Evolution of asexual queen succession in *Reticulitermes* termites

by

Kenji Matsuura
Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University,
Kyoto 606-8502 Japan

Abstract

The evolution and maintenance of sexual reproduction is believed to involve important tradeoffs. The queens of social insects are faced with a dilemma over the costs and benefits of sexual and asexual reproduction. Asexual reproduction by a queen doubles her contribution to the gene pool. However, overuse of asexual reproduction reduces the genetic diversity of the offspring and thus the ability of the colony to adapt to environmental stress. Recent research suggests that queens of some *Reticulitermes* termites can solve this tradeoff by the conditional use of sexual and asexual reproduction, whereby queens produce the next generation of queens by parthenogenesis but use sexual reproduction to produce workers. This reproductive system, so called AQS (Asexual Queen Succession) has been reported in *R. speratus*, *R. virginicus* and in *R. lucifugus*, indicating that it has evolved multiple times independently in *Reticulitermes* termites. I discuss possible physiological mechanisms and genetic background underlying the asexual queen succession system in these termites. In addition, the AQS system and consequent sex-asymmetric inbreeding provides an ideal opportunity to test kin selection in diploid organisms, although a strong test of the theory has proven difficult in diploid social insects because they lack the relatedness asymmetry found in the Hymenoptera.

Key words: thelytoky, caste differentiation, genetic diversity, queen succession, sex ratio

Introduction

The capability of parthenogenesis in Isoptera was first reported by Light in 1944. However, the reproductive biology of termite parthenogenesis, until recently, has not been examined in detail beyond its notional documentation. This is largely because parthenogenetic reproduction has been regarded as an unusual case with little adaptive significance in nature. Even after the adaptive significance of termite parthenogenesis was recognized, researchers believed that the function of parthenogenesis in termites was no more than “the best of a bad job”, that is, females used parthenogenesis only when they failed to mate with males (Matsuura and Nishida, 2001; Matsuura *et al.,* 2002).

Recently, however, it was revealed that parthenogenesis in some termite species plays a much greater role than has been previously understood. In the Japanese subterranean termite *Reticulitermes speratus* (Matsuura *et al.,* 2009), the North American subterranean termite *R.*

virginicus (Vargo *et al.,* 2012) and Italian subterranean termite *R. lucifugus* (Luchetti *et al.,* 2013), queens exclusively use parthenogenesis to produce secondary (neotenic) queens, while queens produce workers and alates by outcrossing with the primary king. This asexual queen succession (AQS) or conditional use of sexual and asexual reproduction can be studied to understand the advantages and disadvantages of thelytoky in termites.

Mechanism of termite parthenogenesis

Research on chromosome numbers of Isoptera showed that higher termites (Termitidae) are karyotypically uniform (2n = 42), while lower termites are more variable, with diploid numbers ranging from 28 to 56 (Bergamaschi *et al.,* 2007). *Reticulitermes* termites have a fixed number of chromosomes (2n = 42). Karyotypic chromosome observations showed that parthenogenetic offspring are diploid, with 2n = 42 chromosomes in *R. speratus*. Although termite sex determination mechanisms are not completely understood, males commonly appear heterogametic in termites (Roisin, 2001). Interchange multiples (chains or rings of chromosome) in male meiosis is common in termites (Syren and Luykx, 1977). Chromosome multivalents have been observed in male meiosis in *R. speratus*, generating a multiple-X, multiple-Y system (Matsuura, 2002). Because of the XY sex determination system, termite parthenogenesis produces only female progeny.

The genotypes of parthenogenetic offspring depend on the mode of parthenogenesis (Templeton, 1982). Thelytokous parthenogenesis can be categorized into two major cytological divisions, “apomixis (ploidy stasis)” and “automixis (ploidy restoration).” In apomictic parthenogenesis, known as clonal reproduction in aphids, the features of meiosis are either entirely or partially lacking. Only one maturation division takes place in the egg and this division is equational. The offspring retain the genetic constitution of the mother (excluding mutations), and heterozygosity is maintained in subsequent generations.

