

REVIEW

Evolution of the asexual queen succession system and its underlying mechanisms in termites

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ABSTRACT

One major advantage of sexual reproduction over asexual reproduction is its promotion of genetic variation, although it reduces the genetic contribution to offspring. Queens of social insects double their contribution to the gene pool, while overuse of asexual reproduction may reduce the ability of the colony to adapt to environmental stress because of the loss of genetic diversity. Recent studies have revealed that queens of some termite species can solve this tradeoff by using parthenogenesis to produce the next generation of queens and sexual reproduction to produce other colony members. This reproductive system, known as asexual queen succession (AQS), has been identified in the subterranean termites *Reticulitermes speratus*, *Reticulitermes virginicus* and *Reticulitermes lucifugus* and in the Neotropical higher termites *Embitermes neotenicus* and *Cavitermes tuberosus*. The studies presented here have uncovered the unusual modes of reproduction in termites and have aimed to identify their underlying mechanisms. The study of AQS, the mixed use of sexual and asexual reproduction, is of fundamental importance as it may provide a key to solve the evolutionary paradox of sex.

KEY WORDS: Thelytoky, Caste differentiation, Genetic diversity, AQS, Queen pheromone, Sex ratio, Social insect, Kin selection, Parthenogenesis, Genetic conflict

Introduction

The evolution and maintenance of sexual reproduction presents a paradox to evolutionary biology because asexual populations have a twofold fitness advantage over their sexual counterparts (Williams, 1975; Maynard Smith, 1978). In order for sex to be evolutionarily advantageous, it must confer a significant increase in the fitness of offspring. One of the most widely accepted explanations for the advantage of sex lies in the generation of genetic variation (Bell, 1982; Barton and Charlesworth, 1998; Hamilton et al., 1990). In social insects, both individual-level and colony-level genetic diversity are important for colony growth, survival and reproduction. One of the best solutions to this dilemma over the costs and benefits of sexual and asexual reproduction is to use both modes of reproduction so as to experience the advantages of both.

Unusual modes of reproduction, in which queens use sex for somatic growth but use parthenogenesis for germline production, have been uncovered both in the ants *Cataglyphis cursor* (Pearcy et al., 2004), *Wasmannia auropunctata* (Fournier et al., 2005), *Vollenhovia emeryi* (Ohkawara et al., 2006), *Paratrechina longicornis* (Pearcy et al., 2011), *Cataglyphis hispanica* (Leniaud et al., 2012), *Cataglyphis velox*, *Cataglyphis mauritanica* (Eyer

et al., 2013) and *Cardiocondyla kagutsuchi* (Okita and Tsuchida, 2016), and in the termites *Reticulitermes speratus* (Matsuura et al., 2009), *Reticulitermes virginicus* (Vargo et al., 2012), *Reticulitermes lucifugus* (Luchetti et al., 2013), *Embitermes neotenicus* (Fougeyrollas et al., 2015) and *Cavitermes tuberosus* (Fournier et al., 2016).

The capacity for parthenogenesis in termites (Isoptera) was first reported by Light (1944). However, the adaptive function of parthenogenesis in termite life history had not been examined in detail until recently. This is likely because parthenogenetic reproduction has been regarded as an unusual case with little adaptive significance in nature. Even after the finding of colony foundation of female–female pairs by parthenogenesis, researchers still believed that the function of parthenogenesis was no more than ‘the best of a bad job’ as females used parthenogenesis only when they failed to mate with males (Matsuura and Nishida, 2001; Matsuura et al., 2002). However, our understanding of the importance of parthenogenesis in termites dramatically changed after the finding of asexual queen succession (AQS) in 2009 (Matsuura et al., 2009).

Termite parthenogenesis

Studies of termite chromosomes showed that higher termites (Termitidae) have a fixed number of chromosomes ($2n=42$), while the diploid number of chromosomes in lower termites varies from 28 to 56 (Bergamaschi et al., 2007). *Reticulitermes* termites have $2n=42$ chromosomes. In *R. speratus*, parthenogenesis produces diploid offspring (Matsuura et al., 2004). Termite parthenogenesis is thelytokous, producing only female offspring because of the XY sex determination system. In termites, males are commonly heterogametic (Roisin, 2001) and interchange multiples are observed in male meiosis, generating a multiple-X, multiple-Y system (Syren and Luykx, 1977; Matsuura, 2002).

The genotypes of parthenogenetic offspring depend on the mode of parthenogenesis (Templeton, 1982). Until recently, it was believed that phylogenetic constraints explain the difference in the cytological mechanism of ploidy restoration between termites and social Hymenoptera because all known termite thelytoky involved automixis with terminal fusion (Fig. 1A) and thelytoky in the social Hymenoptera involved automixis with central fusion (Fig. 1B) (reviewed by Matsuura, 2011; Wenseleers and Van Oystaeyen, 2011). Therefore, it was astonishing that the mode of thelytoky in a higher termite, *E. neotenicus*, was found to be most like automixis with central fusion (Fougeyrollas et al., 2015). This is not the case for thelytoky of another higher termite, *C. tuberosus*, where parthenogens are completely homozygous, suggesting gamete duplication (Fournier et al., 2016).

