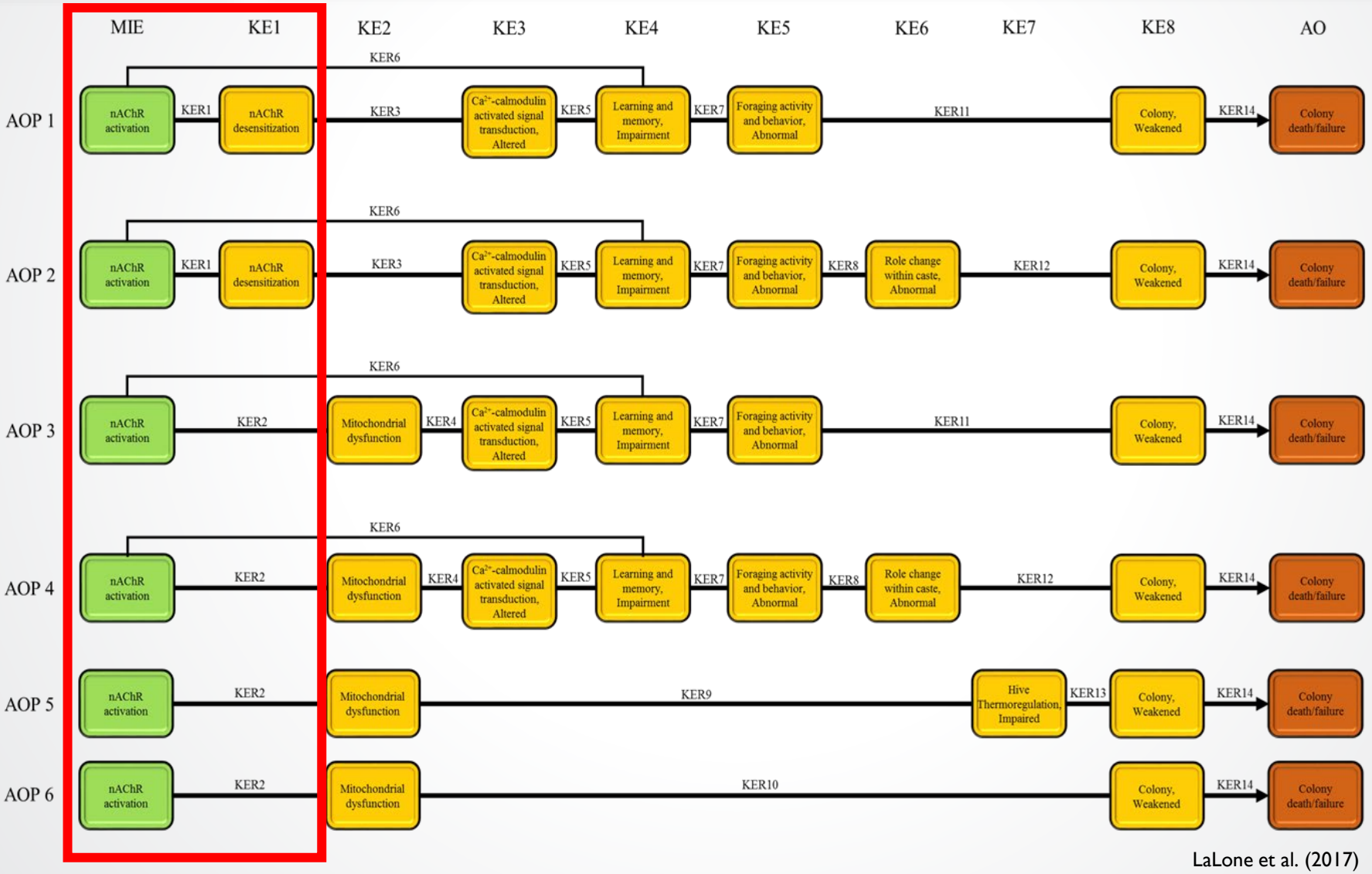
- Evolution
- Structure and Function



Protein in MIE or KE for species used to develop the AOP

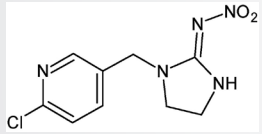
Compare to Millions of Proteins From Thousands of Species

Molecular Initiating Event (MIE) and Key Event 1 (KE1)



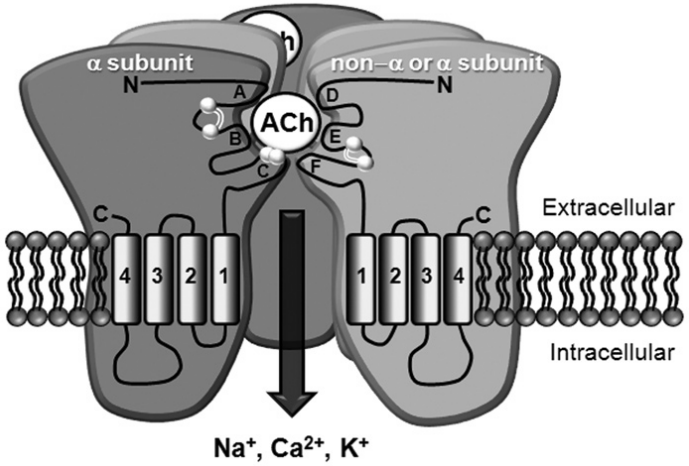
LaLone et al. (2017)