Importantly, parthenogenesis of termites is not clonal. Thelytoky in termites, such as *R. speratus* and *R. virginicus*, is accomplished by automixis with terminal fusion, in which two haploid pronuclei that divide at meiosis II fuse (Fig.1). Thus, offspring are homozygous for a single maternal allele at all loci that did not crossover, whereas offspring have the same genotype as their mother at loci where crossover occurred. The result is a rapid reduction of heterozygosity (Matsuura et al., 2004).

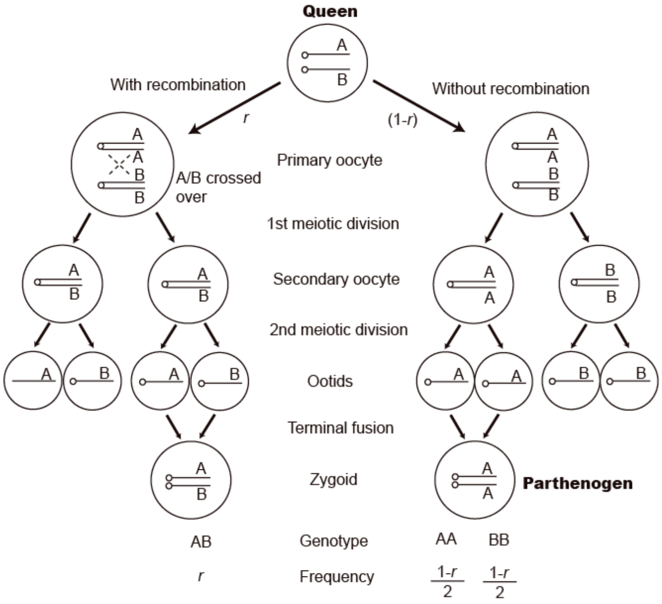

Fig. 1. Scheme of ploidy restoration by automixis with terminal fusion. This type of parthenogenesis results in rapid reduction of heterozygosity *r* is the recombination rate.

Composition of reproductives in field colonies

Reticulitermes speratus is the most common termite in Japan. To date, we have collected more than 1,000 nests in the field to obtain reproductives from a sufficient number of natural colonies. We successfully found the royal chambers, where reproductives and young brood were protected, of 47 colonies (Matsuura et al., 2009). In nearly all cases, primary kings were continuously present (Fig. 2a), but primary queens had been replaced by an average of 64.4（± 16.1SE）secondary queens (Fig. 2b). The largest colony had 676 secondary queens but had only a single primary king. These results indicate that primary kings live much longer than primary queens; replacement of the primary king is rare, whereas replacement of the primary queen is the rule at a certain point in colony development. The rare occurrence of secondary kings in natural colonies shows that colonies terminate soon after the death of primary kings. In addition, secondary reproductives always differentiate from nymphs but never from workers in natural colonies of *R. speratus* (n = 3029).