AQS

AQS is a mode of reproduction whereby workers, soldiers and alates (dispersing reproductives) are produced sexually while neotenic queens (non-dispersing queens) arise through

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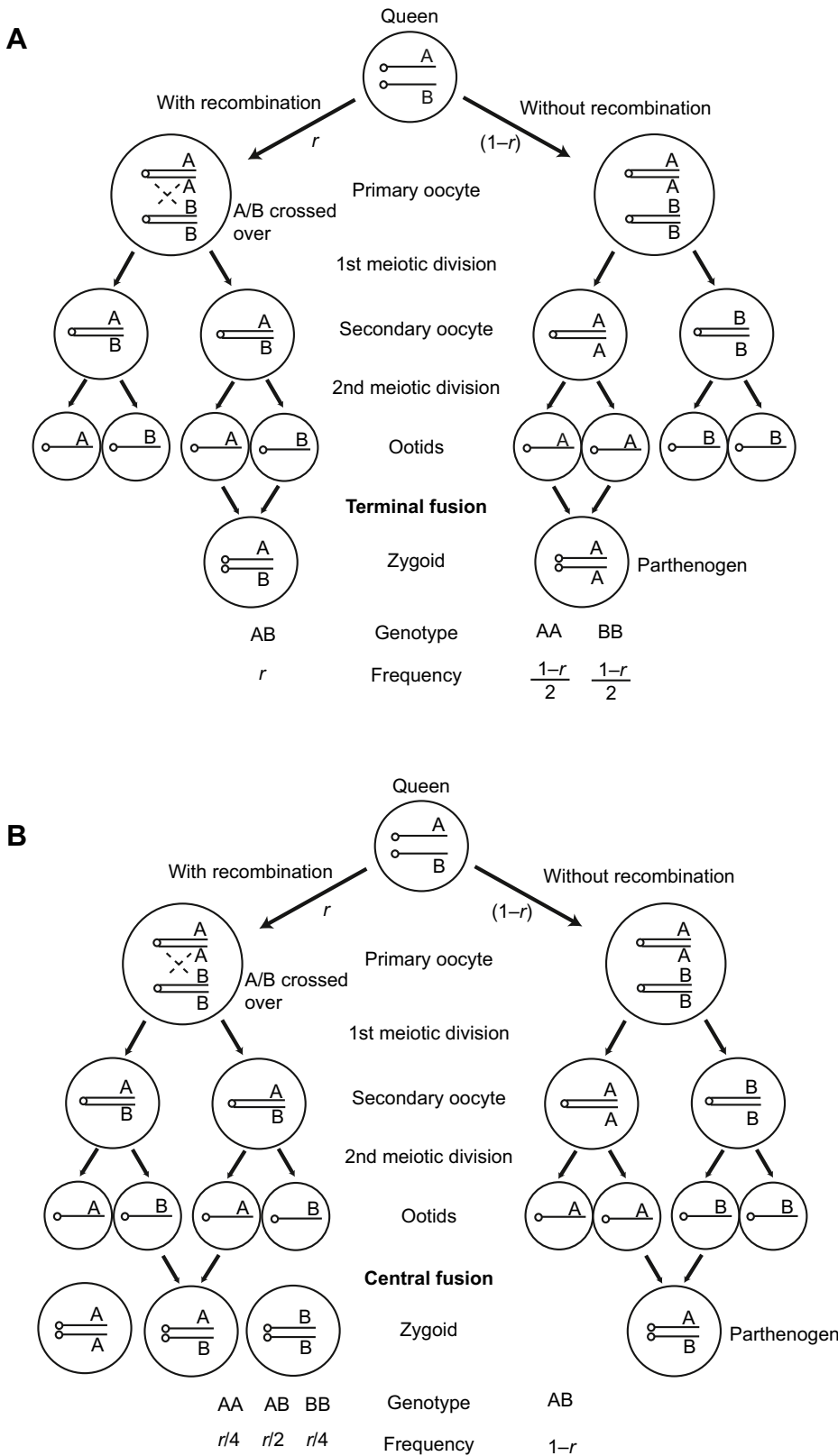


Fig. 1. Schematic diagram of cytological mechanisms of termite thelytoky. (A) Ploidy restoration by automixis with terminal fusion, which occurs in asexual queen succession (AQS) in *Reticulitermes* species and a higher termite, *Cavitermes tuberosus*. This type of parthenogenesis results in a rapid reduction of heterozygosity. (B) Ploidy restoration by automixis with central fusion, which occurs in an AQS higher termite, *Embiratermes neotenicus*, and some social Hymenoptera (e.g. *Cataglyphis cursor*, *Cerapachys biroi*, *Wasmannia auropunctata*, *Vollenhovia emeryi*, *Pristomyrmex punctatus* and *Apis mellifera capensis*). Transition to homozygosity is very low in central fusion but it depends on recombination rate (r).

thelytokous parthenogenesis and eventually replace the old queens (Fig. 2). The neotenic queens are then replaced by subsequent cohorts of asexually produced neotenic queens. By using this system, founding queens are able to boost reproduction without reducing the transmission rate of their genes to future generations; that is, the queens are genetically immortal as long as the colonies

survive. This system also seems advantageous to the colony to promote its growth rate without losing genetic diversity in workers.

