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Preadaptation for parthenogenetic colony foundation in subterranean termites *Reticulitermes* spp. (Isoptera: Rhinotermitidae)

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Abstract Thelytokous (all-female producing) parthenogenesis, in some cases, involves reproductive advantages against obligate sexual reproduction. However, the completion of parthenogenesis takes multiple steps without the help of males, and thus preadaptation that meets those requirements will be an important factor for the evolution of parthenogenesis. The Japanese subterranean termite, *Reticulitermes speratus*, is known to have the ability of parthenogenetic colony foundation, where females that failed to mate with males found colonies cooperatively with partner females and reproduce by parthenogenesis. In this study, we compared the parthenogenetic ability and the colony initiation behavior among six *Reticulitermes* species in Japan. All species other than *R. speratus* were not able to reproduce parthenogenetically. Nevertheless, females of these species without the parthenogenetic ability performed homosexual female–female colony initiation and produced eggs without fertilization. In addition, in one species without parthenogenetic reproduction, *R. kanmonensis*, female–female pair initiated founding behavior as quickly as a heterosexual pair. These results suggest that female–female colony initiation and virgin egg-laying are predominant characters among the genus *Reticulitermes* and provide a preadaptive condition for parthenogenetic colony foundation in *R. speratus*.

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Introduction

Because a given animal strategy usually involves a set of morphological, molecular, and behavioral traits, its evolution hypothetically takes multiple and complicated pathways. In such cases, considering conditions of preadaptation is an informative way to understand the evolution of animal strategies. A preadaptation is a trait that acquires a new biological role when organisms undergo a change in their environment (Koehl 1996), and it often provides a condition for promoting the evolution of a different trait, which is a neutral or maladaptive one under the previous environment (Bock 1959; Scharloo 1991). This evolutionary process is termed canalization, and diverse strategies or organs are considered to evolve through step-wise mutations of distinct traits (Waddington 1942). In this study, from the viewpoint of the preadaptation argument, we investigated prerequisite conditions for parthenogenetic colony foundation in subterranean termites of the genus *Reticulitermes* (Isoptera: Rhinotermitidae).

Parthenogenesis is a form of asexual reproduction and thus it theoretically has many advantages in reproduction against obligate sexual reproduction, such as the well-known twofold demographic advantage of thelytoky or producing all-female offspring (Maynard Smith 1978). The prevalence of sexual reproduction in eukaryotes despite its disadvantages has been considered one of the most challenging problems in evolutionary biology (Otto 2009), and many theoretical and empirical studies have tackled the problem (e.g., Kondrashov 1988; Hamilton et al. 1990).

In some cases, however, the completion of parthenogenesis takes multiple steps and thus organisms should have to simultaneously meet those requirements in order to experience its reproductive advantage. For example, it has been argued that some characters should set the stage for the evolution of parthenogenetic reproduction in the stick insect genus *Bacillus*. From a cytological point of view, the lack of sperm contribution of the centriole presents a barrier for an unfertilized egg in most animals to accomplish parthenogenetic development, because the organelle is essential to build the first embryonic spindle but lost from the maternal gamete during oogenesis (Schatten 1994). However, the ability of the egg pro-nucleus to fully organize a functional spindle without the help of the sperm organelle is a widespread trait in stick insects (Marescalchi et al. 2002) and appears to represent preadaptive condition for the evolution of facultative or obligate parthenogenesis in the genus (Scali et al. 2003; Scali 2009). As in this case, any preadaptation would have played an important role for the evolution of parthenogenetic colony foundation in the genus *Reticulitermes* for the following reasons.

A few species of *Reticulitermes* termites are known to have the ability of parthenogenetic reproduction (Matsuura 2011). For example, a queen of *R. speratus* (Kolbe) parthenogenetically produces daughters as replacement reproductive but initially reproduces sexually when she founds the colony with a male (Matsuura and Nishida 2001; Matsuura et al. 2002). Importantly, those termites have to follow several distinct steps in order to found colonies and perform successful reproduction (Shellman-Reeve 1999; Matsuura and Nishida 2001); after swarming during the day-time, an alate breaks off its wings and runs on the ground until it encounters a potential partner with which to form a tandem running pair (formation of tandem running). Then the pair locates suitable nest wood, and they excavate the nest wood and cooperatively construct the incipient cell (colony initiation). That is, to accomplish parthenogenetic reproduction, termite females successfully initiate and complete these steps without a male partner. In addition, a previous study found that although a female alate is able to initiate founding behavior with or without partners, the founding success of a single female unit is significantly lower than that of homosexual female–female pair and normal male–female pair (Matsuura and Nishida 2001; Matsuura et al. 2004). This result indicates that a female of *Reticulitermes* termites relies on the cooperation by the other partner for successful colony foundation.

