

Expression Patterns of Bark Beetle Cytochromes P450 During Host Colonization: Likely Physiological Functions and Potential Targets for Pest Management

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Introduction

Cytochromes P450 family genes (P450s) are found in a diverse array of organisms ranging from bacteria to mammals to plants to arthropods. Although there are exceptions to this rule, organisms generally contain a fairly large number of P450 genes and pseudogenes in their genomes. For instance, among arthropods whose genomes are well characterized, the mosquito, *Anopheles gambiae*, has about 100; the fruit fly, *Drosophila melanogaster*, has about 89; and the honeybee, *Apis mellifera*, has about 46.

While there are a very large number of P450s in nature, the general biochemistry of most P450s is similar. That is, P450s are typically involved in NADPH-mediated oxidative attack on various substrates. In practice, however, because specific P450s metabolize only certain substrates, they are involved in a diverse array of biologically important metabolic pathways. In insects, the functions of P450s include, but are not limited to, detoxification of plant secondary metabolites, detoxification of pesticides, biosynthesis of pheromone components and hormones, degradation of pheromone components and hormones, and omega oxidation of fatty acids. In tree-killing bark beetles, it is possible that P450s function in degradation of pheromone and kairomone components in antennae during host searching (Maibèche-Coisne and others 2004), rapid detoxification of constitutive and induced plant secondary metabolites during host colonization, production of aggregation pheromone components, hormonal and other metabolic changes related to reproduction, and larval survival while feeding in host tissues.

Because of the importance of this group of genes and their associated proteins to insect survival, they are ideal targets for research into the development of novel pest management tools. For instance, because certain substances are capable of altering the activity of specific P450 enzymes (for example, Pelkonen and others 1998), an understanding of the biochemical and ecological functions of P450s in bark beetles may allow for the development of species-specific pesticides that disrupt important aspects of bark beetle host location, colonization, and development.

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More complete information pertaining to cytochromes P450 in insects can be found in reviews by Feyereisen (1999) and Omura (1999).

General Approach

Using degenerate PCR, and other PCR-based methods, we were able to isolate fragments of 13 P450s from the California fivespined ips, *Ips paraconfusus*. We separately fed newly emerged males and females fresh ponderosa pine, *Pinus ponderosa*, tissue for 0h, 8h, and 12h. We then assessed transcript levels in the beetles in a 12-replicate quantitative PCR experiment using TaqMan® primers and probes unique for each of the 13 novel P450s.

We found increased levels of transcripts of some P450s in males only, or in both sexes, following feeding. We also found decreased levels of transcripts of some other P450s in males only, or in both sexes, following feeding. We found that the transcript levels of one gene increased in females following feeding but decreased in males. The transcript levels of one gene remained constant in both sexes no matter their feeding state. These observations allowed us to make predictions as to putative biochemical and ecological functions for these P450s, and they will also guide further functional characterization and gene expression studies.

Further Research

Current and future work includes:

- Discovery of more P450 genes in *I. paraconfusus*, *Dendroctonus ponderosae*, and other bark beetles.
- Further exploration of expression patterns of P450s in *I. paraconfusus*, *D. ponderosae*, and other bark beetles under ecologically relevant conditions.
- Functional characterization of the protein products of P450 genes in bark beetles.
- Similar work with lepidopteran defoliators and seed orchard pests.

References

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