AQS was first identified in a lower termite species, *R. speratus* (Matsuura et al., 2009), and later confirmed in two other species of the same genus, *R. virginicus* (Vargo et al., 2012) and *R. lucifugus*

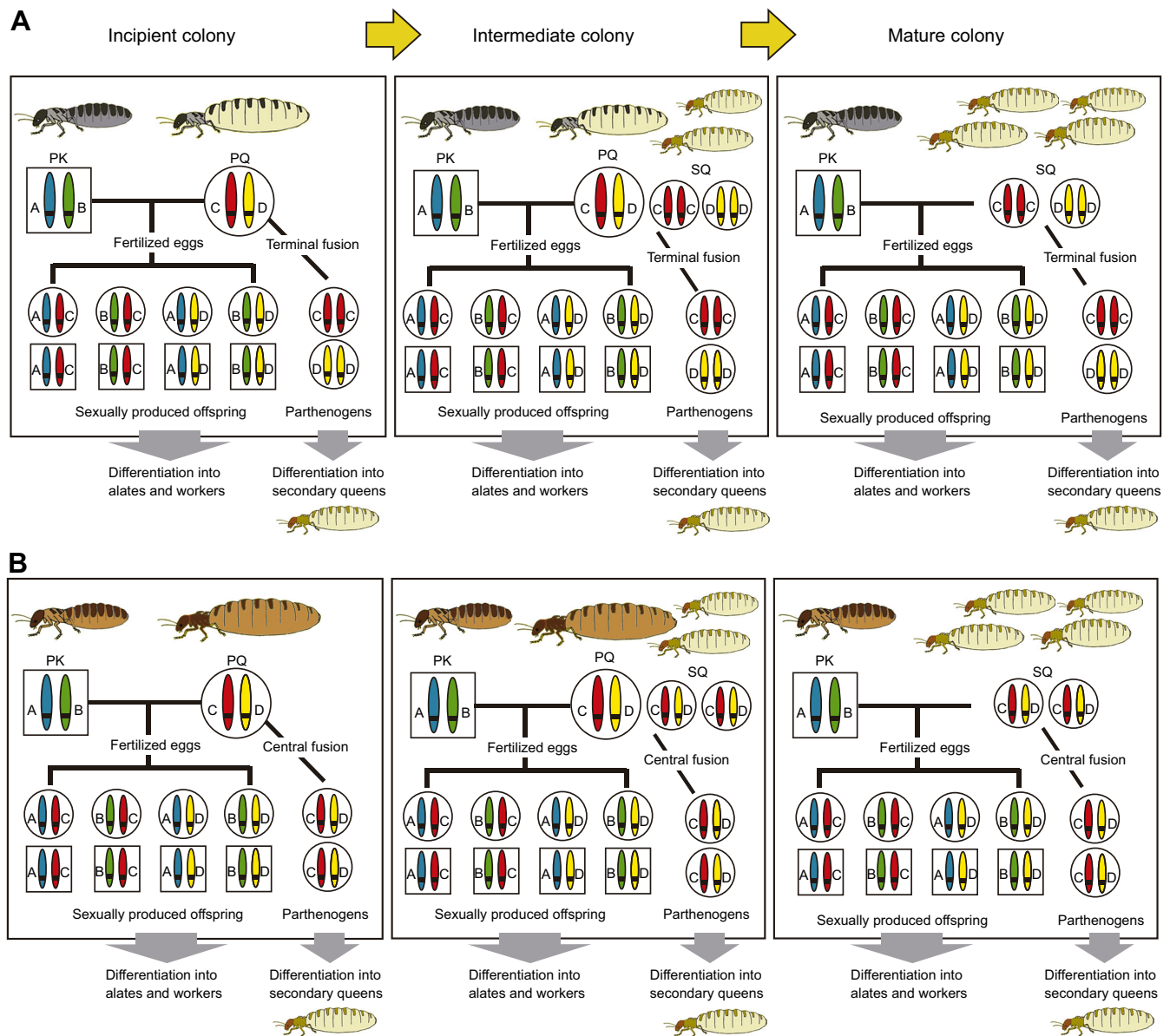


Fig. 2. AQS models. (A) AQS in *Reticulitermes* termites and the higher termite *C. tuberosus*. Secondary queens produced asexually (automixis with terminal fusion in *R. speratus* and automixis with gamete duplication in *C. tuberosus*) by the primary queen differentiate within the colony and supplement egg production, eventually replacing the primary queen. Modified from Matsuura et al. (2009). (B) AQS in the higher termite *E. neotenicus*. Secondary queens produced asexually (automixis with central fusion) by the primary queen differentiate within the colony and eventually replace the primary queen. Illustration based on the data of Fougeyrollas et al. (2015). These breeding systems enable the primary queens to maintain their full genetic contribution to the next generation, while avoiding any loss in genetic diversity from inbreeding. PK, primary king; PQ, primary queen; SQ, secondary queen.

(Luchetti et al., 2013), and was recently identified in two species of higher termite (Termitidae), *E. neotenicus* (Fougeyrollas et al., 2015) and *C. tuberosus* (Fournier et al., 2016) (Table 1).