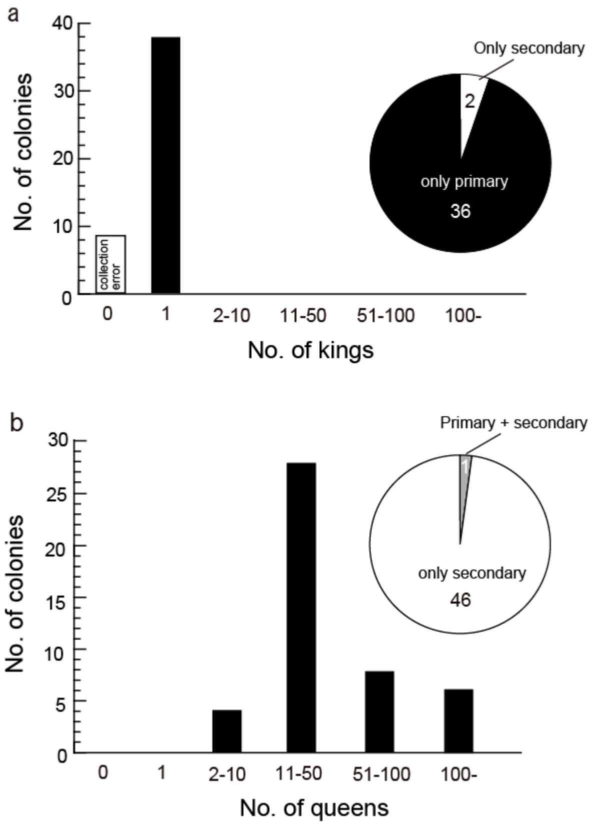

Fig. 2. Number of reproductives in field colonies of *Reticulitermes speratus*. (a) Number of kings in a colony. The pie chart shows that primary kings were present in most colonies. (b) Number of queens in a colony. Primary queens had been replaced by secondary queens in nearly all field colonies.

The paradox of the king-daughter inbreeding hypothesis

Sexual reproduction can lead to important conflicts between sexes and within genomes. In monogamous termites, conflicts between the primary king and queen can arise over parental investment and genetic contribution to offspring. Our finding that primary queens are replaced much earlier than primary kings in *R. speratus* (Fig. 2) leads to a paradox if the secondary queens are the daughters of the primary king. King-daughter inbreeding should result in uneven genetic contribution to the secondary offspring (offspring of secondary queens) by the primary king and queen (Fig. 3).

Let g_{pq} and g_{pk} be the proportion of genes in alates derived from the primary queen and primary king, respectively. Then, in cases of king-daughter inbreeding, the pair producing the F2 offspring consists of the primary king and his F1 daughter (Fig. 3). Under this mating system $g_{pk} = 0.75$, but $g_{pq} = 0.25$ so that the primary king contributes three times as many genes as the primary queen. Therefore, colonies would bias alate (new primary reproductives that disperse) production in favor of males. Contrary to this prediction under this breeding system, the alate sex ratio is slightly but significantly female-biased in this species (numerical ratio of males = 0.43 ± 0.02SE). Because of the larger size of females relative to males, the biomass sex ratio was even more biased toward females (Investment ratio of males: 0.415 ± 0.02SE). This inconsistency between king-daughter inbreeding and sex investment ratio in alate production suggests that there is a different breeding system in which the king and queen have more equal genetic contributions to offspring.

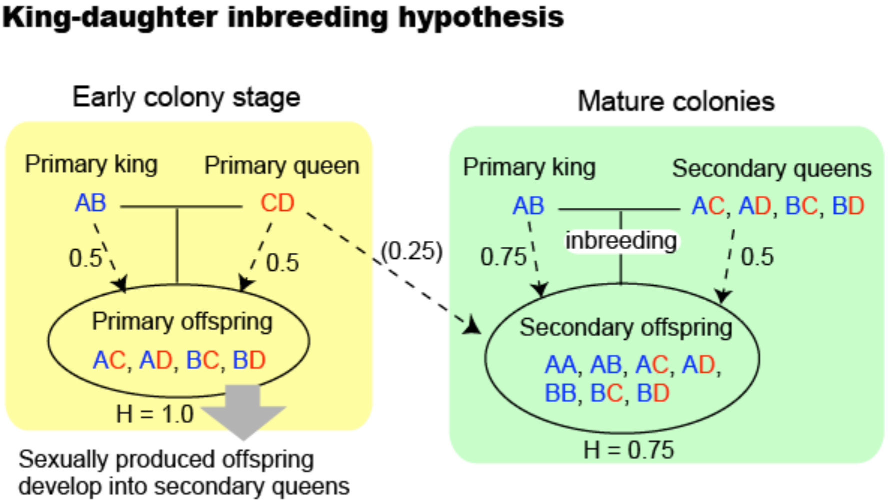

Fig. 3. Paradox of king-daughter inbreeding hypothesis. Under this system, heterozygosity of colony members decreases after queen replacement. The primary queen cannot retain her full genetic contribution to descendants after she is replaced. This sexual conflict predicts male-biased alate sex ratios. Nevertheless, female-biased alate sex ratios are observed in this termite.

