Journey to the Insect Society

Uncovering the Secrets of Termite Queens

Forefronts of Research at Kyoto University

Termites in the black box

The word "termite" is generally associated with horrible house pests and major wood decomposers in forest ecosystems. The total number of termites on the planet is estimated to be roughly 240,000,000,000,000,000. This abundance of termites can be attributed to their overwhelming reproductive capacity, which is based on their sophisticated social life. Isoptera (termites) belong to the group of highly eusocial insects which also includes Hymenoptera (ants, bees, and wasps). Termites have often been compared to ants. However, the origins of these two social insect groups are quite distinct; termites are basically social cockroaches, whereas ants evolved from wasps. Compared to the rapid progress in ant studies since Hamilton first proposed kin selection theory in 1964, many aspects of termite biology still remain unexplored. Even the reproductive system of the termite *Reticulitermes speratus*, the most common species in Japan, was misunderstood until only recently.

Opening the black box

In most termite species, colonies are typically founded by a monogamous pair of primary reproductives (one king and one queen). It was long believed that the inbreeding cycles of generations of neotenic reproductives (colony offspring) propagated the colony after the death of the primary king and queen, and evidence for inbreeding depression in termites is mounting. Like most subterranean termites, *Reticulitermes* species have cryptic nesting habits with transient, hidden royal chambers found underground or deep inside wood, making it difficult to reliably collect reproductives (kings and queens). Therefore, the breeding system of subterranean termites has been estimated primarily by genotyping workers or culturing laboratory colonies rather than from census data from field colonies. This is one reason why the reproductive systems of some termites have been completely misunderstood in the past.

To obtain reproductives from a sufficient number of natural colonies, we collected more than 1,000 nests in the field, successfully identifying reproductives from 55 of them. In nearly all cases, the primary kings were present, whereas the primary queens had been replaced by a number of secondary queens (neotenic queens differentiated within the colonies). These results indicate that primary kings live much longer than primary queens and that replacement of primary kings is rare, whereas replacement of primary queens is the rule in the colony's development.

Asexual queen succession

Our genetic analysis of the reproductives and other colony members has uncovered an extraordinary mode of reproduction. Whereas workers and alates are produced by means of sexual reproduction, secondary queens are exclusively produced parthenogenetically by founding primary queens.

The production of secondary queens through conditional parthenogenesis effectively extends the reproductive life of the primary queen, greatly expanding her reproductive capacity. This process of queen succession allows the colony to boost its size and growth rate without suffering any loss in genetic diversity or diminishing the transmission rate of the queen's genes to her grand offspring. In actuality, termites are not produced by inbreeding. On the contrary, they avoid inbreeding completely by using parthenogenesis.

King-queen conflict over parthenogenesis

In the evolution of parthenogenesis, males and females are usually considered to be in conflict over genetic transmission because this reproduction method enhances female reproductive output but prevents any genetic contribution from the males. For males, any trait that coerces females into sexual reproduction should increase their fitness. In haplodiploid social Hymenoptera (ants, bees, and wasps), unfertilized eggs become males while fertilized eggs produce females, giving queens a potentially powerful mechanism for controlling fertilization. However, in diploid insects, including termites, sperm release is generally activated through a neural loop whenever an egg passes the genital chamber, with no control over fertilization occurring. This raises an important question: how do termite queens produce parthenogenetic offspring even in the presence of kings?

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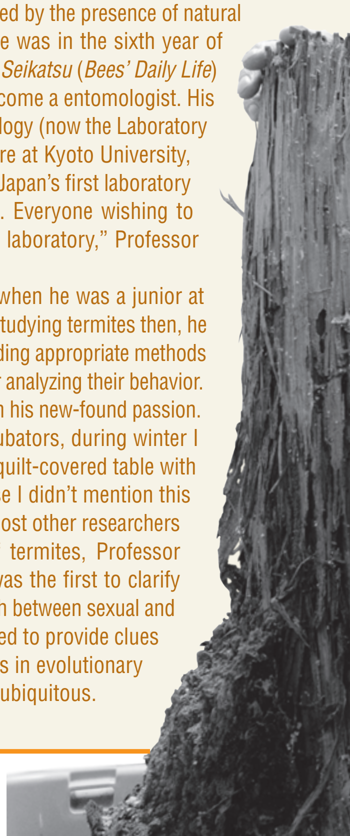
Research on termites could offer clues to research that benefits the future of humans.