AQS in *Reticulitermes* termites

Earlier studies of the breeding system of *Reticulitermes* termites relied primarily on estimation by genotyping workers or culturing laboratory colonies rather than making censuses of field colonies. This is likely because of the difficulties in collecting reproductives from the nests of *Reticulitermes* termites, which have cryptic nesting habits with transient, hidden royal chambers underground or deep inside wood. This practice has concealed their true reproductive systems. *Reticulitermes speratus* is the most common termite in Japan. By collecting more than 1000 nests in the field

and sampling royal chambers, where reproductives and young brood were protected, reproductives were recovered from 54 natural colonies. In nearly all colonies, primary kings were continuously present but primary queens had been replaced by an average of 57.6 (ranging from 5 to 676) secondary queens (updated after Matsuura et al., 2009; see Yashiro and Matsuura, 2014, for details). Among the 54 colonies, only two colonies had primary queens, indicating that queen replacement is the rule at a certain point of colony development. In contrast, only three colonies had secondary kings; that is, replacement of the primary king is rare, suggesting much greater longevity of primary kings than primary queens. The neotenic queens differentiated almost exclusively from nymphs (99.97%, $n=3110$), while ergatoid queens (i.e. a neotenic reproductive differentiated from a

Table 1. Asexual queen succession in termites across the world

Family	Species	Distribution	Feeding	Timing of AQS origin ^a	Mode of ploidy restoration	Type of neotenic queens	Alate sex ratio	References
Rhinotermitidae	<i>Reticulitermes speratus</i>	EA	Xylophagous	7.8–6.9 Ma	TF	Nymphoid	F>M ^b	Matsuura et al. (2009)
	<i>Reticulitermes virginicus</i>	NA	Xylophagous	<14.1 Ma	TF	Nymphoid	F>M ^b	Vargo et al. (2012)
	<i>Reticulitermes lucifugus</i>	WE	Xylophagous	10.2–4.7 Ma	TF	Nymphoid	F>M ^c	Luchetti et al. (2013)
Termitidae	<i>Embiratermes neotenicus</i>	SA	Humivorous	Unknown	CF	Nymphoid	Unknown	Fougeyrollas et al. (2015)
	<i>Cavitermes tuberosus</i>	SA	Soil-feeding	Unknown	GD	Nymphoid	Unknown	Fournier et al. (2016)

EA, East Asia; NA, North America; WE, West Europe; SA, South America.

Ploidy is restored by automixis with terminal fusion (TF), central fusion (CF) or gamete duplication (GD).

^aTiming estimation is based on the global time-scaled phylogeny of *Reticulitermes* termites (Dedeine et al., 2016). ^bKin selection predicts that alate sex ratios are biased toward female (F>M) in asexual queen succession (AQS) species, which is supported by the data for *R. speratus* and *R. virginicus* (Kobayashi et al., 2013). ^cThe Italian AQS species *R. lucifugus* shows a female-biased alate sex ratio (85%, $n=194$) and the non-AQS species *R. urbis* shows an equal sex ratio (50%, $n=298$) (Luchetti et al., 2013).

worker) are of extremely rare occurrence in field colonies (Fig. 3A).

Genetic analysis using microsatellites showed the surprising result that secondary queens were parthenogenetic daughters, and workers and alates were sexual offspring of the primary queens (Fig. 2A). The relatedness (r) of the primary queens to workers ($r=0.49$) and to alates ($r=0.58$) is not significantly different from 0.5, indicating that the primary queens retain their genetic contribution to descendants even after replacement. This system enables the colonies to undergo queen succession without inbreeding (Fig. 2A). Queen-replaced colonies showed no significant reduction of heterozygosity in workers and alates.

After the first identification of AQS in *R. speratus*, this process was found in the North American subterranean termite *R. virginicus* (Vargo et al., 2012) and in the Italian subterranean termite *R. lucifugus* (Luchetti et al., 2013). The global time-scaled phylogeny of *Reticulitermes* termites showed that the three *Reticulitermes* species exhibiting AQS belong to three distinct phylogenetic lineages, i.e. from East Asia, North America and West Europe, suggesting that AQS was originally absent in the ancestral lineage of *Reticulitermes* but evolved at least three times independently during the diversification of the genus (Dedeine et al., 2016).

AQS in higher termites

Embiratermes neotenicus

The recent finding of AQS species in higher termites (Termitidae) was a big step forward in understanding the evolution of termite reproductive systems. In the Neotropical higher termite *E. neotenicus* (Termitidae, Syntermitinae), multiple neotenic queens (35–170 individuals, $n=24$ colonies) frequently accompany a single primary king (Fougeyrollas et al., 2015). This is a key characteristic of AQS species in *Reticulitermes* termites and suggests that *E. neotenicus* is a prime candidate for AQS, although *Embiratermes* and *Reticulitermes* are phylogenetically distant.

Microsatellite analysis of *E. neotenicus* colonies including reproductives demonstrated that neotenic queens carry only maternal alleles, never paternal alleles, confirming asexual queen succession (Fougeyrollas et al., 2015). The relatedness among neotenic queens ($r=0.982$) and between neotenic queens and their inferred mothers ($r=0.991$) was significantly different from 0.5 expected under the assumption of sexual origin of the neotenic queens. By contrast, workers, soldiers and male nymphs carry both

maternal and paternal alleles, indicating that they were sexually produced. The relatedness among workers and soldiers ($r=0.575$) did not significantly differ from 0.5. Female nymphs contained individuals produced sexually as well as those produced parthenogenetically. It remains to be investigated whether sexually produced nymphs are destined to become alates and asexually produced nymphs are destined to develop into neotenic queens, as known in AQS species of *Reticulitermes*.