Molecular Initiating Event (MIE) and Key Event 1 (KE1)



Molecular Initiating Event

Nicotinic acetylcholine receptor (nAChR)

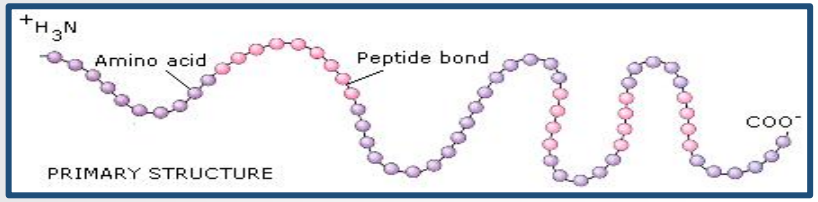


Adverse Outcome

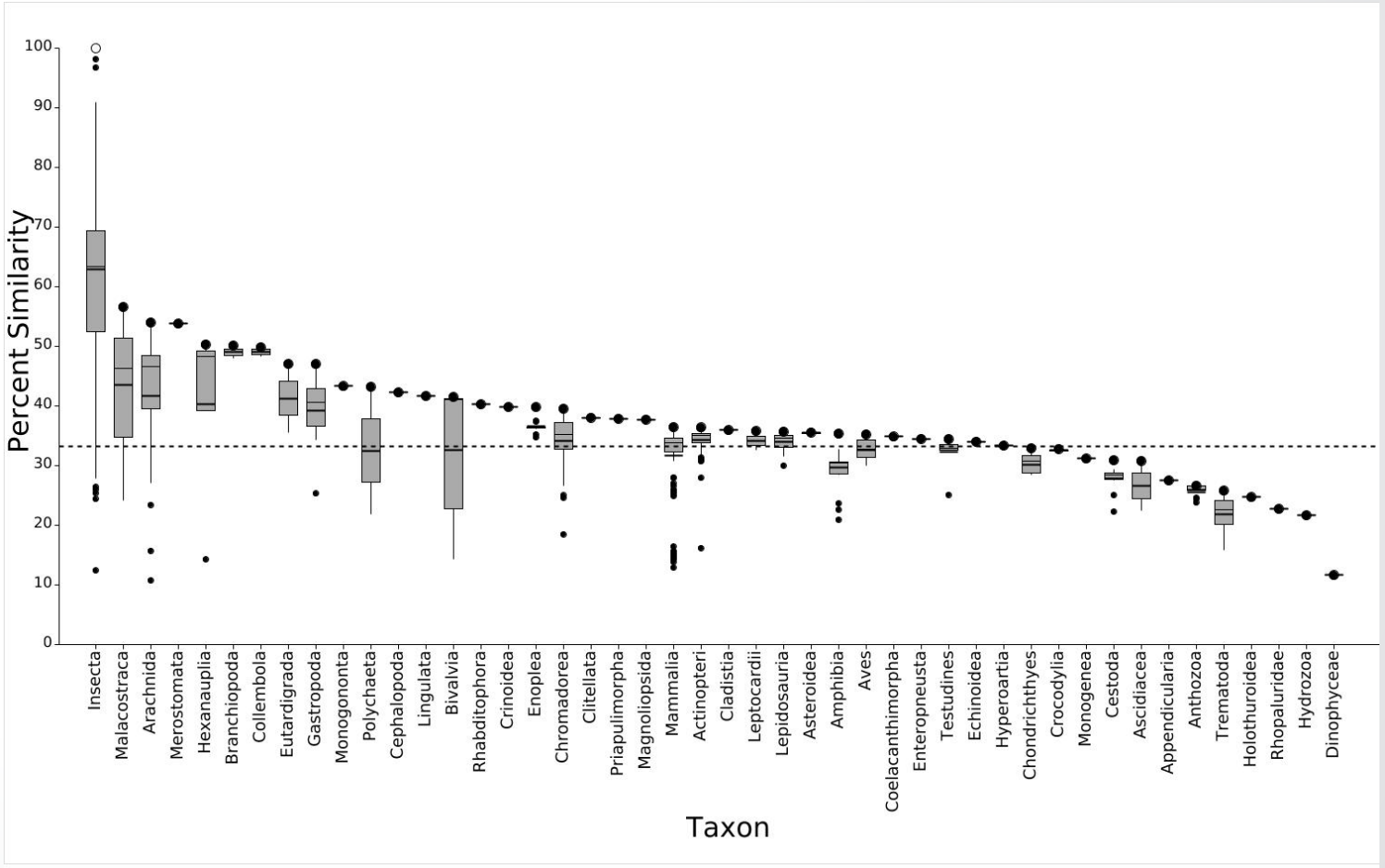
Taken from: Jones and Sattelle, 2010

Level 1 SeqAPASS Results: MIE and KE1

Primary Amino Acid Sequence Alignment

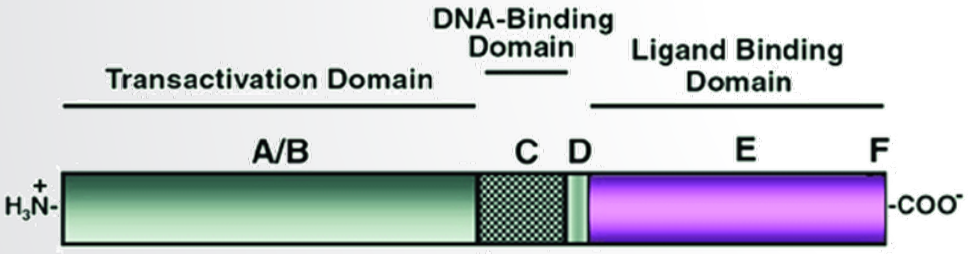


Nicotinic Acetylcholine Receptor α1 Subunit



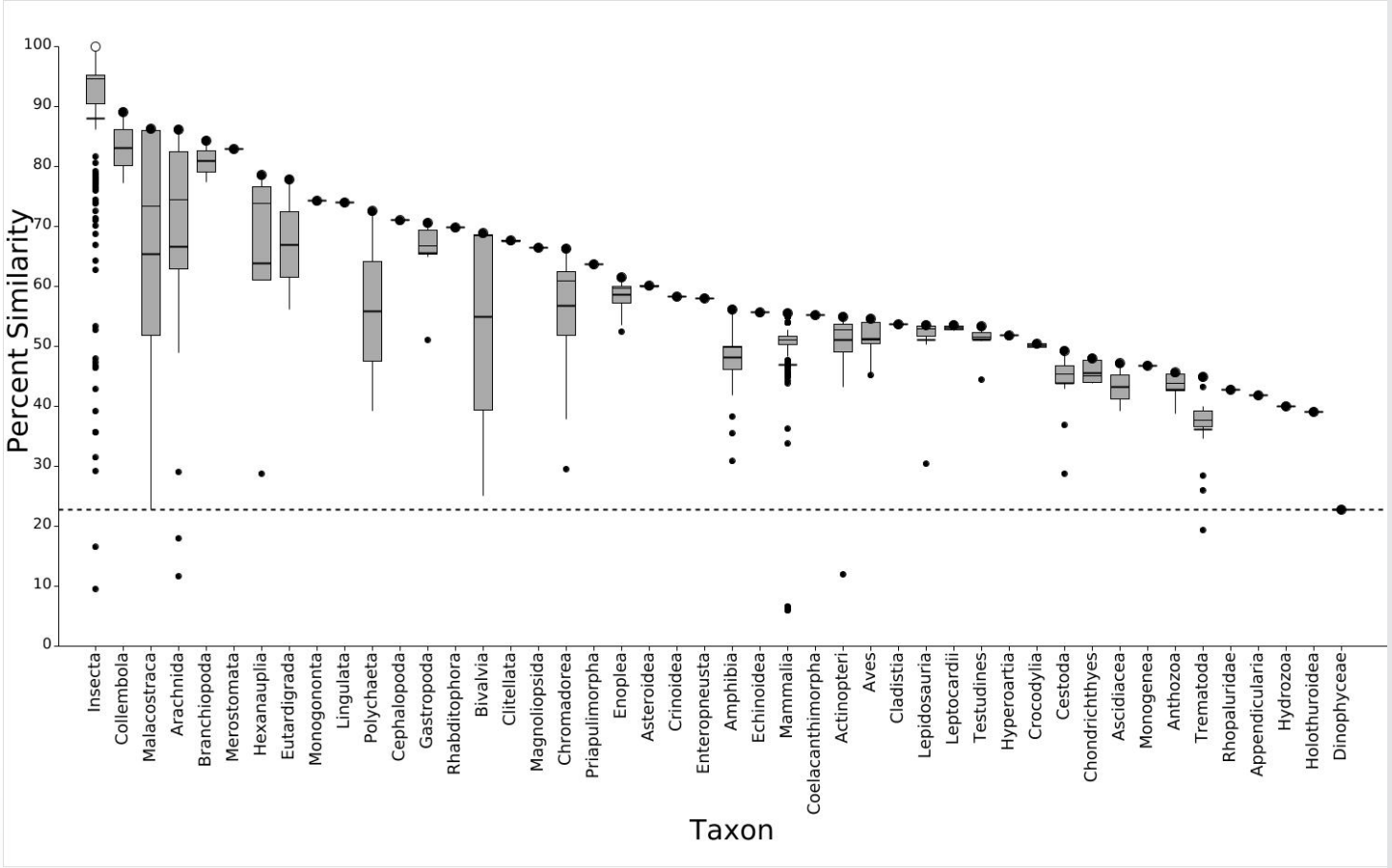
Level 2 SeqAPASS Results: MIE and KE1

Conserved Functional Domain Alignment

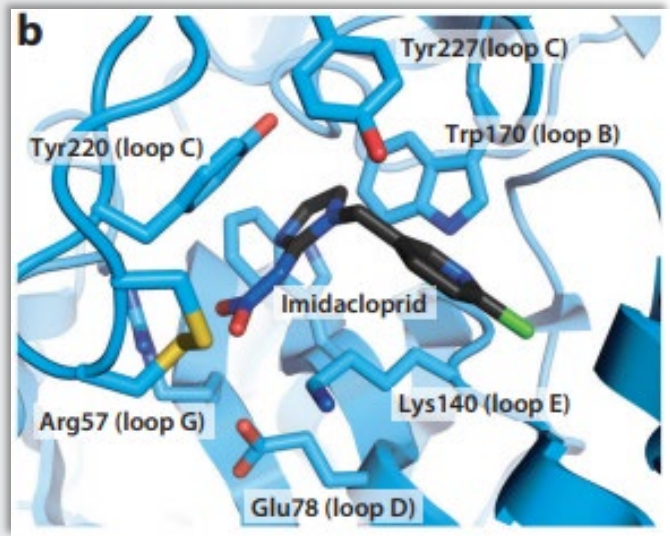


Nicotinic Acetylcholine Receptor α1 Subunit

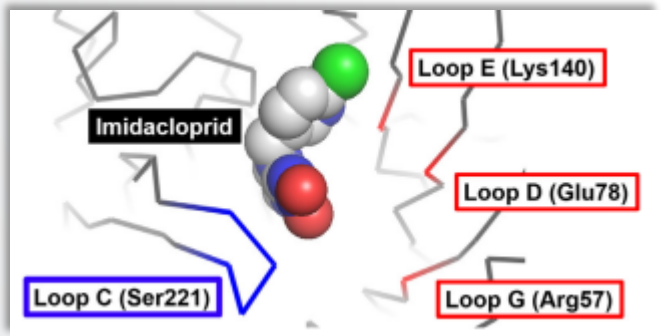
Neurotransmitter-gated ion-channel ligand binding domain



Level 3 SeqAPASS Results: MIE and KE1



Matsuda, Kazuhiko, et al. "Neonicotinoid Insecticides: Molecular Targets, Resistance, and Toxicity." *Annual Review of Pharmacology and Toxicology*, vol. 60, no. 1, 2020, pp. 241–255., doi:10.1146/annurev-pharmtox-010818-021747.



