

authors identified a tyrosinase-like enzyme, NspF, that was homologous to tyrosinase-like GriF in grixazone biosynthesis. Tyrosinases contain a binuclear copper cluster and have known functions as mono- and diphenol oxidases (for example, in melanin production), in keeping with the elucidated role of GriF. However, because a traditional N-oxidase was absent from the 4,3-HNBAm gene cluster, the authors further hypothesized that NspF was a nitrosation copper enzyme similar to tyrosinases. Indeed, NspF catalyzed nitrosation of its proposed biosynthetic substrate 3-amino-4-hydroxybenzamide to 4,3-HNBAm *in vitro*, and NspF single and double mutants in which one or two conserved histidines essential to copper binding were replaced by asparagines showed decreased and abolished nitroso-forming activity, respectively. Additionally, a copper chaperone, NspE, which might provide copper to the NspF active site, was required for *in vivo* activity.

An interesting question regarding the presented nitrosation pathway is how overoxidation of the nitrogen is prevented by NspF. A plausible mechanism for the controlled nitrosation of 3-amino-4-hydroxybenzamide (3,4-AHBAm) that would prevent overoxidation is shown in **Figure 1b**. In this mechanism, the active site contains a peroxo-dicopper(II) complex that is coordinated by the phenol

substrate to one copper atom during the oxidation mechanism<sup>8,9</sup>. The nitrosation mechanism would involve a radical recombination of the amine with both oxygens in the peroxo-dicopper(II) complex, leading to intermediate B (**Fig. 1b**), and subsequent elimination would result in water and the formation of stable 4,3-HNBAm; the necessity for the reactive oxygen species to be regenerated would then facilitate release of the product at the nitroso oxygenation stage. Finally, the enzyme is positioned to pick up another oxygen molecule for the next catalytic cycle. Further comparison of NspF and GriF, as well as NspF and the previously identified RubN8, should yield additional insights into this mechanism as well as the discrimination afforded by these enzymes.

This study presents an exciting addition to the existing N-oxidation mechanisms, as both the first reconstitution of a secondary metabolic pathway to a nitroso natural product and the concomitant discovery of a new copper-dependent N-oxidase, NspF. Ultimately, the characterization of NspF fills in a gap in our understanding of how biology decorates aromatic rings with nitric oxide. Unlike any other pathways described to date, NspF carries out controlled aromatic C-nitrosation biochemistry, introduces new copper-dependent biocatalysis and, in so doing, implies that there is broader versatility among copper enzymes in the biosynthesis

of biologically active natural products than had previously been demonstrated. Aromatic C-nitrososynthases, much like tyrosinases, may soon be used extensively in synthetic reactions that carry out aromatic C-nitrosations.

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#### Competing financial interests

The authors declare no competing financial interests.

## CHEMICAL ECOLOGY

# Reprogramming a termite monarchy

Subterranean termite colonies are founded by a single king and queen. However, the king generally outlives the queen, and an optimal number of secondary termite queens must be produced to meet the reproductive needs of the colony. A recent study explains the chemical basis of this biological process.

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Termites of the genus *Reticulitermes* are social insects characterized by a structured but flexible reproductive division of labor. To maintain an optimal balance within the colony between reproductive and nonreproductive members, the presence of a fertile queen must be communicated to suppress the differentiation of additional queens. However, the mechanism by which this process occurs has remained elusive. Using a combination of termite bioassays and analytical chemistry, Matsuura *et al.* have now identified the volatile inhibitory

termite queen pheromone, produced by secondary queens and their eggs, that both inhibits the differentiation of new reproductive individuals (**Fig. 1a**) and induces workers to care for eggs. Astonishingly, the pheromone that exerts this complex behavioral and developmental control is a simple binary blend of two small molecules, the alcohol 2-methyl-1-butanol and the ester *n*-butyl-*n*-butyrate<sup>1</sup>.

Termites live in complex societies organized into castes comprising reproductive queens and kings and nonreproductive male and female nymphs,

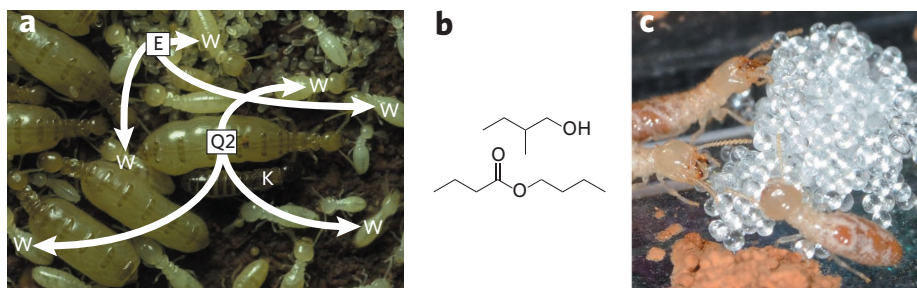
workers and soldiers who care for eggs and carry out the other work of the group<sup>2,3</sup>. Upon the death of the primary queen, the colony must respond by inducing nymphs or workers to differentiate into secondary queens, also referred to as 'neotenic' royals<sup>2</sup>. Although all female termites have the potential to differentiate into queens, only some do so, and even then only when the primary queen dies. It has been assumed that the presence of a fertile queen suppresses the differentiation of secondary queens. However, although recent studies have identified substances specific to

reproductive individuals, none of these substances has an inhibitory effect on the differentiation of additional queens<sup>4–6</sup>.