While *E. neotenicus* shows striking similarities of breeding structures with AQS *Reticulitermes* termites, the astonishing difference is that the cytological mechanism of parthenogenesis in *E. neotenicus* is automixis with central fusion (Fig. 2B). In automixis with central fusion, the two central polar nuclei fuse and give rise to the parthenogens (Fig. 1B). If the primary queen is heterozygous, all parthenogens will be heterozygous, assuming that there is no recombination. If there is recombination at a locus, the reduction of heterozygosity in parthenogens depends on the recombination rate (Fig. 1B). In cases where heterozygosity is advantageous, selection favors reduced recombination rates, as known for *Drosophila mangabeirai* (Suomalainen et al., 1987) and the Cape honeybee (Baudry et al., 2004). In the higher termite *E. neotenicus*, the rates of transition to homozygosity (2–8%) differ among loci, probably depending on the position on the chromosome (Fougeyrollas et al., 2015).

Cavitermes tuberosus

In the soil-feeding higher termite *C. tuberosus* (Termitidae, Termitinae), colonies frequently contain numerous neotenic females (2–667 individuals, $n=28$ colonies) usually with a single primary king (Fournier et al., 2016), suggesting a high probability of AQS. This species is ecologically close to the AQS higher termite *E. neotenicus*, but belongs to a different subfamily of the Termitidae.

As for other AQS species, genetic analyses demonstrated that most of the female neotenic (81.95%, $n=277$) were produced asexually by thelytokous parthenogenesis. The parthenogenetically produced neotenic were consistently homozygous and half-clones of their primary queen mother but were unrelated to the primary king. By contrast, all workers ($n=595$), all soldiers ($n=149$) and almost all female alates or alate-destined fifth instar nymphs (93.5%, $n=281$) were produced by sexual reproduction.

Although the main use of parthenogenesis is to produce neotenic queens, it is also important to note that 18 of 281 female alates and

