Defining the Taxonomic Domain of Applicability of an Adverse Outcome Pathway Network Using Bioinformatics

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Introduction

- The adverse outcome pathway (AOP) framework captures and organizes existing biological pathway knowledge to guide research efforts and aid in risk assessment (Ankley et al., 2010).
- In 2017, an AOP was developed linking activation of the nicotinic acetylcholine receptor (nAChR) to colony death/failure in *Apis mellifera* (Figure 1; LaLone et al., 2017)
- While there have been growing concerns regarding the impact of chemical stressors on *Apis mellifera*, there is also concern for other pollinator species, including non-*Apis* bees.
- Defining the taxonomic domain of applicability (tDOA) for this AOP can provide insight relevant to filling research gaps for the protection of *Apis* and non-*Apis* bees.
- Bioinformatics approaches, such as the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool, may provide evidence to define the tDOA more broadly.
- Structural conservation of proteins identified in the key events and key event relationships of the AOP (Table 1) were evaluated using SeqAPASS to define the tDOA.

Objective: To describe how to utilize bioinformatics approaches to define the tDOA of an AOP and components of the AOP, specifically focusing on adding lines of evidence for structural conservation across species using SeqAPASS

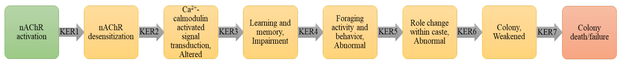


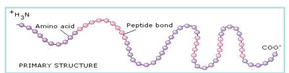
Figure 1. Adverse outcome pathway (AOP) used for the case study of defining the plausible taxonomic domain of applicability (tDOA) that links the activation of the nicotinic acetylcholine receptor (nAChR) to the adverse outcome of colony death/failure.

Table 1. Identified proteins within the key events (KEs), respective KE IDs of adverse outcome pathway 89 (AOP 89, https://aopwiki.org/aops/89), and associated KEs from Figure 1. These nine proteins were evaluated using SeqAPASS to identify structural conservation across species.

Protein	AOP-Wiki Key Event ID	Associated Key Event
Nicotinic acetylcholine receptor	559, 663	MIE, KE1
Calmodulin	1243	KE2
Adenylyl cyclase	1243	KE2
Protein kinase A	1243	KE2
Calcium calmodulin-dependent protein kinase II	1243	KE2
cAMP-responsive element-binding protein	1243	KE2
Vitellogenin precursor	1108	KE5
Juvenile hormone acid O-methyltransferase	1108	KE5
Methyl farnesoate epoxidase	1108	KE5

SeqAPASS

Level 1: Primary Amino Acid Sequence Alignments



Level 2: Conserved Functional Domain Alignments

Level 3: Critical Amino Acid Residue Queries

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

Defining tDOA of AOP

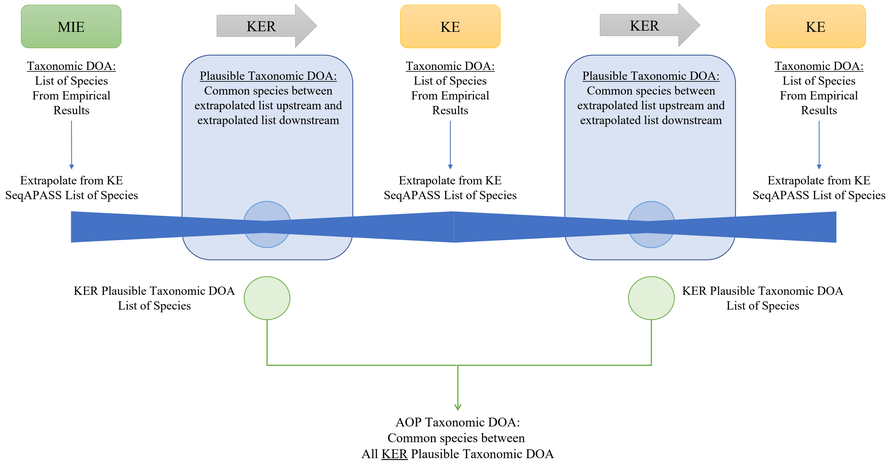


Figure 2. Outline of how to determine the taxonomic domain of applicability (tDOA) for key events (KEs), key event relationships (KERs), and the adverse outcome pathway (AOP).

- An AOP is made up of different components: a molecular initiating event (MIE), key events (KEs), key event relationships (KERs) and an adverse outcome (AO).
  - An AOP begins with a MIE, in which a chemical interacts with a molecular target to generate a perturbation
  - KEs represent measurable biological changes that span multiple levels of biological organization
  - Biological plausibility and empirical data are gathered to support causal linkages between KEs, represented by KERs
  - KERs can be causal, mechanistic, inferential, or correlation based, and can be supported by in vitro, in vivo, or computational data.
  - An AO is at a biological level of organization relevant to risk assessment (e.g., organism or population responses)

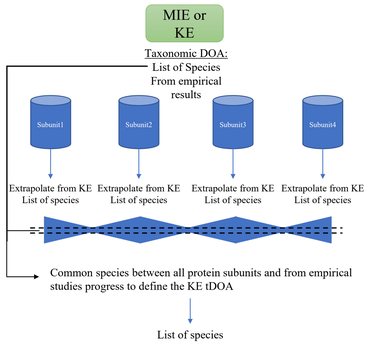


Figure 3. Outline of how to determine the taxonomic domain of applicability (tDOA) for a molecular initiating event (MIE) or key event (KE) that contains a protein with multiple subunits