In his childhood, Professor Kenji Matsuura loved to play outdoors in the fields. One autumn day, when he was in the third year of elementary school, he overturned a plywood board left in a field and found countless termites wiggling in the nest they had made in the board. He discovered that there were many types of termites that looked different and had different roles to play. Despite his young age, he was deeply impressed by the presence of natural laws that guided the termites' lives. When he was in the sixth year of elementary school, he read the book *Hachi no Seikatsu* (*Bees' Daily Life*) by Kunio Iwata, and this convinced him to become a entomologist. His intention was to join the Laboratory of Entomology (now the Laboratory of Insect Ecology) in the Faculty of Agriculture at Kyoto University, where the book's author had studied. "It was Japan's first laboratory of insect ecology, established 90 years ago. Everyone wishing to study entomology was eager to enroll in the laboratory," Professor Matsuura explained.

He began full-fledged studies of termites when he was a junior at Kyoto University. Since few researchers were studying termites then, he had to develop everything from scratch – including appropriate methods of keeping termites and the best procedures for analyzing their behavior. This meant that he spent a great many hours on his new-found passion. "Since students were not allowed to use incubators, during winter I kept termites underneath the *kotatsu* (small quilt-covered table with a heater underneath) at my lodging. Of course I didn't mention this to my landlord," he said with a smile. While most other researchers took biological approaches to the study of termites, Professor Matsuura took an ecological approach. He was the first to clarify the mechanism by which termite queens switch between sexual and asexual reproduction. His findings are expected to provide clues to resolving one of the most enduring puzzles in evolutionary biology: why sexual reproduction is virtually ubiquitous.



Closing the sperm gates of eggs

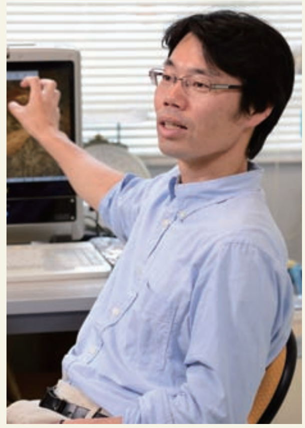
To identify a mechanism for controlling fertilization in termites, we focused on the micropyles of eggs. The micropyle is a channel of sperm entry into a mature oocyte. Since termite queens are always attended by kings, the simplest and most effective mechanism to produce parthenogenetic eggs would be to close the micropyles of eggs in order to prevent sperm entry.

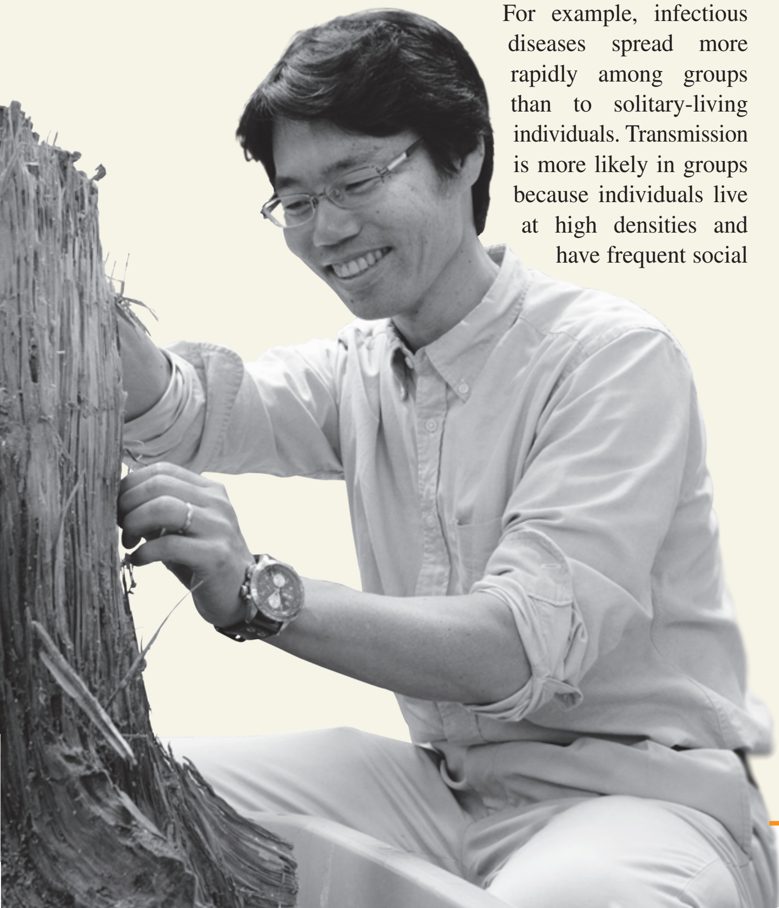
Observations of eggs from *R. speratus* showed that the number of micropyles varies greatly, even within a single colony. If a certain proportion of eggs lack micropyles (sperm gates), their fertilization would be mechanically impossible, regardless of whether or not a king was present. Genetic analysis has demonstrated that the embryos of eggs lacking micropyles develop parthenogenetically, whereas embryos from eggs with micropyles are fertilized and develop sexually. Analysis of eggs has shown that queens begin to lay eggs lacking micropyles when they are older, and thus must produce their replacements parthenogenetically. We found that *R. speratus* queens alter the number of micropyles in their eggs over time, thereby producing eggs lacking micropyles in order to generate their replacements asexually.