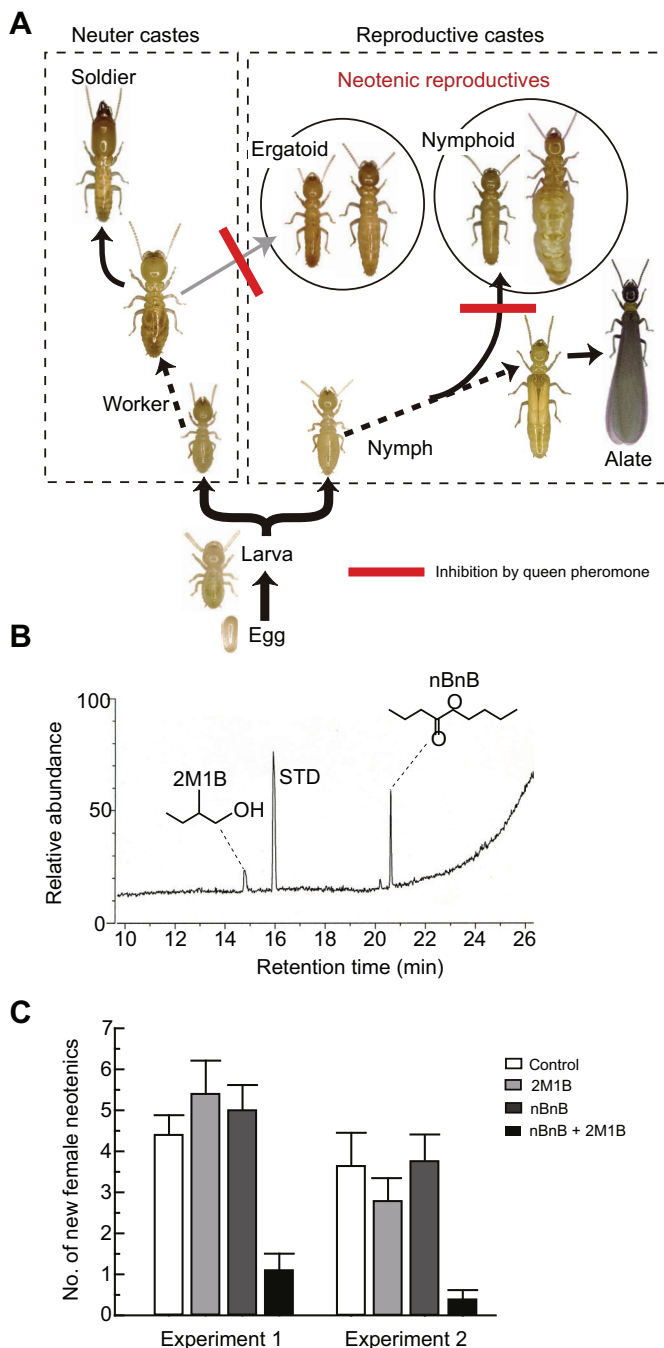


Fig. 3. Identification of the termite queen pheromone. (A) Differentiation pathways of primary and secondary (neotenic) reproductives in *Reticulitermes* termites. Primary reproductives (primary king and primary queen) derive from alates and found new colonies. Neotenic queens differentiate within the colony and supplement egg production, eventually replacing the primary queen. The presence of physogastric queens sufficient for egg production suppresses the differentiation of neotenic queens. Nymphoid: neotenic reproductives differentiated from nymphs. Ergatoid: neotenic reproductives differentiated from workers. Although workers retain totipotency to develop into neotenic, secondary reproductives almost exclusively differentiate from nymphs in field colonies. (B) Headspace GC-MS analysis of the volatiles of female neotenic. Physogastric queens emit the volatiles *n*-butyl-*n*-butyrate (nBnB) and 2-methyl-1-butanol (2M1B), while workers and nymphs do not have these compounds. STD: toluene as an internal standard. (C) Suppression of the differentiation of new neotenic females by a blend of synthetic nBnB and 2M1B. A 2:1 mixture of nBnB and 2M1B suppressed the differentiation of new female neotenic but each compound on its own did not. Modified from Matsuura et al. (2010).

alate-destined nymphs were parthenogenetically produced, and four of 65 primary queens were completely homozygous at all 17 loci, indicating parthenogenetic origin. This clearly demonstrates that the fully homozygous females produced by parthenogenesis have the ability to become successful founders.

The parthenogenesis of *C. tuberosus* differs from that in the higher termite *E. neotenicus* in terms of the ploidy restoration mechanism. Because parthenogens of *C. tuberosus* were completely homozygous at all 17 loci (even at loci heterozygous in their mother), the cytological mechanism seems most likely to be gamete duplication (Fig. 2B). However, the actual mechanism of ploidy restoration in *C. tuberosus* remains to be identified by cytological observation because complete homozygosity of the offspring can also be explained by terminal fusion without recombination. Regardless, as the mechanism of parthenogenesis is different in the two AQS higher termites, it is likely that AQS evolved independently in *Embiratermes* and *Cavitermes* (Fournier et al., 2016). To date, five independent origins of AQS have been suggested, including three independent origins in *Reticulitermes* termites.

Purging: another benefit of AQS

One of the major costs of asexual reproduction is a faster accumulation of deleterious mutations (Muller, 1964; Felsenstein, 1974). In haplodiploid organisms, deleterious alleles in the haploid males are directly exposed to selection without the masking effect of dominance and are thus rapidly purged from a population (Goldstein, 1994).

The termite AQS may also function as a genetic purging system (Matsuura, 2011). In the AQS *Reticulitermes* species, parthenogens are homozygous for a single maternal allele at almost all loci as a result of terminal fusion. Parthenogens carrying homozygous recessive deleterious alleles should not be able to survive or develop into functional secondary queens. Thus, deleterious recessive genes are exposed to selection in homozygous parthenogens as in the haploid males of Hymenoptera. This purge process is especially efficient in the AQS higher termite *C. tuberosus*, where parthenogens are completely homozygous, likely because of gamete duplication (Fournier et al., 2016). In *C. tuberosus*, primary queens of parthenogenetic origin were found in mature colonies, which demonstrates the ability of parthenogens to become successful founders (Fournier et al., 2016). The high viability of full homozygotes might be attributed to the strong purging system in gamete duplication-type AQS. In the AQS higher termite *E. neotenicus*, the rate of transition to homozygosity is very low because of central fusion (Fougeyrollas et al., 2015). Therefore, the purging of deleterious alleles through asexual queen replacement would be slower in central fusion- and gamete duplication-type AQS.

Mechanism of switching from sexual to asexual reproduction

A theoretical model of the evolution of parthenogenesis predicted that females would probably be unable to switch from sexual to asexual reproduction unless isolated from males, if male counter-adaptations interfere with parthenogenesis (Kawatsu, 2013). How can queens of AQS termites control egg fertilization? In haplodiploid social Hymenoptera, queens are able to control fertilization (Ratnieks and Keller, 1998; Aron et al., 2011), producing unfertilized male eggs and fertilized female eggs (Crozier and Pamilo, 1996). However, females of diploid insects cannot control fertilization because sperm release is generally