In this study, we investigated whether the evolution of parthenogenetic colony foundation in *R. speratus* beforehand requires conditions that a female alate forms a tandem pair even with a homosexual one and lays eggs without sexual fertilization. Specifically, we hypothesized that the formation of a homosexual female–female pair and

subsequent virgin egg laying are more widespread than the capacity for parthenogenetic reproduction. To test this hypothesis, we examined the ability of parthenogenesis and the occurrence of homosexual female–female colony initiation of six different *Reticulitermes* species in Japan by comparing incipient colony success and the founding behavior between different pair-units (single females, female–female pairs, and male–female pairs).

Materials and methods

Sample collection

The genus *Reticulitermes* is widely distributed in the northern temperate zone, and throughout Japan from Hokkaido to the Ryukyus (Takematsu 1999). We chose six of the seven species of *Reticulitermes* distributed in Japan (Takematsu 1999) as the study species for their availability. Specifically, *R. speratus* and *R. kanmonensis* are distributed in central Japan, and the other four species (*R. amamianus*, *R. miyatakei*, *R. okinawanus*, *R. yaeyamanus*) are distributed in the southern part of Japan. Only *R. speratus* is known to be a facultatively thelytokous species (Matsuura and Nishida 2001; Matsuura et al. 2002), but the other species have not been investigated with respect to colony initiation behavior or the ability to reproduce parthenogenetically.

Sample collections of termites used in the experiments were performed as follows. We collected termite colonies of each termite species with nest wood, just before the swarming season; for *R. kanmonensis*, sample collection was performed in March 2006 from sites near the Kanmon Strait, Yamaguchi prefecture; colonies of *R. speratus* were collected in May 2006 at three sites located in the northern suburbs of Kyoto prefecture; colonies of *R. amamianus* and *R. miyatakei* were collected in May 2006 on Amami Island and Tokunoshima Island, Kagoshima prefecture; colonies of *R. okinawanus* were collected in February 2007 on the main island of Okinawa, Okinawa prefecture; and colonies of *R. yaeyamanus* were collected in April 2007 on Ishigaki Island and Iriomote Island, Okinawa prefecture. All sampled colonies contained alates, and the colonies were maintained separately in plastic boxes (200 × 350 × 200 mm) with nest wood and moist filter paper at 25 °C in the laboratory, until the experiments were carried out.

Comparison of foundation success between different founding units in the collected species

We performed colony founding experiments using the following procedures. First, dealated termites were randomly chosen from each stock colony, and assigned to one

of the three colony initiation units: single females (F), female–female pairs (FF), and female–male pairs (FM). We conducted the colony founding experiments using three colonies (A, B, and C) of each species. That is, F-units consisted of three different colonies (F = A, B, and C); FF-units consisted of six different combinations (FF = AA, AB, AC, BB, BC, and CC); FM-units consisted of nine different combinations (FM = AA, AB, AC, BA, BB, BC, CA, CB, and CC). We set up five replicates for each unit combination, except for *R. miyatakei*, *R. okinawanus*, and *R. speratus* owing to small sizes of the sampled colonies. Dealates of each unit were placed in a plastic Petri dish (ca. 90 × 20 mm) that contained mixed sawdust bait blocks (Matsuura and Nishida 2001) at 25 °C under constant darkness. After 100 days, the bait block were carefully dissected to determine the number of surviving primary reproductive, the number of eggs, and the number of hatching larvae.

Observation of initiation behavior of species without the ability of parthenogenesis

The previous study reported that F-units took the longest time to start colony initiation (evaluated by the time until nest excavation) compared to the other two (FF and FM) units in *R. speratus* (Matsuura and Nishida 2001). To study the relationship between parthenogenetic reproduction and colony initiation behavior in detail, we performed the observation experiments using the following procedure. First, we inferred the presence or absence of parthenogenetic capacity for *Reticulitermes* species other than *R. speratus* from the colony founding experiments described above. Then, we chose a species without parthenogenetic capacity and observed the time until nest excavation of the species. The observations were performed as follows. As in the above method, three colonies (A, B, and C) were used for the observations. Each unit (F, FF, and FM) was observed once every hour during the first 6 h, and then every 6 h until all individuals had bored into the nest bait. Then the time until excavation was recorded following the methods of a previous study (Matsuura and Nishida 2001). We performed two replicates for each unit combination.