Why sexual reproduction?

Termite queens limit the use of parthenogenesis to generating replacements, although asexual reproduction by a queen doubles her contribution to the gene pool. Why do the queens still use sexual reproduction to generate most of the colony members, including workers, soldiers, and alates? Social insect queens face a dilemma regarding the costs and benefits of sexual vs. asexual reproduction. One proposed major advantage of sexual reproduction is that it promotes genetic variability across generations, facilitating adaptation to local ecological conditions. Overuse of asexual reproduction reduces the genetic diversity of the offspring and thus potentially reduces the ability of the colony to adapt to environmental stress.

Living in social groups also has drawbacks as well as advantages. For example, infectious diseases spread more rapidly among groups than to solitary-living individuals. Transmission is more likely in groups because individuals live at high densities and have frequent social contact. In addition, group members are close relatives and are therefore susceptible to the same parasitic infections. This can be understood using an analogy to human agriculture. Selective breeding of crops for desirable traits and against undesirable ones leads to monocultures – entire farms of plants that are nearly genetically identical. Little to no genetic diversity makes crops extremely susceptible to widespread disease.





The near-ubiquity of sexual reproduction is one of the most enduring puzzles in evolutionary biology, because all else being equal, asexual populations have a two-fold fitness advantage over their sexual counterparts and should therefore rapidly outnumber sexual populations. Termite queens which conditionally switch between sexual and asexual reproduction may hold the key to a better understanding this enigma.

References

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2) **PNAS** *107*, 12963-12968, 2010.
3) **Nature Comm.** *4*, 2048, 2013.
4) **PNAS** *111*, 17212-17217, 2014.

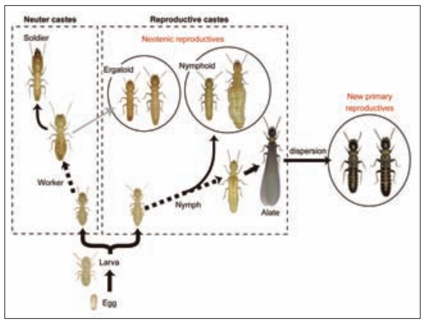

Fig. 1 Differentiation pathways of primary and secondary (neotenic) reproductives in *Reticulitermes* termites. Primary reproductives (queens and kings) are derived from alates found in new colonies. Ergatoids are neotenic reproductives differentiated from workers, and nymphoids are neotenic reproductives differentiated from nymphs. PNAS *111*, 934–938, 17212-17217, 2014.

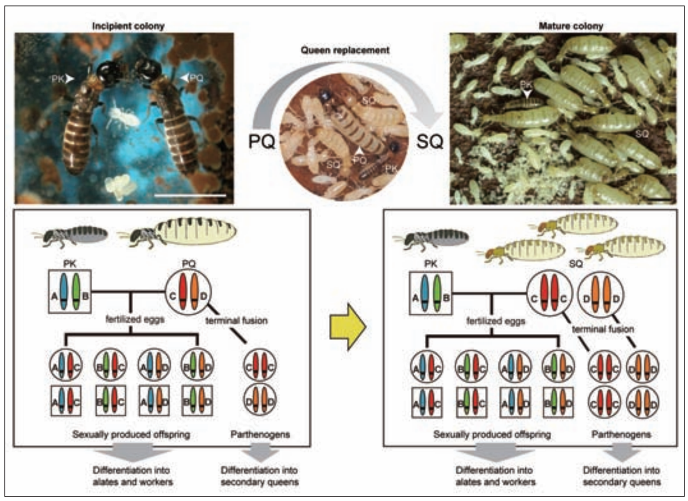

Fig. 2 Colony development and asexual queen succession in the termite *R. speratus*. As the primary queen senesces, secondary queens produced asexually by the primary queen differentiate within the colony and supplement egg production, eventually replacing the primary queen. Squares indicate males and circles represent females. PK, primary king; PQ, primary queen; SQ, secondary queen. (Scale bars: 3 mm.)
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