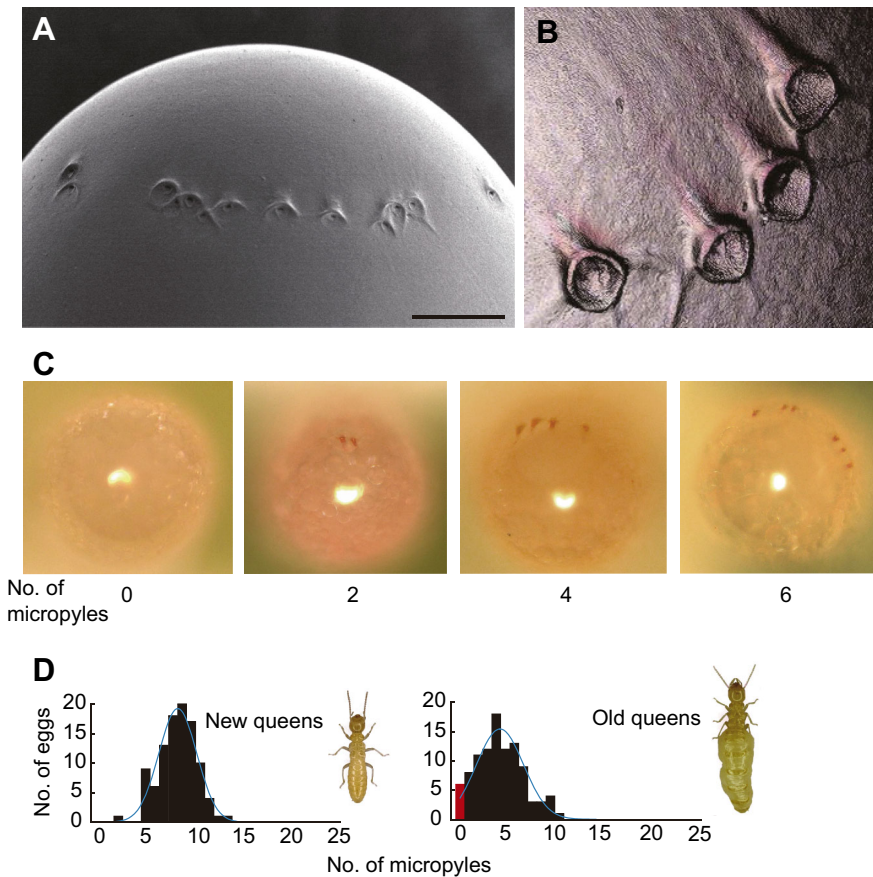


Fig. 4. Micropyle and parthenogenesis. (A) Scanning electron microscope image of the posterior end of an egg with micropyles (ventral view). Scale bar, 50 μm . (B) Confocal scanning laser microscope image of micropyles (close-up view). (C) Posterior views (dorsal up) of representative eggs with different numbers of micropyles. Micropyles were visualized by eosin Y staining. (D) Frequency distribution of the number of micropyles on eggs laid by young (left) and old queens (right). Micropyle-less eggs are indicated by red bars. Blue lines show the fitted normal distributions. Modified from Yashiro and Matsuura (2014).

activated through a neural loop whenever an egg passes the genital chamber.

Termite eggs have tiny openings, called micropyles, on their surfaces for sperm entry (Fig. 4A). A recent study demonstrated that termite queens produce parthenogenetic offspring by closing the micropyles on their eggs (Yashiro and Matsuura, 2014). This mechanism enables queens to produce parthenogenetic eggs even in the presence of kings. In *Reticulitermes* termites, the funnel-shaped micropyles form an arc on the posterior end of the egg (Fig. 4A,B). The number of micropyles per egg ranged from 0 to 33, with an average number of 9.48 ($n=6000$). Genetic analysis showed that embryos in the micropyle-less eggs are parthenogenetic and the other eggs are fertilized. Comparison of the number of micropyles on eggs among queens of different age groups demonstrated age-dependent production of micropyle-less eggs (Fig. 4D).

The variation in the number of micropyles can be attributed to the differing number of micropylar channel-forming cells (MCFCs) because a single cytoplasmic projection from each MCFC forms each micropyle in insects (Yamauchi and Yoshitake, 1984; Kubrakiewicz et al., 2005). The most parsimonious explanation of the evolutionary origin of AQS in termites may be the age-dependent reduction in the number of MCFCs, which automatically results in the production of parthenogenetic eggs by old queens.

Termite queen pheromone and AQS

Reproductive division of labor is a hallmark of eusocial insects, where reproduction is primarily monopolized by queens (and kings in termites), while workers specialize on the other tasks required for colony growth and survival. The pheromones produced by queens profoundly influence the behavior, development and physiology of

colony members. In the subterranean termites, secondary queens (neotenic females), are produced from within the colony to supplement and eventually replace primary queens. These neotenic females can differentiate either from nymphs to become 'nymphoid' queens with wing pads or from workers to become 'ergatoid' queens without wing pads (Fig. 3A). Physogastric queens of *R. speratus* produce the queen pheromone, which is the prime factor inhibiting the differentiation of new reproductive individuals. The active volatile compounds of the queen pheromone were identified as *n*-butyl-*n*-butyrate (nBnB) and 2-methyl-1-butanol (2M1B) (Matsuura et al., 2010) (Fig. 3B). An artificial pheromone blend consisting of these two compounds had a strong inhibitory effect similar to that of live neotenic females, although each single compound did not (Fig. 3C).