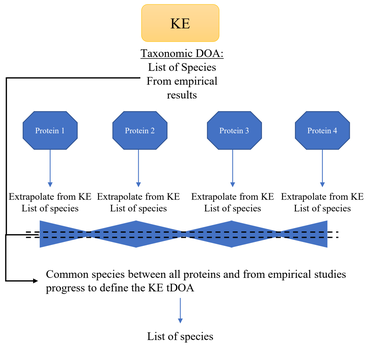


Figure 4. Outline of determining taxonomic domain of applicability (tDOA) for a key event (KE) that contains multiple proteins

Case Study Results

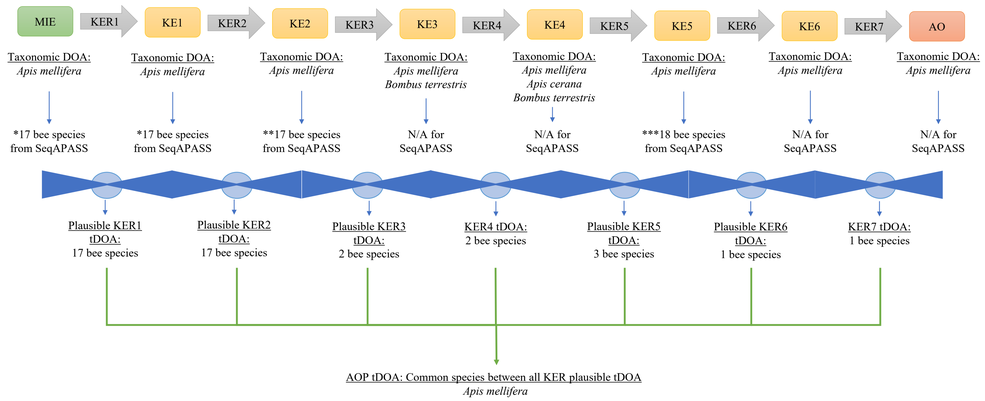


Figure 5. Results of the case study's KE tDOAs, KER tDOAs, and AOP tDOA. In this case study, the AOP tDOA was determined to be defined by *Apis mellifera*. Early KEs contained more broadly defined plausible tDOAs, with 17 bee species represented. For more downstream KEs, the plausible tDOAs were more narrowly defined. The bee species identified in SeqAPASS evaluations for the MIE, KE1, KE2, and KE5 are listed on the right.

***MIE, KE1**
17 bee species from SeqAPASS

Apis mellifera	*Bombus vosnesenskii*
Apis cerana	*Ceratina calcarata*
Apis dorsata	*Dufourea novaeangliae*
Apis florea	*Eufriesea mexicana*
Bombus bifarius	*Habropoda laboriosa*
Bombus impatiens	*Megachile rotundata*
Bombus terrestris	*Nomia melanderi*
Bombus vancouverensis nearcticus	*Osmia bicornis bicornis*
	Osmia lignaria

****KE2**
17 bee species from SeqAPASS

Apis mellifera	*Ceratina calcarata*
Apis dorsata	*Dufourea novaeangliae*
Apis florea	*Eufriesea mexicana*
Bombus bifarius	*Habropoda laboriosa*
Bombus impatiens	*Megachile rotundata*
Bombus terrestris	*Megalopta genalis*
Bombus vancouverensis nearcticus	*Nomia melanderi*
Bombus vosnesenskii	*Osmia bicornis bicornis*
	Osmia lignaria

*****KE5**
18 bee species from SeqAPASS

Apis mellifera	*Bombus vosnesenskii*
Apis cerana	*Ceratina calcarata*
Apis cerana cerana	*Dufourea novaeangliae*
Apis dorsata	*Eufriesea mexicana*
Apis florea	*Habropoda laboriosa*
Bombus bifarius	*Megalopta genalis*
Bombus impatiens	*Nomia melanderi*
Bombus terrestris	*Osmia bicornis bicornis*
Bombus vancouverensis nearcticus	*Osmia lignaria*

Discussion

- SeqAPASS evaluations revealed conservation across *Apis* and non-*Apis* bees at the MIE and early KEs of the AOP. The tDOA for the entire AOP is defined by *Apis mellifera* only (Figure 5).
- Because the downstream KEs describe apical and species-specific endpoints, those KEs are expected to differ across bee species depending on factors such as colony structure or lack thereof or foraging strategies.
- The SeqAPASS tool can only be applied when there are proteins identified in the KEs of the AOP. This case study highlights the need for new tools/methods to extrapolate or further define species relevance for KEs that do not include genes/proteins.
- Using computational predictive approaches (i.e., SeqAPASS) allows for description of hundreds of untested organisms to be considered in the tDOA. In this case study, the focus was purposely narrowed to bee species in Level 3 SeqAPASS analyses, however, it is important to note that this process can be performed for any AOP to extrapolate beyond the model organisms typically defined during AOP development.
- As more genomes are sequenced and annotation improves, it is expected that the use of bioinformatic approaches will continue to enhance tDOA descriptions.

References

1. Ankley et al. (2010). Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry, 29*(3), 730-741.
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