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The Unusual Neotenic System of the Asian Dry Wood Termite, *Neotermes koshunensis* (Isoptera: Kalotermitidae)

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Abstract

In most lower termites, colonies are headed by neotenic reproductives of both sexes after the primary reproductives (i.e., the queen and king) are lost. The production of a neotenic sexual is inhibited by the presence of a primary reproductive of the same sex.

We found an exception in the caste system of the dry wood termite *Neotermes koshunensis* (Kalotermitidae). The neotenic caste is exclusively male. Moreover, production of male neotenics is completely inhibited not only by the presence of a king but also by the presence of a queen. Therefore, it is likely to be difficult for *N. koshunensis* colonies to replace their reproductive pairs.

Introduction

Termites are highly eusocial insects that show reproductive division of labor among morphologically distinguishable castes (Weesner, 1969). The reproductive castes of termites are classified broadly into primary reproductives, namely the queen and king, which are the dealates that found new colonies after a swarming, and the secondary reproductives, which are recruited within an established colony (Roisin, 2000). Depending on circumstances of the emergence of secondary reproductives, they are classified into either replacement reproductives, which emerge after the death (or disappearance) of the queen or the king, or both, and supplementary reproductives, which emerge even in the presence of the primary reproductives (Roisin, 2000). On the basis of their ontogenetic origin, secondary reproductives are classified into neotenics, which are reproductives raised from immature individuals (they are more specifically called nymphoids, ergatoids, or pseudergatoids, depending on their origin from nymphs, workers or pseudergates), and adultoids, which are dealate imagoes and are therefore indis-

tinguishable from the primary reproductives (Roisin, 2000). The neotenic castes are widespread in various termite taxa, whereas adultoids are rare (Myles, 1999). Termite society is in principle bisexual, and males and females in most species perform the same social tasks (Roisin, 2000). The neotenic castes in termites are also often bisexual, although a biased numerical sex ratio is reported in neotenics of some species (Myles, 1999).

The termite genus *Neotermes* (Kalotermitidae) shows an interesting diversity in the neotenic caste system. In *Neotermes connexus* and *Neotermes papua* (Kalotermitidae) neotenics are found only in males (Myles & Chang, 1984; Roisin & Pasteels, 1991), whereas in *Neotermes tectonae* and *Neotermes jouteli* the common pattern of bisexual neotenic production is observed (Kalshoven, 1930 cited by Myles, 1999; Nagin, 1972). To understand the biological reasons for this variation in the neotenic system more species should be studied in this genus.

Lüscher (1961) proposed the currently well-acknowledged model of sex-specific inhibition of neotenic production in termites, i.e., the emergence of male and female neoten-

ics is inhibited by the presence of the primary reproductive of the same sex. In *Kalotermes flavicollis* (Kalotermitidae), these inhibitory effects are lost when the transmission of anal excretions from royal reproductives to pseudergates is blocked, suggesting that there is a pheromone in the anal excretions (Lüscher, 1961; Miller, 1969).

Here, we report an unusual neotenic system in the Asian dry wood termite, *Neotermes koshunensis* (Kalotermitidae); the production of male neotenics is totally inhibited by the presence of a queen. Although partial inhibition of male neotenic production by the presence of a queen was reported in a related species, *N. jouteli* (Nagin, 1972), the system found in *N. koshunensis* is exceptional and differs markedly from the model proposed by Lüscher (1961).

Materials and Methods

Termite

N. koshunensis is distributed from Taiwan to Okinawa (Ikehara, 1966). Colonies nest in dead branches of living trees and in dead trees that serve as sources of both food and shelter (i.e., the termites are of the "one-piece" type, sensu Abe, 1987); this enabled us to collect entire colonies. This species has a linear caste development pathway: all castes, including neotenics (pseudergatoids) differentiate from pseudergates (older larvae of the functional worker caste in termite species with a linear caste development pathway) after molting (Roisin, 2000; Katoh et al., 2007).