Asexual queen succession (AQS) in *Reticulitermes speratus*

While examining genotypes within nests of *R. speratus*, we uncovered an extraordinary mode of reproduction. Secondary queens are almost exclusively produced parthenogenetically by the founding primary queens, whereas workers and alates were produced by sexual reproduction (Fig. 4). By using parthenogenesis to produce secondary queens, primary queens are able to retain the transmission rate of their genes to descendants while maintaining genetic diversity in the workers and new primary reproductives even after the primary queen is replaced (Fig. 5). The relatedness of the primary queens to workers ($r = 0.49$, $SE_{jackknife} = 0.04$) and to alate nymphs ($r = 0.58$, $SE_{jackknife} = 0.079$) is not significantly different from 0.5, the value expected between a female and her sexual offspring. This is twice the expected genetic contribution queens would make to colony members under king-daughter inbreeding ($r_{\text{primary queen to king-daughter offspring}} = 0.25$).

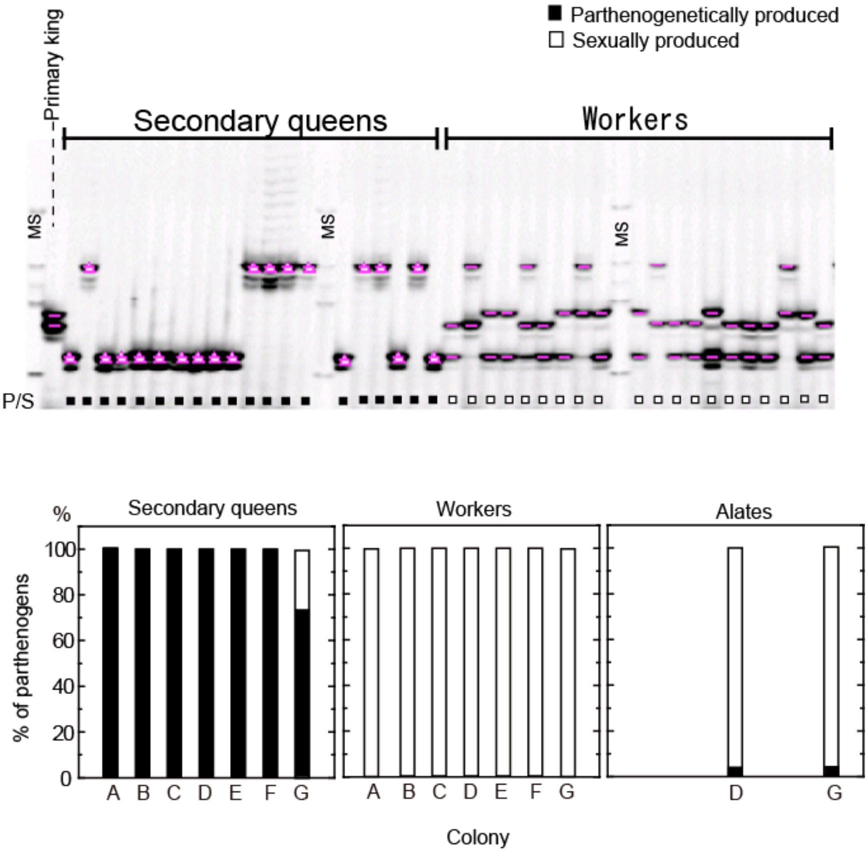

Fig. 4. Proportion of parthenogenetically produced offspring (black) and sexually produced offspring (white) in secondary queens, workers and alates. Secondary queens have only maternal (primary queens') alleles, while workers and alates have both maternal and paternal alleles indicating conditional use of sexual and asexual reproduction. MS: molecular standard.

Parthenogenetic production of secondary queens allows *R. speratus* to undergo queen succession without inbreeding. Heterozygosity of workers in colonies headed by secondary queens was as high (*Ho* = 0.733) as that expected for offspring produced by outcrossing of the primary king and primary queen (*He* = 0.736). Likewise, there was no significant reduction of heterozygosity in nymphs produced in colonies with secondary queens. Further evidence of the lack of inbreeding in *R. speratus* colonies is provided by the low inbreeding coefficient of workers, which did not differ significantly from zero (F_{IT} = 0.014, SE jackknife = 0.048, over all loci). The lack of consanguineous mating in this breeding system may also benefit primary kings. The offspring produced by outcrossing between the king and parthenogenetic queens may have greater fitness than those produced by king-daughter inbreeding.