Statistical analysis

In the colony founding experiments, the founding success of each unit was evaluated by the survival rate of a female, the number of total progeny (eggs and hatching larvae) from a survival colony, and the number of hatching larvae from a survival colony. The results were not significantly different between the inter- and intra-colony combinations, and the effect of inbreeding (or outbreeding) was not found in the all studied species (GLM, $P > 0.05$). Also, in the

observation experiments, the results were not different between the colony combinations (GLM, $P > 0.05$). Therefore, the data were pooled for the analyses. The results of the foundation success and the time until nest excavation were analyzed with GLM and then compared between different units by Tukey–Kramer HSD test. All analyses were done with JMP 8.0 (SAS Institute, Japan).

Results

In the colony founding experiments, survivability of a female significantly differed among the different unit types in each *Reticulitermes* species ($P < 0.01$, GLM). Specifically, although a female in FF-units had a statistically similar survivability to that in FM-units in all species, the survivability in F-units was significantly lower than that in the FF- and FM-units in all species, except for *R. miyatakei* and *R. speratus* (Tukey–Kramer HSD-test, $P < 0.05$; Fig. 1a). For the number of total progeny, although some females in F-units survived in *R. kanmonensis* (Fig. 1a), no oviposition was observed in any of the replicates (Fig. 1b); on the other hand, some oviposition was observed in homosexual FF-units in all species (Fig. 1b) in spite of the significant difference in the number of progeny between FF-units and FM-units in *R. kanmonensis* and *R. miyatakei* (Tukey–Kramer HSD test, $P < 0.05$). For the number of hatching larvae, survived larvae were observed in FF-units of *R. speratus* and there was no significant difference between the units (Tukey–Kramer HSD test, $P > 0.05$), indicating that this species has the ability to reproduce parthenogenetically. This result was consistent with previous studies (Matsuura and Nishida 2001; Matsuura et al. 2002); however, for the other species, successful hatching of unfertilized eggs did not occur in any of the replicates in surviving FF-units (Fig. 1c). That is, the other five species of Japanese *Reticulitermes* have no ability to reproduce parthenogenetically.

Based on the results of the foundation experiments, we chose *R. kanmonensis* as the species without the ability of thelytokous parthenogenesis for the observation experiments. As a result, there was significant difference in the time until nest excavation between different unit types ($P > 0.05$, GLM; Fig. 2). Specifically, F-units took a significantly longer time to initiate the nest-excavation than the other two units (Tukey–Kramer HSD test, $P < 0.05$), but there was no significant difference in the time between FF- and FM units (Tukey–Kramer HSD test, $P > 0.05$).