Effect of the presence of primary reproductives on the emergence of neotenics

We collected 22 colonies of *N. koshunensis* on the main island of Okinawa during 2007–2010. Collected individuals were classified into the following castes according to Katoh et al., (2007): small larvae, pseudergates, nymphs, pre-alate nymphs, alates, pre-soldiers, soldiers, the queen (we found a maximum of one individual in each colony) and the king (also a maximum of one individual in each colony). From each colony, we established subcolonies receiving two or three of the following four treatments (note that it was impossible to apply treatments i and ii, or i and iii, simultaneously in the same colony).

(i) Normal colony: 50 haphazardly sampled pseudergates + a queen and a king (n = 7),

(ii) Queen colony: 50 haphazardly sampled pseudergates + a queen (n = 9),

(iii) King colony: 50 haphazardly sampled pseudergates + a king (n = 13),

(iv) Orphaned colony: 50 haphazardly sampled pseudergates (n = 22).

Each subcolony was put into a petri dish (90 mm diameter × 16 mm height) with a piece of damp filter paper, which served as a source of both food and moisture. The subcolonies were then kept in the laboratory (25 ± 1 °C) in the dark for 6 weeks. The caste composition of each subcolony was investigated every 7 days, and the filter paper was replaced at the same time. All statistical tests were performed using R version 2.12.2 (R Development Core Team, 2011).

Results

Neotenics were produced only in the orphaned subcolonies: the presence either a queen or a king, or both, led to total inhibition of neotenic production (Fig. 1). The proportion of subcolonies that produced neotenics differed significantly between the orphaned and any other treatments (all pairs P < 0.001, Fisher's exact test followed by Bonferroni correction). This result holds true after controlling for the possible confounding effect of colonies: in the likelihood ratio test after applying the generalized linear model (GLM) with binomial error structure (dependent variable = the proportion of subcolonies producing male neotenic(s), explanatory variable = treatment and colony, link function = logit), a significant effect of treatment was detected ($\chi^2 = 58.958$, P < 0.0001), but the effect of colony was non-significant ($\chi^2 = 17.526$, P = 0.6788).

Moreover, all of the neotenics that emerged in these orphaned subcolonies were males (Fig. 1). At the end of the experiment, we carefully checked the contents of all petri dishes. We found eggs in the normal and queen subcolonies,
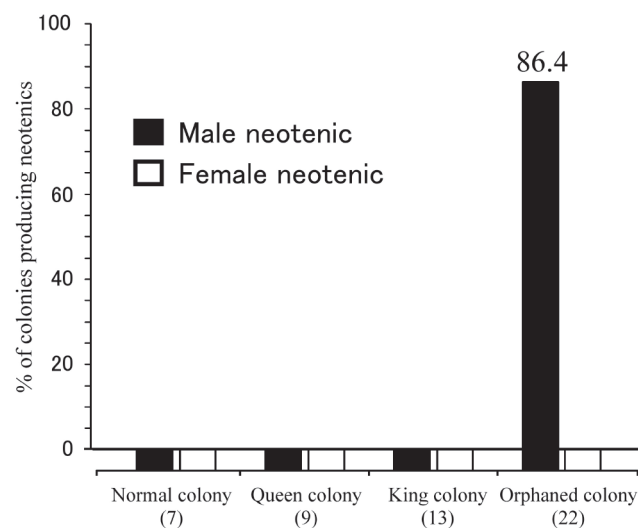
Figure 1. Effects of the presence of either a king or a queen, or both, on the production of neotenics in *Neotermes koshunensis*. Normal colony: subcolonies with both a queen and a king; Queen colony: subcolonies with a queen but no king; King colony: subcolonies with a king but no queen; Orphaned colony: subcolonies without reproductives. Numbers in parentheses denote sample sizes, i.e., the number of subcolonies of each type formed.

whereas eggs were absent in the orphaned and king subcolonies. Immediately after the experiment, we haphazardly chose 4 of the 19 orphaned subcolonies that had produced at least one neotenic and dissected all individuals to observe their gonads. In these colonies, the numerical sex ratio in females was 25% (10 of 40), 26% (10 of 39), 36% (16 of 45) and 40% (18 of 45), but no individuals except male neotenics had clearly developed gonads. In 16 of the 19 orphaned subcolonies, each subcolony produced a maximum of one male neotenic during the entire experimental period. In 3 subcolonies, a maximum of two male neotenics were found at the same time, however only one individual survived after 1-2 weeks. Emergence of a neotenic was apparent by day 7 in 1 of the 19 orphaned subcolonies, by day 14 in 15 subcolonies, and by day 21 in 3 subcolonies.