Matsuda, Kazuhiko. "Robust Functional Expression of Insect Nicotinic Acetylcholine Receptors Provides New Insights into Neonicotinoid Actions and New Opportunities for Pest and Vector Control." *Pest Management Science*, 2020, doi:10.1002/ps.6182.

Total Match
Partial Match
Not a Match
Susceptible Yes
Susceptible No

Nicotinic Acetylcholine Receptor α1 Subunit

Scientific Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5	Amino Acid 6	Amino Acid 7
Drosophila melanogaster	Y	57R	78E	140K	170W	220Y	221S	227Y
Apis mellifera	Y	53R	74E	136K	166W	216Y	217I	223Y
Apis cerana	Y	79R	100E	162K	192W	242Y	243I	249Y
Apis florea	Y	79R	100E	162K	192W	242Y	243I	249Y
Habropoda laboriosa	Y	53R	74E	136K	166W	216Y	217I	223Y
Osmia bicornis bicornis	Y	79R	100E	162K	192W	242Y	243I	249Y
Osmia lignaria	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vancouverensis nearcticus	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus bifarius	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vosnesenskii	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus terrestris	Y	79R	100E	162K	192W	242Y	243I	249Y
Megachile rotundata	Y	79R	100E	162K	192W	242Y	243I	249Y