The production of secondary queens through conditional parthenogenesis effectively extends the reproductive life of the primary queen, greatly expanding her reproductive capacity (Fig. 5). This process of queen succession allows the colony to boost its size and possibly its growth rate without suffering any loss in genetic diversity or diminishing the transmission rate of the queen's genes to her grand offspring, feats that would not be possible if secondary queens were produced by normal sexual reproduction.

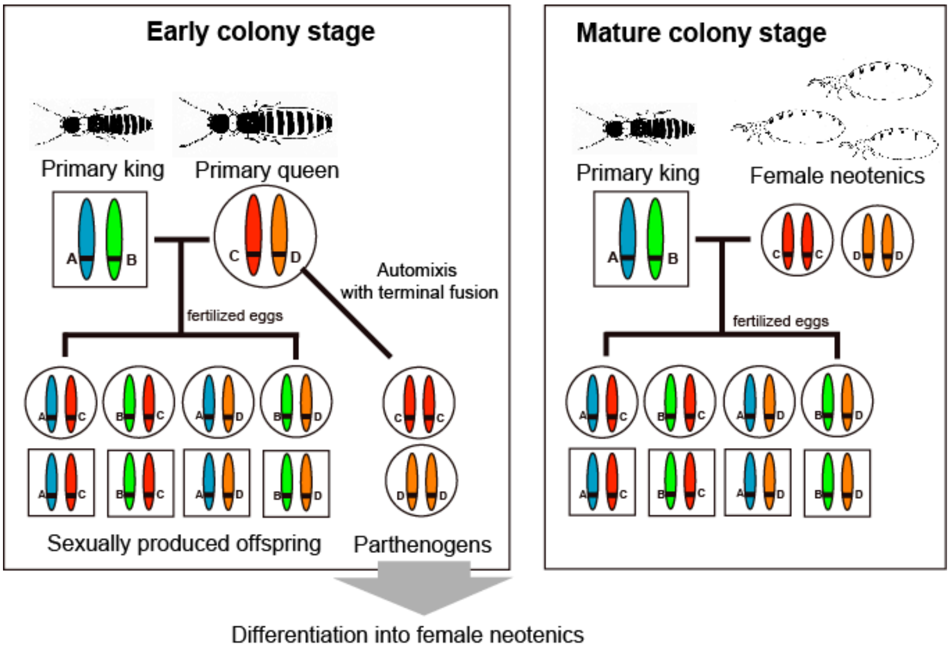

Fig. 5. Scheme for the breeding system with asexual queen succession in termites. This breeding system enables the primary queen to maintain her full genetic contribution to the next generation, while avoiding any loss in genetic diversity from inbreeding.

Purging: another benefit of AQS

A faster rate of accumulation of deleterious mutations is a major cost of asexual reproduction (Kondrashov 1993). In haplodiploid organisms, deleterious alleles are directly exposed to selection each generation in the haploid males, and there is no masking effect of dominance. Therefore, purging selection will cause a more rapid decrease in the frequency of deleterious alleles at haplodiploid loci than at comparable loci in diploid organisms (Goldstein 1994).

In termites, paradoxically, asexual queen succession can function to purge deleterious mutations. Parthenogenetic offspring are homozygous for a single maternal allele at most loci due to terminal fusion under a low recombination rate. Therefore, deleterious recessive genes are exposed to selection in homozygous parthenogens. Parthenogens carrying homozygous recessive deleterious alleles should not be able to survive or develop into functional secondary queens. The obligate occurrence of parthenogenesis in the normal life cycle of this species can eliminate recessive deleterious genes in every generation, much like the genetic purging that haploid males of Hymenoptera undergo, eliminating the transmission of deleterious recessive alleles to the sexual offspring.

