



## Identification of a queen and king recognition pheromone in the subterranean termite, *Reticulitermes flavipes*

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Chemical communication is fundamental to success in social insect colonies. Species-, colony-, and caste-specific blends in cuticular hydrocarbons (CHCs) and other chemicals have been well documented as pheromones mediating important behavioral and physiological aspects of social insects. More specifically, royal pheromones used by queens (and kings in termites) enable workers to recognize and care for these vital individuals and maintain the reproductive division of labor. In termites, however, no royal recognition pheromones have been identified to date. In the current study, solvent extracts of the subterranean termite *Reticulitermes flavipes* were analyzed to assess differences in cuticular compounds among castes. We identified a royal-specific hydrocarbon – heneicosane – and several previously unreported and highly royal-enriched long chain alkanes. When applied to glass dummies, heneicosane elicited worker behavioral responses identical to those elicited by live termite queens, including increased vibratory shaking and antennation. Further, the behavioral effects of heneicosane were amplified when presented with nestmate termite workers' cuticular extracts, underscoring the importance of chemical context in termite royal recognition. Thus, heneicosane is a royal recognition pheromone that is active in both queens and kings of *R. flavipes*. The use of heneicosane as a queen and king recognition pheromone by termites suggests that CHCs evolved as royal pheromones ~150 million years ago, ~50 million years before their first use as queen recognition pheromones in social Hymenoptera. We therefore infer that termites and social Hymenoptera convergently evolved the use of these ubiquitous compounds in royal recognition.