Dufourea novaeangliae	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus impatiens	Y	79R	100E	162K	192W	242Y	243I	249Y
Nomia melanderi	Y	79R	100E	162K	192W	242Y	243I	249F
Eufriesea mexicana	Y	79R	100E	162K	192W	242Y	243I	249Y
Polistes dominula	Y	79R	100E	162K	192W	242Y	243I	249Y
Megalopta genalis	N	--	8E	70K	100W	150Y	151I	157F
Orussus abietinus	Y	80R	101E	163K	193W	243Y	244I	250Y
Nasonia vitripennis	Y	53R	74E	136K	166W	216Y	217I	223Y
Fopius arisanus	Y	53R	74E	136K	166W	216Y	217I	223Y
Diachasma alloeum	Y	79R	100E	162K	192W	242Y	243I	249Y
Microplitis demolitor	Y	53R	74E	136K	166W	216Y	217S	223Y
Trichogramma pretiosum	Y	179R	200E	262K	292W	342Y	343I	349Y
Belonocnema treatae	N	--	8E	70K	100W	150Y	151V	157Y
Copidosoma floridanum	Y	109R	130E	192K	222W	272Y	273I	279Y
Polistes canadensis	Y	79R	100E	162K	192W	242Y	243I	249Y
Apis dorsata	Y	57K	78E	140K	170W	220Y	221T	227Y
Ceratina calcarata	Y	61K	82E	144K	174W	224Y	225T	231Y

Level 3 SeqAPASS Results: MIE and KE1

MIE and KE1 Conserved Among:

Apis
Bombus
Habropoda
Osmia
Megachile
Dufourea
Nomia
Eufriesea
Polistes
Orussus
Nasonia
Fopius
Diachasma
Microplitis
Trichogramma
Copidosoma
Ceratina