Clues to find new AQS species

AQS is, so to speak, a 'cryptic parthenogenesis'. Termites with AQS avoid inbreeding even after queen replacement and thus maintain colony genetic structure for almost the entire life of the colony. Therefore, it is impossible to identify AQS by genotyping workers. Analyses of colony breeding structure based only on worker genotyping could wrongly identify a colony with secondary queens and a primary king as a simple family, if the species has AQS.

The difficulties in finding royal chambers from termite nests have concealed AQS. A reasonable clue to assist in finding AQS is the alate sex ratio. AQS system balances the genetic contribution to the next generation from the primary king and primary queen as long as the primary king is alive. However, inbreeding is inevitable after the primary king's death, and should result in uneven genetic contribution to the offspring by the queen. Based on kin selection theory, it can be predicted that sex-asymmetric inbreeding biases sex allocation (Kobayashi et al. 2013). That prediction matches well with the empirical sex allocation of *Reticulitermes* termites where the primary king can be replaced by a son. The AQS species, *R. speratus*, *R. virginicus* and *R. lucifugus* have significantly female-biased alate sex ratios, whereas a non AQS species such as *R. flavipes* should show an equal alate sex ratio (Fig. 6; Kobayashi et al. 2013). Besides the genus *Reiculitermes*, I can also predict that an East African termite *Schedorhinotermes lamanianus* should be an AQS species because it has been reported that AQS species have significantly female-biased alate sex ratios (reviewed by Jones et al. 1988). Although finding royal chambers in the field is difficult, biases in alate sex ratio can be a good clue to identify AQS.

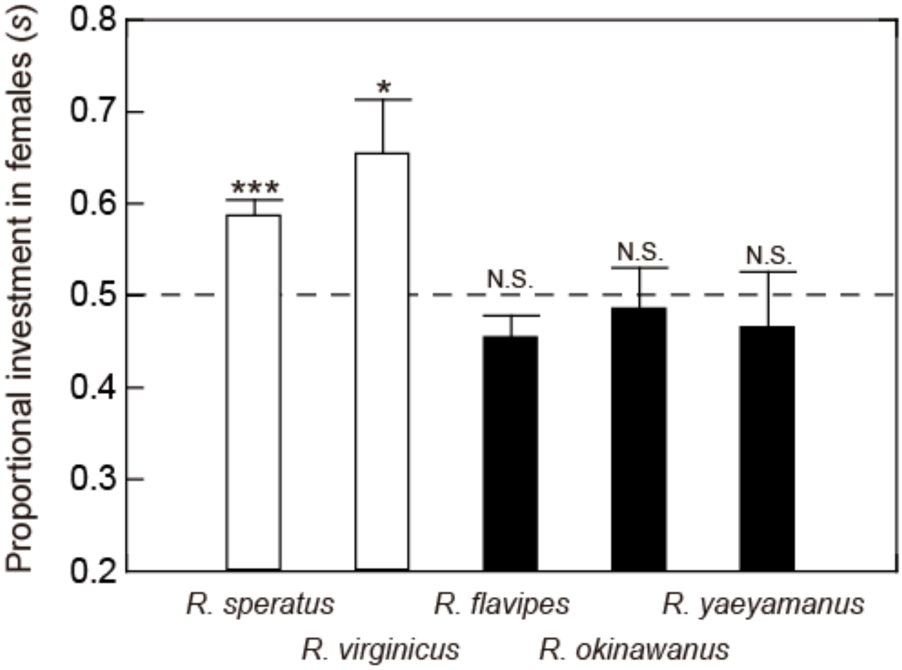

Fig. 6. Comparison of the observed proportional investment in female alates between species with and without AQS. The species with AQS are *Reticulitermes speratus* and *R. virginicus* and those

without AQS are *R. flavipes*, *R. okinawanus* and *R. yaeyamanus*. The AQS species (open bars) showed significantly female-biased investment in comparison with non-AQS species (closed bars) (GLM, P < 0.0001). Error bars indicate standard errors. The statistics on the bars indicate the significance level of the deviation from equal allocation (two-tailed *t*-test: ***P < 0.001; *P < 0.05).

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