Discussion

In summary, the results of this study confirm the prediction that both the homosexual colony initiation and the virgin
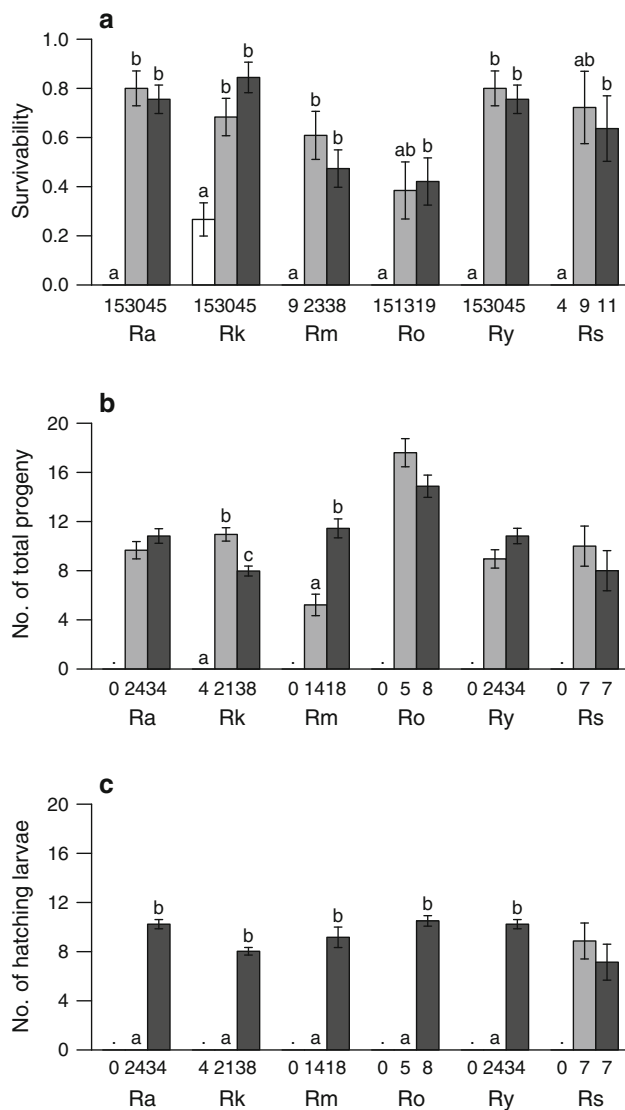
Fig. 1 Results at 100 days after foundation success in F-, FF-, and FM-units of six *Reticulitermes* spp. **a** Survival rate of a founding female; **b** number of total progenies in a survived unit; **c** number of hatching larvae in a survived unit. *White*, *gray*, and *black* columns in the panels indicate the mean of F-, FF-, and FM-units, respectively. *Numbers below each column* represent the replicates for the unit in each study species. *Bars* indicate standard error. *Labels* of *x* axis represent different *Reticulitermes* species (Ra, *R. amamianus*; Rk, *R. kanmonensis*; Rm, *R. miyatakei*; Ro, *R. okinawanus*; Ry, *R. yaeyamanus*; Rs: *R. speratus*). *Letters* (*a–c*) designate significant differences between the units in each species (*P* < 0.05, Tukey–Kramer HSD-test). *Dots* above the replicate numbers represent unavailability of the data for statistical analysis
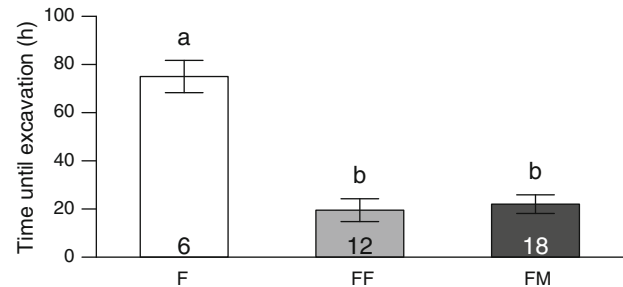

Fig. 2 Comparison of the time until excavation between the three unit combination (F-, FF-, and FM-unit) in the species without parthenogenetic ability, *R. kanmonensis*. *Columns* and *bars* indicate mean and standard error, respectively. *Numbers in each column* represent the replicates for the unit. *Letters* (*a*, *b*) designate significant differences between the units (*P* < 0.05, Tukey–Kramer HSD-test)

non-viable and the cooperation of a partner is necessary for successful colony initiation. This is because termite societies are strongly threatened by the risk of pathogen infection. The termite nest is usually in a confined and damp environment, which will promote microbial growth and facilitate the transmission of infection (Rosengaus et al. 2003). In such a pathogen-rich environment, many tasks associated with colony foundation will exhaust single females without partners and decrease their immunity against pathogen infection. Furthermore, unlike ants, the only body part that termites can effectively clean through self-grooming is the antenna; therefore, allo-grooming from nest-mates is essential to their survival (Matsuura et al. 2002; Yanagawa and Shimizu 2005).

Second, we found that parthenogenetic reproduction is a rare ability, whereas virgin egg laying is prevalent among the genus *Reticulitermes*. This is because, in spite of the occurrence of virgin egg laying in all the study species (Fig. 1b), no hatching larvae were observed in FF-units of species other than *R. speratus* (Fig. 1c). In addition, the result of the foundation experiments implied that, when a male partner is unavailable, a female alate of *Reticulitermes* termites forms a homosexual tandem pair and builds the nest cooperatively with the female partner as often as the species with the parthenogenetic ability, *R. speratus*. This conclusion was supported by the results of the observation experiments: as shown in the previous study using *R. speratus* (Matsuura and Nishida 2001), a homosexual female–female pair initiated cooperative nest excavation as quickly as a bisexual male–female pair, even in the species without the parthenogenetic capacity, *R. kanmonensis* (Fig. 2). These results suggest that the acquisition of the two characters, i.e., homosexual colony initiation and virgin egg laying, precedes the evolution of the ability to reproduce parthenogenetically and would provide the preadaptive condition for parthenogenetic colony foundation in *R. speratus*. This suggestion is supported by the inferred phylogeny of the genus *Reticulitermes* including the studied

egg-laying are common characters in the genus of *Reticulitermes* for the following reasons. First, the foundation experiments have shown that most of the females of F-units died 100 days after the start of the experiments but those of both homosexual FF-units and bisexual FM units survived equally in all the study species (Fig. 1a). The results indicated that, in *Reticulitermes* termites, an alate alone is

species (Park et al. 2006): a phylogenetic tree indicates that *R. speratus* is a derived species and thus it is most parsimonious to infer that the parthenogenetic capacity is acquired after the evolution of homosexual colony initiation and virgin egg laying.