Total Match
Partial Match
Not a Match
Susceptible Yes
Susceptible No

Nicotinic Acetylcholine Receptor α1 Subunit

Scientific Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5	Amino Acid 6	Amino Acid 7
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Osmia lignaria	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vancouverensis nearcticus	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus bifarius	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus vosnesenskii	Y	79R	100E	162K	192W	242Y	243I	249Y
Bombus terrestris	Y	79R	100E	162K	192W	242Y	243I	249Y
Megachile rotundata	Y	79R	100E	162K	192W	242Y	243I	249Y
Dufourea novaeangliae	Y	79R	100E	162K	192W	242Y	243I	249Y
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Eufriesea mexicana	Y	79R	100E	162K	192W	242Y	243I	249Y
Polistes dominula	Y	79R	100E	162K	192W	242Y	243I	249Y
Megalopta genalis	N	--	8E	70K	100W	150Y	151I	157F
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Fopius arisanus	Y	53R	74E	136K	166W	216Y	217I	223Y
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Trichogramma pretiosum	Y	179R	200E	262K	292W	342Y	343I	349Y
Belonocnema treatae	N	--	8E	70K	100W	150Y	151V	157Y
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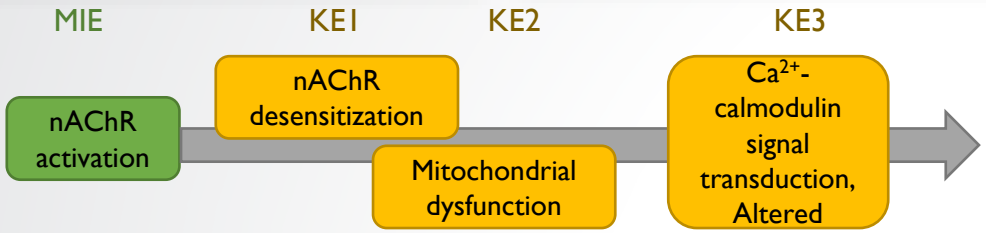
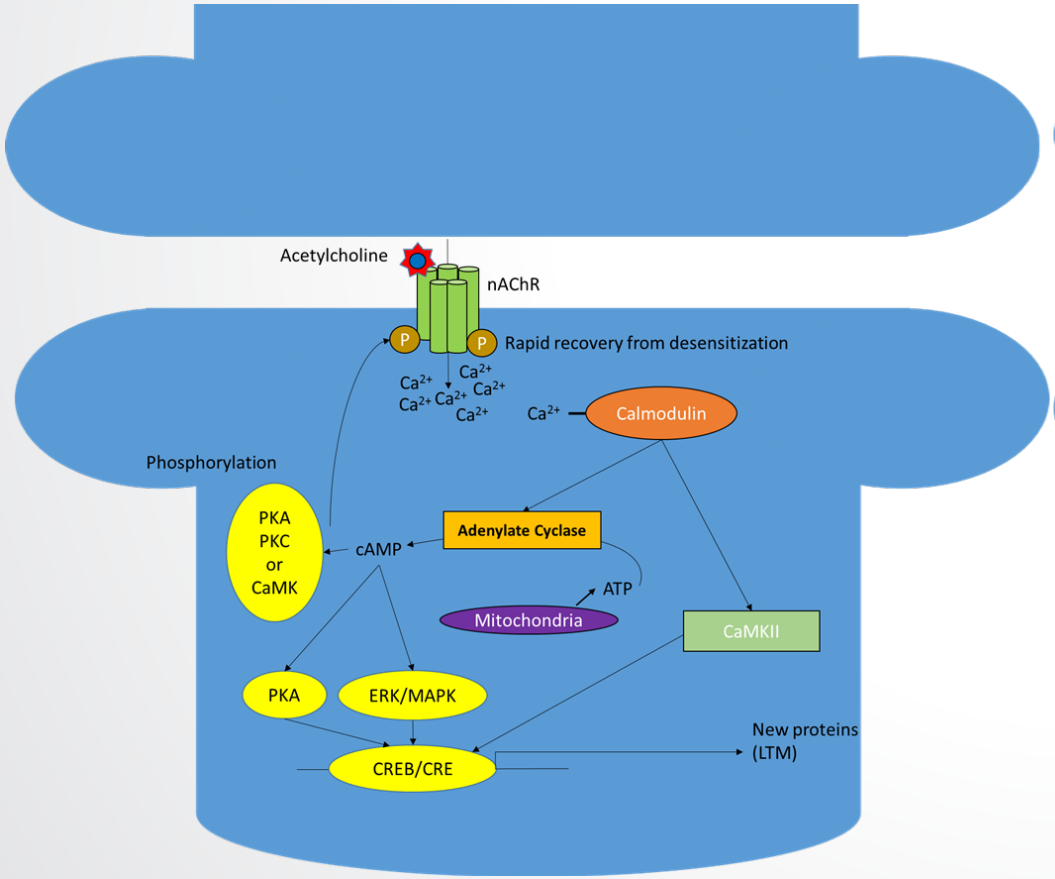
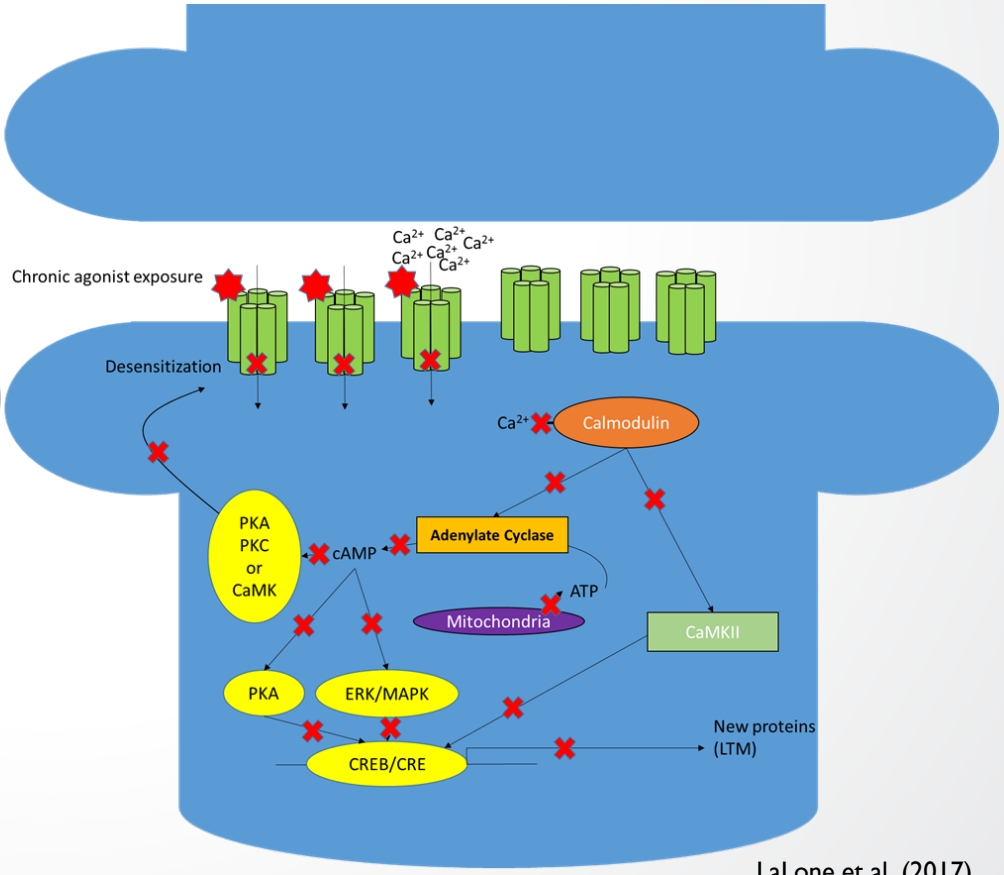
Polistes canadensis	Y	79R	100E	162K	192W	242Y	243I	249Y
Apis dorsata	Y	57K	78E	140K	170W	220Y	221T	227Y
Ceratina calcarata	Y	61K	82E	144K	174W	224Y	225T	231Y

