The Evolution of Geadephagan Chemical Defense

Genetic basis, biosynthetic pathways and molecular evolution of carabid quinone production

Carabid beetles are the largest clade of organisms to use a single homologous gland system to produce at least 18 distinct classes of defensive chemical compounds.

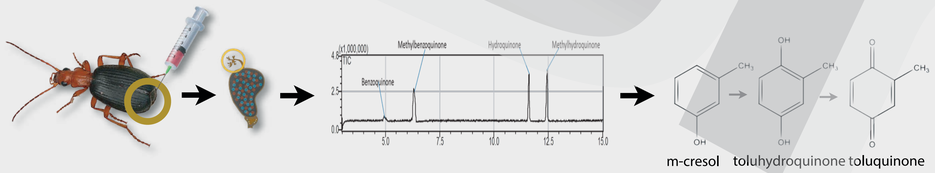
The chemical composition and delivery mechanisms of carabid defensive compounds are well understood, but little is known about the biosynthetic pathways, genetic architecture, and evolutionary history underlying their production.

We aim to develop an understanding of defensive chemical evolution by linking genes, chemicals, biosynthetic pathways and phylogeny in carabid beetles.

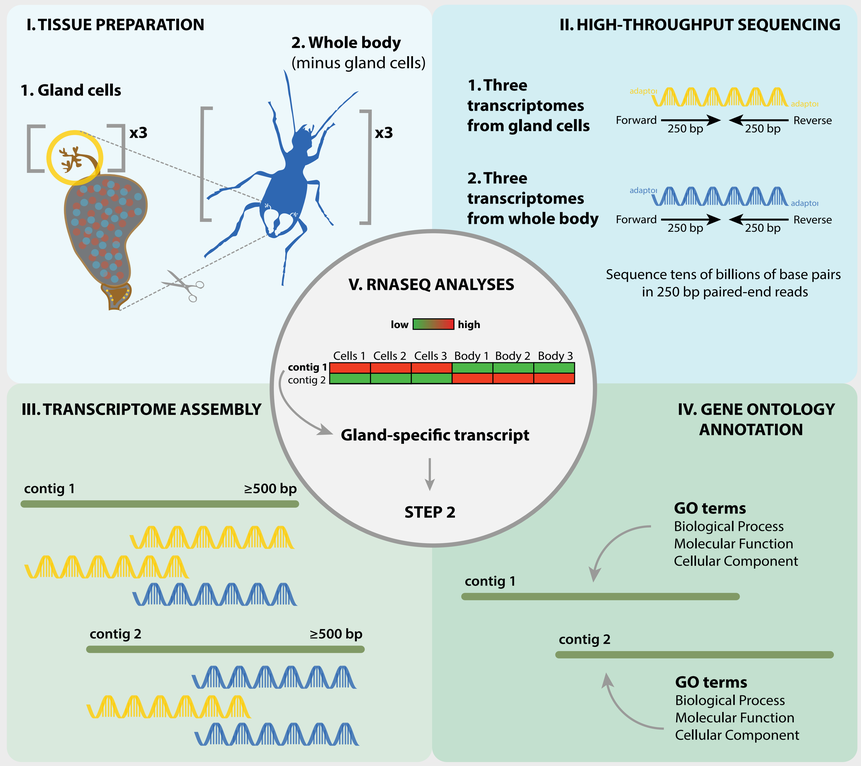
Carabid

What biosynthetic pathways and which genes are used for quinone production?

Chemical assays . Beetles are injected with labelled chemical precursors that are incorporated into gland products during synthesis. Using GC-MS the labelled products are analyzed to infer biosynthetic pathways. Steps in the pathways suggest enzymes likely to be involved, clarifying the evolution of biosynthesis at the chemical level and guiding the search for genes underlying quinone production.