To address whether queens inhibit differentiation, Matsuura *et al.* carried out field collections of *R. speratus* in western Japan and tested groups of nymphs and workers for differentiation into new reproductives in either the presence or absence of secondary queens. The presence of reproductive females—with or without their eggs—inhibited the differentiation of new reproductives from both nymphs and workers. The substance causing inhibition is volatile because female neotenic in a metal mesh cage still inhibited the production of neotenic. The introduction of 100 eggs per day without the neotenic queen was sufficient to suppress differentiation, though introducing 20 eggs a day had no effect. The authors concluded that both fertile females and their eggs must broadcast the putative inhibitory pheromone signal but that the system is tuned to monitor the fecundity of the queen as measured by absolute egg number.

To identify the inhibitory substance, the authors performed GC-MS analysis of volatiles produced by groups of female neotenic. Remarkably, only two volatiles, 2-methyl-1-butanol and *n*-butyl-*n*-butyrate (Fig. 1b), were detected, and they were specific to secondary queens and their eggs. To demonstrate that these two small molecules are sufficient to suppress the differentiation of reproductives, the authors impregnated an unglazed ceramic ball with synthetic 2-methyl-1-butanol and *n*-butyl-*n*-butyrate in the 2:1 ratio found in the GC-MS experiments and monitored the effect on the production of neotenic. The blend, but not either compound alone, was sufficient to suppress the differentiation of new reproductive termites. When the synthetic blend is applied to dummy eggs made of glass beads coated with an egg-recognition protein<sup>7</sup>, worker egg-gathering behavior is strongly enhanced (Fig. 1c). Thus, the same simple chemical blend that prevents nymphs and workers from becoming reproductives drives them to perform the child care of the colony.

The discoveries by Matsuura *et al.* open several new avenues for study. It remains to be seen how the nonreproductive termites receive the inhibitory signal and how their differentiation into reproductive neotenic is triggered when the pheromone is absent. Further, what pathways in the queen and



**Figure 1** | Chemical suppression of reproductive development by a simple binary pheromone blend of 2-methyl-1-butanol and *n*-butyl-*n*-butyrate. **(a)** *R. speratus* colony with king (K), fertile female neotenic (Q2), workers/nymphs (W) and eggs (E) indicated. Inhibitory arrows (white) indicate the volatile inhibitory pheromone produced by E and Q2 suppressing reproductive development of W individuals. For clarity, not all members of a given caste are labeled. **(b)** Chemical structures of the newly identified volatiles. **(c)** A pile of dummy eggs consisting of glass beads coated with termite egg recognition pheromone<sup>7</sup> and the inhibitory pheromone 2-methyl-1-butanol and *n*-butyl-*n*-butyrate attracts the attention of workers. The dummy eggs are carried into the colony and placed on egg piles. Photos courtesy of K. Matsuura.

her eggs produce these compounds, and how are they released? The fact that the inhibitory pheromone is produced by both secondary queens and their eggs may ensure that the termite queen pheromone is an 'honest' signal. If 2-methyl-1-butanol and *n*-butyl-*n*-butyrate are simple metabolic byproducts of egg production, then nonreproductive workers would be unable to produce a fake signal. In addition, a living queen who has lost her fecundity would not be able to suppress the production of neotenic reproductives effectively. It would be interesting to know whether the queen herself is sensitive to the pheromone blend and whether it has any impact on her biology and behavior, reproductive or otherwise.

Many previously characterized insect pheromones are chemically complex compounds not previously found in nature<sup>8</sup>. In contrast, both of the volatiles that compose the termite inhibitory pheromone are extremely simple chemically and are also familiar odors available from any chemical supply house. *n*-Butyl-*n*-butyrate is a fruity ester commonly used as a flavor and fragrance additive, whereas 2-methyl-1-butanol is a pungent alcohol. The effective concentration of the two components remains to be explored: how far can the formula deviate from the 2:1 ratio used here and still suppress the differentiation of neotenic, and why are nymphs and workers unaffected by each of the components alone? Further work can also determine whether other termite

species use the same or related compounds to modulate the composition of their colonies. As *Reticulitermes* termites cause an estimated \$50 billion dollars in estimated annual economic damage worldwide<sup>2</sup>, this work has immediate practical applications. The identification of this simple and inexpensive chemical suppressor of termite reproduction opens up the possibility of using termite queen pheromone to decrease the numbers of these fascinating but destructive social insects. Perhaps new homes of the future will be delivered with a faint fruity odor.

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