KE2 and KE3



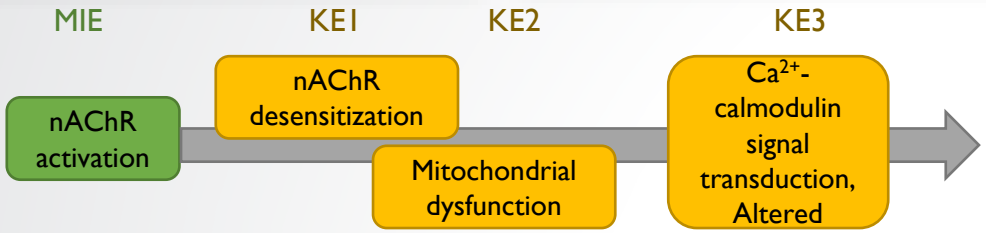
Biological Plausibility: Strong





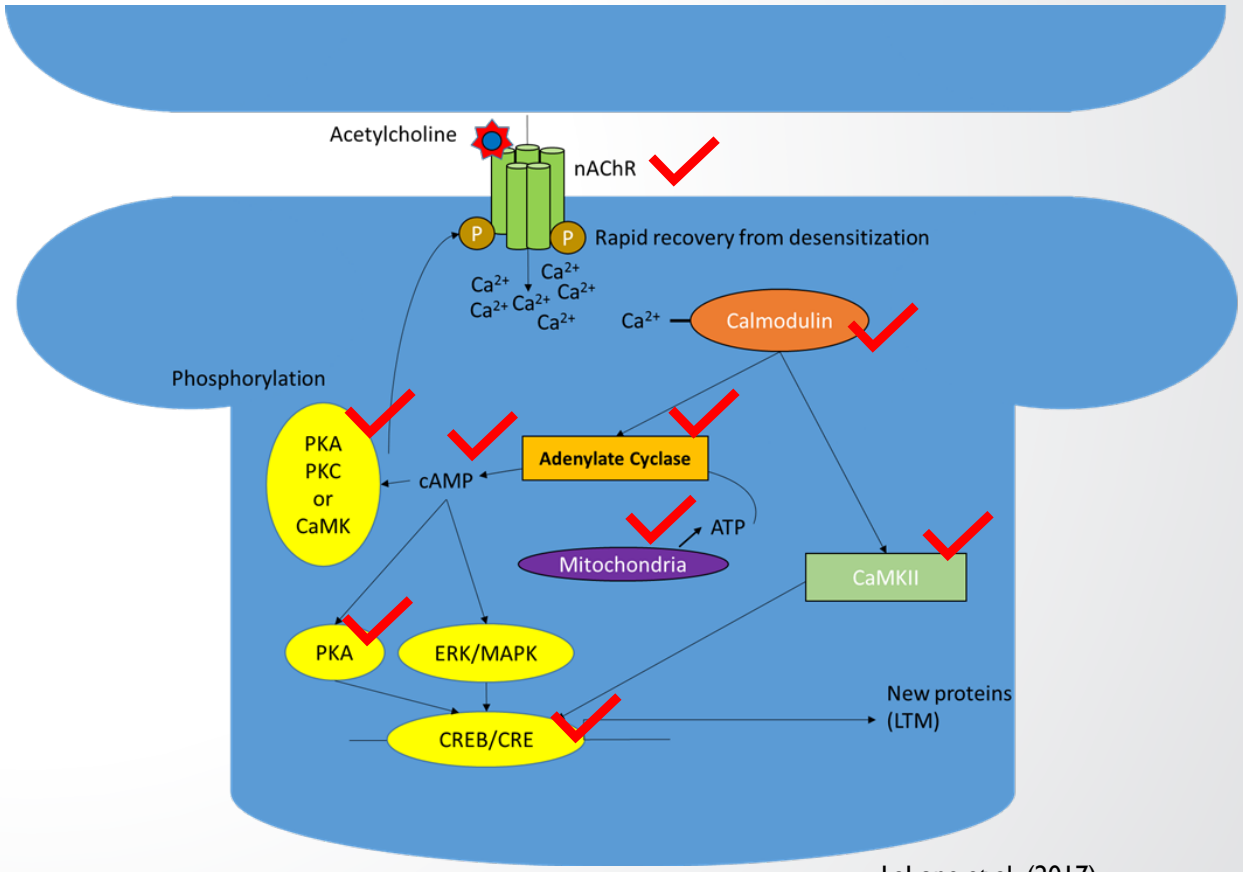
LaLone et al. (2017)

SeqAPASS Results: KE2 and KE3



KE2 and KE3 Conserved Among:

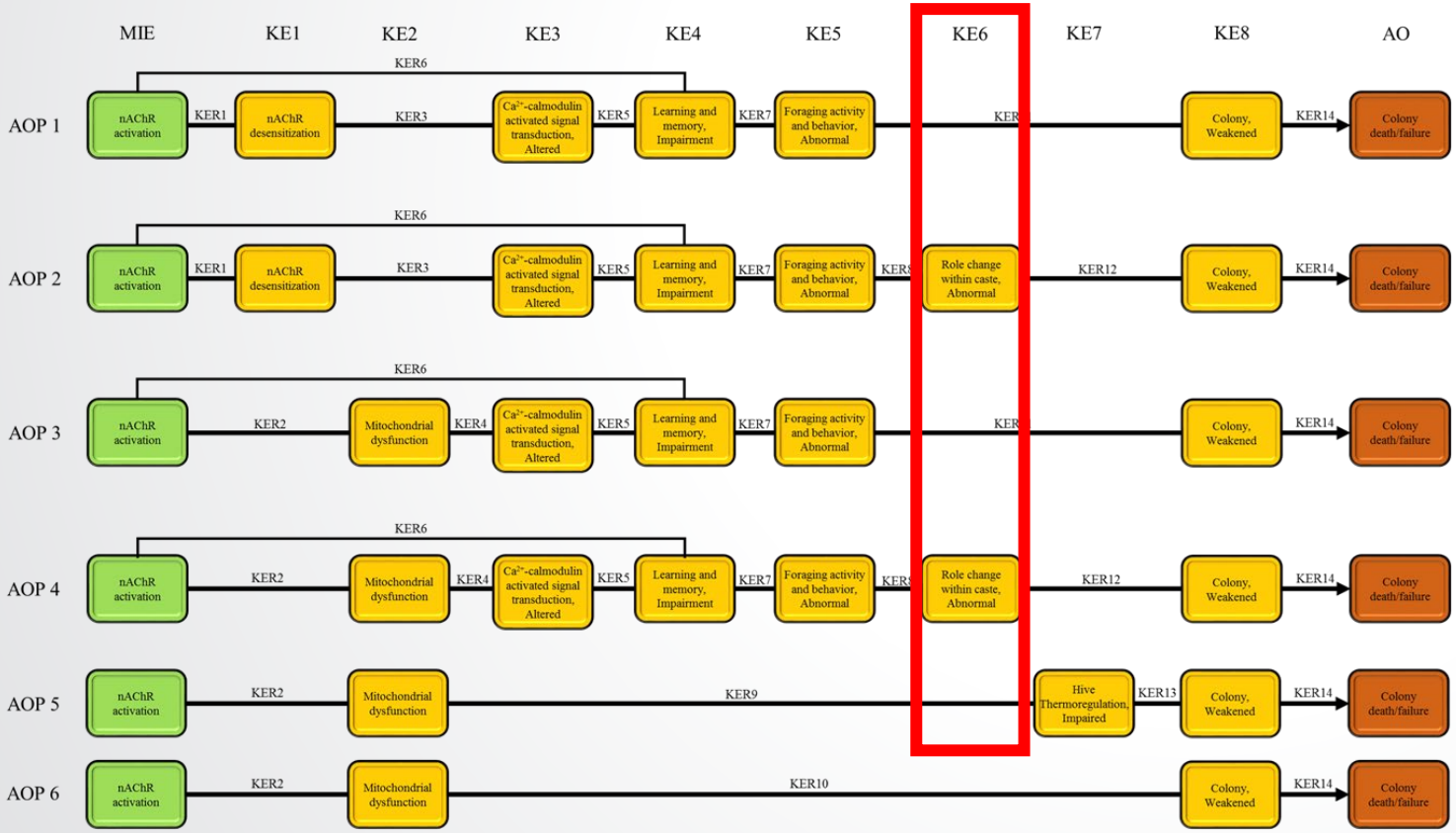
Apis	*Habropoda*
Eufriesea	*Diachasma*
Megachile	*Fopius*
Nomia	*Orussus*
Bombus	*Belonocnema*
Osmia	*Nasonia*
Megalopta	*Microplitis*
Ceratina	*Trichogramma*
Polistes	*Ceratosolen*
Dufourea	*Copidosoma*