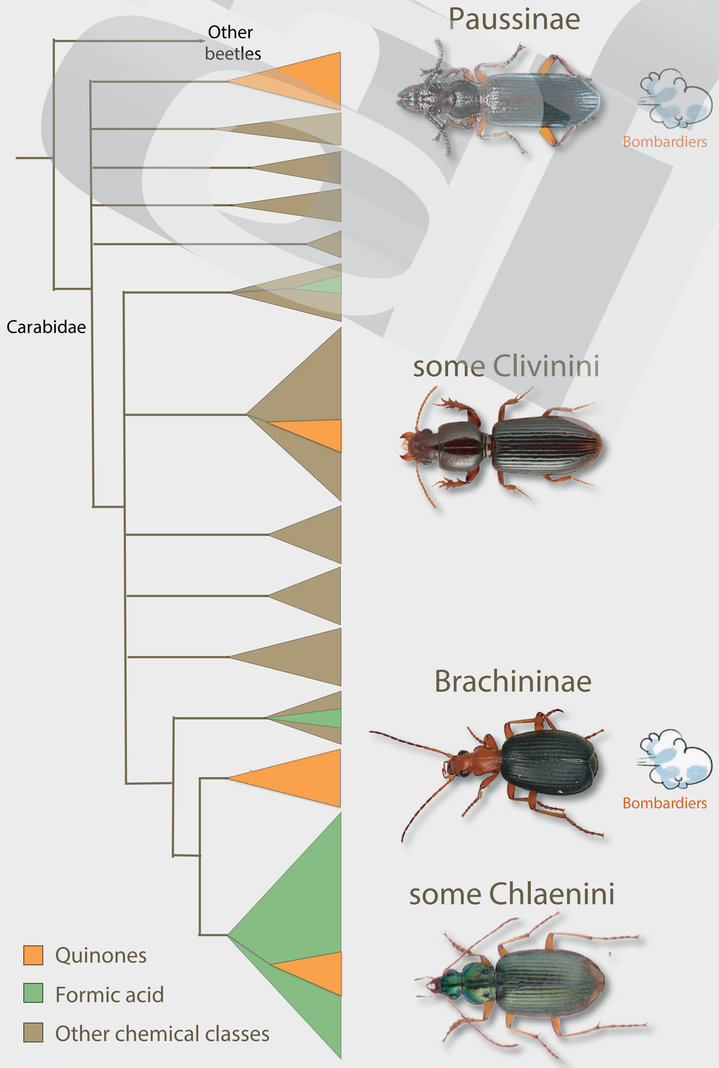


Transcriptome sequencing. Gland-only transcriptomes are compared to transcriptomes from whole bodies (minus gland) to identify genes expressed in secretory gland cells during chemical synthesis. Transcripts are functionally annotated and assigned to known metabolic pathways. This information is combined with expression patterns, biosynthesis assays, and data from *Tribolium* and other systems to identify candidate genes critical for quinone production.



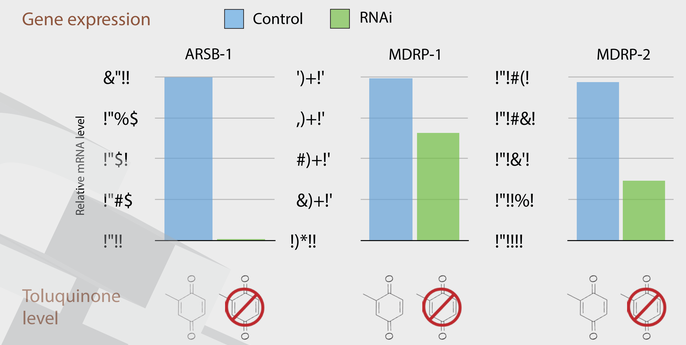
Quinone production: independently evolved or ancestrally conserved?

Phylogenetic analysis. Candidate genes will be identified in four pairs of species, each representing one of four lineages of quinone producers. Candidates will be grouped by whether they're shared among all four lineages, unique to specific lineages, or shared among all bombardiers. This will allow us to examine the extent of independent versus conserved evolution, identify possible patterns of convergence and exaptation, and infer the evolutionary history of quinone production e.g. birth-death dynamics, patterns of selection, and variation in evolutionary rates.



Functional validation of candidate genes using RNAi

Functional assays. Candidate gene expression is knocked down using gene silencing (RNAi) and the resulting gland products are examined, identifying experimentally validated genes with quinone-less or quinone-limited RNAi phenotypes. In a pilot study on Brachinus, knock down of three transcripts reduced expression and resulted in reduced quantities of quinones.



RNAi knockdown of three Brachinus genes. Genes were identified based on homology with two genes known to be essential to quinone production in *Tribolium castaneum*. Knockdown of genes resulted in reduced expression measured via qPCR and absence of defensive toluquinones. ARSB = Arylsulfatase B; MDRP = multi-drug resistance protein, isoform 1 and 2.

Seeking graduate students to study carabid chemical defense evolution!

tinyurl.com/hlq99ru

Check out the KQED DeepLook video about our NSF research.

tinyurl.com/zfeqj52

Authors & Funding

Kipling Will, UC Berkeley
Wendy Moore, U. of Arizona
Aman Gill, UC Berkeley
Tanya Renner, San Diego State U.
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