



## Multifunctionality of soldier pheromone in a termite

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Division of labor in eusocial insects is characterized by efficient communication systems based on pheromones. Among eusocial insects, termites have evolved specialized sterile defenders, called soldiers. Although it has been predicted that termite soldiers secrete a primer pheromone inhibiting new soldier differentiation for a long time, there has been no examples of identifying this pheromone using the authentic standard. Recently, we identified a soldier-specific volatile as the soldier pheromone, which acts not only as an inhibitory primer pheromone but also as a worker arrestant pheromone and as a fungistat in a Japanese subterranean termite *Reticulitermes speratus*. Gas chromatography-mass spectrometry analysis and optical rotation analysis revealed that (?)-?-elemene is the major component of soldier extract, and authentic standard of (?)-?-elemene inhibited the differentiation from workers to soldiers and exhibited an arrestant activity to workers. This compound also has inhibitory effects on the growth of entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). Moreover, we revealed that this pheromone is more secreted by old soldiers than young ones, and behavioral tests demonstrated that the soldiers exhibit age polyethism; old soldiers block nest openings as entrance guards, while young soldiers are biased toward choosing central nest defense as royal guards. These results suggest that (?)-?-elemene originally functioned only as a fungistatic agent derived from soldiers in *R. speratus*, and then came to be used as an inhibitory pheromone, a worker arrestant, and a signal indicating age. Our study provides novel evidence supporting the multifunctionality of termite soldier pheromone and provides new insights into the role of soldiers and the evolutionary mechanisms of termite pheromone communications.