LaLone et al. (2017)

Next Steps/Future Work

- Identifying conservation of proteins in late key events of AOP
  - Other considerations to further define taxonomic domain of applicability

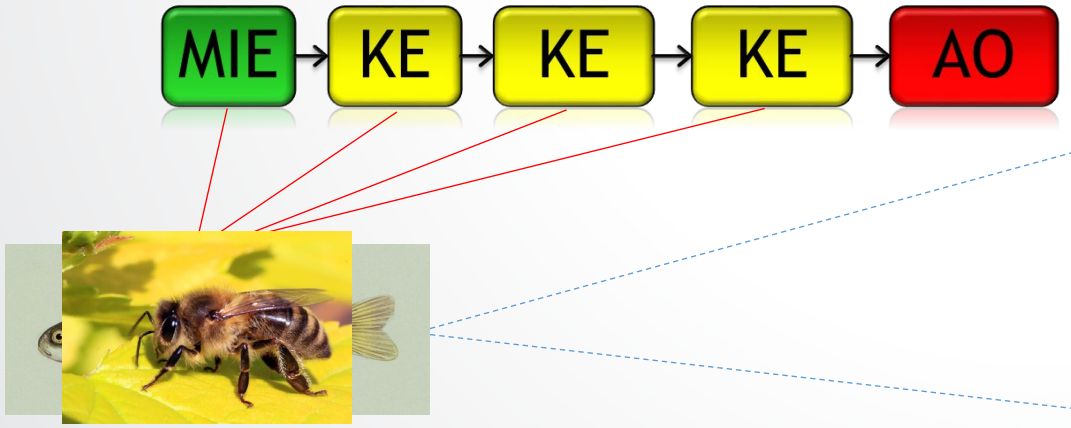


LaLone et al. (2017)

Nicotinic acetylcholine receptor: MIE, KE1
ATP synthase: KE2, KE3
Calmodulin: KE3
Adenylyl cyclase: KE3
Protein kinase A: KE3
Calcium calmodulin dependent protein kinase II: KE3
cAMP-responsive element-binding protein: KE3
Vitellogenin precursor: KE6
Juvenile hormone esterase: KE6
Methyl farnesoate epoxidase: KE6

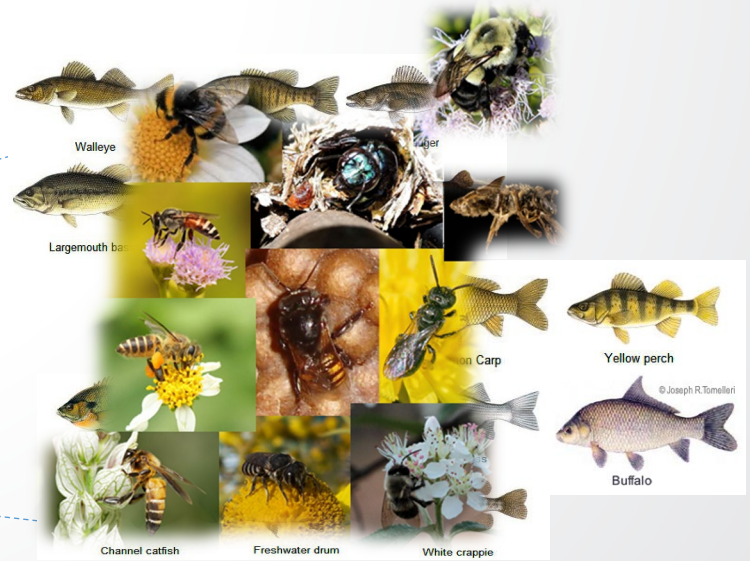
Next Steps/Future Work

- Identifying conservation of proteins in late key events of AOP
  - Other considerations to further define taxonomic domain of applicability
- Can we add evidence to the SeqAPASS predictions?
  - Toolbox: site-directed mutagenesis, in vitro, whole organism studies, molecular modeling/docking
- Use other AOPs!



Species used to develop AOP

?



http://stcroixriverfishing.net/images/fish.species.St.CroixRiver.600pix.jpg

Thanks!
Questions?

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