A Gene Necessary for Reproductive Suppression in Termites

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A major transition in evolution is the origin of a division between reproduction and work among individuals. Nowhere is this divide more striking than in social insects, where workers rarely produce offspring even though they are often capable of reproduction should the queen or king die. The molecular mechanisms that control worker reproduction remain largely unknown (*1*). We used a combination of behavioral assays and RNA interference (RNAi) (*2*) to identify a gene required for the reproductive division of labor between the queen and the workers.

With use of cDNA representational difference analysis (cDNA-RDA), we previously identified genes that are specifically overexpressed in queens but not kings and workers of the lower termite *Cryptotermes secundus* (Fig. 1A) (*3*). One of these, *Neofem2*, is a homolog of a gene involved in communication in the Maderian cockroach, *Leucophaea maderae* (*3*, *4*). Thus, we hypothesized that *Neofem2* plays a critical role in queen-worker communication.

In lower termites, workers are totipotent and can develop into reproductives (Fig. 1) but only when the queen or king dies. Because it is not possible to induce a worker to molt into a new queen within the short functional period of RNAi (preliminary experiments revealed a decline of gene knockdown after 48 hours), we developed a behavioral assay to function as a proxy for the absence of queens. Removing queens from colonies resulted in a single observable effect: an increase in butting behavior among workers (Wilcoxon paired rank test: $Z = -2.43$, $n = 9$ pairs, $P = 0.015$) (Fig. 1B, fig. S1, table S1, and movie S1). Butting is associated with reproductive dominance, and workers that go on to replace the king or queen display more butting than workers that do not change caste (figs. S2 and S3). These observations are consistent with those of bees, ants, and wasps, where aggressive interactions among workers increase before, or associated with, worker reproduction (*5*). Therefore, we used the frequency of butting interactions received by a focal worker as an indicator of the queen's absence and the eventual succession of the queen.

We silenced *Neofem2* in queens with RNAi in eight queenright colonies (figs. S4 and S5) and recorded the behavioral repertoire 1 day before and 1 day after silencing. Although silencing had no observable effect on the behavior of the queen herself (Wilcoxon paired rank tests: always $P > 0.200$) (fig. S6 and table S2), workers showed a significant increase in butting behavior (Wilcoxon paired rank test: $Z = -2.52$, $n = 8$ pairs, $P = 0.012$) (Fig. 1C, fig. S7, and table S3) that was not observed in our 24 control colonies (table S4). Thus, we conclude that inhibiting *Neofem2* makes workers behave as though the colony is queenless and that *Neofem2* is necessary for the queen to suppress worker reproduction. *Neofem2*'s putative 532–amino acid gene product suggest that it is a β-glycosidase, a member of the glycosyl hydrolase family 1 (*3*). These enzymes are found among all taxa—including bacteria, fungi, plants, and animals (*6*)—and have a major function in breaking down polysaccharides like cellulose. The closest homolog of *Neofem2* plays a role in wood consumption in the termite *Neotermes koshuensis* (table S5). β-glycosidases also can release volatiles from substrates composed of sugars and a small chemical group (*7*) and may play a role in egg pheromones in *Reticulitermes speratus* and cockroach species (*8*) and in sex-specific signaling in the Maderian cockroach (*4*). All together, these results suggest that *Neofem2* may have evolved from an ancestral role of wood digestion to one of queen-worker pheromonal communication in modern termites. If confirmed, *Neofem2* provides insight into the chemistry of queen pheromone production, something that has been described in honeybees (*9*).

The evolution and maintenance of a reproductive division of labor is predicted to occur only under restricted ecological conditions (*5*). Our data suggest that this major step in social evolution can be achieved through relatively minor changes to preexisting biochemistry. The finding that termite social organization can be influenced by single genes also suggests a novel strategy for insect control: chemical genetic inhibitors that cause anarchy within their societies.

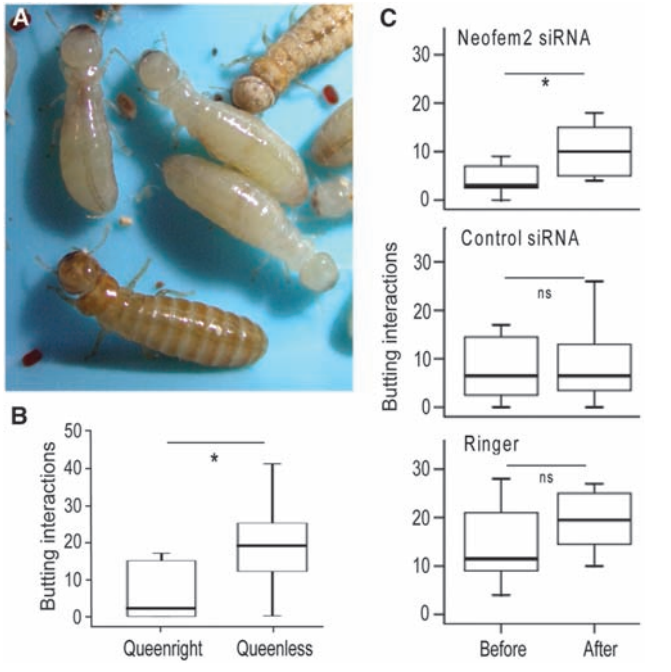

Fig. 1. (**A**) *Cryptotermes secundus* queen (bottom left) together with the king (top right) and workers. (**B**) Frequency of butting interactions among workers in queenright and queenless colonies. (**C**) Frequency of butting interactions before and after treatment of the queen with *Neofem2* small interfering RNA (siRNA), control siRNA, and Ringer's solution. Shown are boxplots with median, quartiles, and minimal and maximal values. Outliers are not shown but were analyzed (tables S1, S3, and S4). *Significant increase in the frequency of butting by workers.

References and Notes

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Supporting Online Material

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Materials and Methods
Figs. S1 to S7
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Movie S1

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