Discussion

The neotenic system of *N. koshunensis* is unusual for two reasons. First, the neotenic caste is male-specific. Second, the inhibitory effect is not sex-specific, i.e., both the king and the queen, whether present individually or together, inhibit the production of male neotenics.

The apparent absence of female neotenics is not explained by the presence of cryptic female neotenics that had no body color change and therefore went unrecognized, because no eggs were found in the orphaned and king subcolonies, which lacked a queen. Furthermore, none of the females in the orphaned subcolonies that produced male neotenics had developed gonads. The experimental period seemed to be long enough at least for males, because the testes size (the width of a testis) of neotenics increased twice that of the testes of pseudergates during the experimental period of 6 weeks (Miyaguni, Y. unpublished data).

There is a rather well-circulated description on the biology of this species by Myles (1999) asserting the existence female neotenics in this species. There is a historical reason why this incorrect information was circulated in the scientific literature. Myles (1999) referred to Takuya Abe's personal communication in which Abe wrote that in late autumn and winter female reproductives (neotenics) were produced in this species. It was also written that female neotenics were not as darkly colored as males but they have mature ovaries. However, Abe's descriptions were all based on the results of examination of gonad size of pseudergates by our coauthor Koji Sugio who was a student of Abe in those days. What Sugio's data showed was only that female pseudergates in late autumn and winter had on average larger ovary size than in summer. This phenomenon is a simple reflection of higher fat storage of individuals in winter (Sugio, K. unpublished data). Unlike in caste differentiation, no specific individual showed more prominent ovarian development than the others. We conclude that Abe simply misinterpreted Sugio's data, which has since been communicated to Myles

(personal communication). Indeed, in addition to the current experimental data, we have much evidence in the field and laboratory strongly indicating the true absence of female neotenics in this species (Miyaguni, Y. and Sugio, K. unpublished data). Male-specific neotenic systems also occur in *N. connexus* and *N. papua* (Myles & Chang, 1984; Roisin & Pasteels, 1991). However, in these two species whether both the queen and the king inhibit male neotenic production remains to be determined.

Our finding that the presence of not only the king but also the queen inhibited the production of male neotenics is not explained by the model of Lüscher (1961), which assumes the sex specificity of pheromonal inhibition (see also Matsuura et al. 2010). However, there are a few known examples that the Lüscher sex-specific inhibition does not fully account for. The possible inhibition of neotenic production by the presence of a reproductive caste of the opposite sex has also been suggested in *Mastotermes darwiniensis* (Mastotermitidae), which has both male and female neotenics (Watson et al., 1975). In *N. jouteli*, the functional queen (female neotenic) and the functional king (male neotenic) inhibit independently the emergence of new neotenics of both sexes, although the inhibition is not as complete as in *N. koshunensis* (Nagin, 1972). These previous data together with our current results suggest that the production of neotenics in some termites is controlled by a non-sex-specific factor.

Our results suggest that it is difficult for the reproductive pairs of *N. koshunensis* colonies to be recovered when colonies lose one or both primary reproductives. One possibility, which occurs in *N. papua*, is the recovery of reproductive castes through the production of both male neotenics and female adultoids in orphaned laboratory colonies (Roisin & Pasteels, 1991).

At this stage, it is difficult to understand the adaptive significance of the production of male-only neotenics that are inhibited by the presence of a queen or king, or both. To answer this question we need more information, especially on the life history of this species and on the reproductive ability of its neotenics.

Acknowledgments

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