However, why are homosexual female–female tandem formation and subsequent colony initiation prevalent among obligately sexual species of the genus *Reticulitermes*? This is because, even without the ability of parthenogenesis, colony initiation of a homosexual pair should be better than that of a single female to some degree for the following reasons. First, tentative pair formation for tandem running would be adaptive as a means to avoid predation. An experimental study using *R. speratus* showed that an alate forming a homosexual or heterosexual tandem avoids the risk of predation by a termite-hunting ant, *Pachycondyla chinensis* (Emery), more effectively than an alate remaining alone (Matsuura et al. 2002). In addition, it is possible that surviving females of a FF-unit might encounter an additional male while excavating wood and building the nest cell. In such a situation, they could adopt the male into the founding group and recover ordinary heterosexuality. Little work has been done on the possibility of the addition of a male after homosexual pair formation among *Reticulitermes* species, and thus further experiments are needed to shed light on the reproductive benefit of homosexual colony foundation in those species without the parthenogenetic ability.

Provided that female–female colony foundation and subsequent virgin egg laying are predominant among *Reticulitermes* termites, how would such conditions have affected the acquisition of the ability of parthenogenetic reproduction in *R. speratus*? A transition from obligately sexual to parthenogenetic reproduction has been hypothesized to take several evolutionary routes (Simon et al. 2003). One such scenario is that the ability of parthenogenesis is gradually enhanced from tychoparthenogenesis, that is, accidental and occasional embryo development of unfertilized eggs (Templeton 1982), and, in fact, it is known that a small fraction of unfertilized eggs occasionally develops into viable zygotes in many insects (Bell 1982; Simon et al. 2003). In the route from tychoparthenogenesis, an increase in the opportunity of successful oviposition by unmated females will be an important condition, because it should select for the capacity of parthenogenetic reproduction. For example, some studies using bisexual *Drosophila* have shown that the ability of thelytokous reproduction can gradually be improved through selection experiments for unfertilized females that reproduce parthenogenetically (Stalker 1954; Carson 1967; Kramer and Templeton 2001). As in those experiments, replicated occurrence of maleless colony initiation might provide the condition for promoting the ability of parthenogenetic reproduction in *R. speratus*.

However, to tackle the unresolved issue of this study, i.e., the reason why parthenogenetic ability evolved only in *R. speratus*, it should be important to consider a different ultimate cause for the evolution of parthenogenesis other than a proximate cause such as the route from preadaptation of an increase in homosexual colony founding. Recently, it has been revealed that parthenogenetic reproduction has another important function specific to societies of some *Reticulitermes* termites (Matsuura et al. 2009; Vargo et al. 2012). In lower termites without parthenogenetic ability, sexually produced offspring (secondary kings and queens) inherit the reproductive position in the colony after the death of the primary king and queen. Therefore, such inbreeding cycles of generations of secondary reproductives will lead to the accumulation of inbreeding depression in the colony (Fei and Henderson 2003; DeHeer and Vargo 2006). On the other hand, for the parthenogenetic species, a primary king has significantly greater longevity than a primary queen, and secondary queens are usually produced through parthenogenesis by the primary queen, whereas sexual reproduction is used for the production of workers and alates (Matsuura et al. 2009). That is, such conditional use of parthenogenesis (asexual queen succession; AQS) enables the primary queen to maintain her full genetic contribution to the next generation, while avoiding any loss in genetic diversity from inbreeding in the colony (Matsuura et al. 2009; Matsuura 2011). Thus, because parthenogenetic reproduction in the AQS system obviously provides the reproductive benefit in the termite colony, the route from the AQS to the established parthenogenetic ability is possible as well as that from the increased occurrence of homosexual female–female colony initiation. However, the present information on the reproductive biology of *Reticulitermes* termites does not reveal the relative significance of AQS and parthenogenetic colony foundation for the establishment of parthenogenesis, and hence further investigation from the distinct viewpoints of the two evolutionary routes will be needed for more complete understanding of the evolution of parthenogenesis in *R. speratus*.

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