Although the queen pheromone emitted by reigning queens suppresses the differentiation of new neotenic queens from nymphs, its inhibitory effect is obviously different between sexual nymphs and parthenogenetic nymphs. The difference of queen pheromone perceptibility may explain why parthenogenetic nymphs exclusively differentiate into secondary queens, while sexual nymphs are prevented from becoming neotenic queens and thus develop into alates in AQS termites. It should be noted that neotenic queen differentiation is a seasonal event in field colonies. In *R. speratus*, simultaneous differentiation of neotenic queens and alates can be observed in May just before swarming (Yashiro and Matsuura, 2014). In some cases, more than 1000 new neotenic queens (all parthenogens) differentiate within a colony all at once even in the presence of reigning queens, despite the fact that most of them are destined to be cannibalized by workers (so-called 'neotenic explosion'). These phenomena suggest that

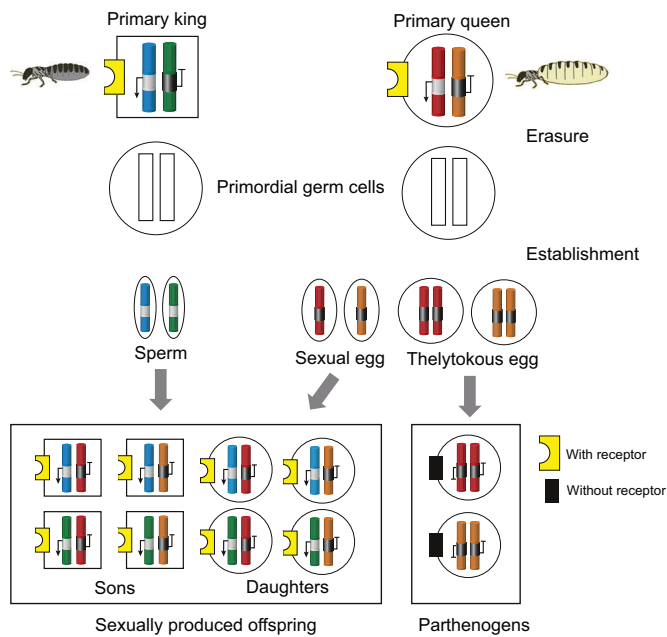


Fig. 5. Genomic imprinting model for the priority of parthenogens to become secondary queens. Maternal imprinting silences the queen pheromone receptor gene. Parthenogens carrying only maternal genes do not have active receptors for the queen pheromone and thus differentiate into neotenic queens in the presence of reigning queens. This model can explain the developmental priority of parthenogens in central fusion-type AQS as well as in terminal fusion-type and gamete duplication-type AQS, while a strictly genetic determination model cannot account for parthenogens in central fusion-type AQS.

parthenogenetic nymphs ignore the queen pheromone or they are at least less susceptible to the inhibitory pheromone in comparison with sexual nymphs.

Genomic imprinting model for AQS

Given that the difference in queen pheromone perceptibility generates the developmental bias between sexual and asexual nymphs, the next question is why parthenogens are biased to develop into neotenic whereas sexual nymphs develop into alates. As described above, a heterozygous primary queen (CD) produces parthenogenetic offspring of near-total homozygosity (CC or DD) and sexual reproduction with a primary king (AB) produces heterozygous offspring (AC, AD, BC or BD) in the AQS species of the genus *Reticulitermes*. Therefore, a multilocus genetic system giving a homozygous advantage of development into secondary queens was hypothesized as a possible explanation for the parthenogens' developmental priority (see Matsuura, 2011, for details). However, the discovery of the AQS higher termite *E. neotenicus* (Fougeyrollas et al., 2015), in which a heterozygous queen (CD) produces heterozygous parthenogens (CD) through automixis with central fusion, clearly indicated that the genetic system of homozygous advantage cannot generally explain the mechanism of AQS.

Instead of the genetic determination model, a genomic imprinting model can reasonably explain the parthenogens' developmental priority in general. The parthenogens inherit only maternal genes, regardless of cytological mechanisms (terminal fusion, central fusion or gamete duplication), whereas sexual offspring have both maternal and paternal genes. Genomic imprinting refers to the differential expression of genes inherited from the mother and father (reviewed in Burt and Trivers, 2006; Kronauer, 2008). Recent studies of genomic imprinting showed that genes can be differentially marked, or

imprinted, in parents and that DNA methylation is a key molecular mechanism of imprinting. During the development of germ cells into sperm or eggs, methylation marks the genes differently in eggs and sperm, leading to differential gene expression. These epigenetic marks are maintained as chromosomes duplicate and segregate in the offspring. Imprints are erased in the germ cells of the offspring and then re-established.

These modifications are inherited by somatic cells of offspring and result in parent-of-origin-specific silencing or upregulation of gene expression. Any parent-specific gene expression of the genes involved in secondary queen differentiation [e.g. queen pheromone receptor, juvenile hormone, hexamerin, fat body endoreduplication (Nozaki and Matsuura, 2016) or vitellogenin synthesis] can explain the parthenogens' developmental priority to be secondary queens. For simplicity, let us assume maternal imprinting (silencing) of the queen pheromone receptor gene (Fig. 5). The parthenogenetically produced offspring have a pair of maternal alleles (both silenced) at the locus of the queen pheromone receptor gene. Hence, parthenogens do not have active queen pheromone receptors, so cannot be suppressed by the presence of reigning queens. In contrast, sexually produced daughters have a set of maternal and paternal alleles (unmethylated) and thus have active receptors of the queen pheromone (Fig. 5).

A high-throughput mRNA sequencing (RNA-seq) demonstrated that several chemoreceptor genes are differentially expressed among castes and between sexes in *R. speratus* (Fig. 6; Mitaka et al., 2016). Future studies on parent-specific expression of chemoreceptor genes involved in the differentiation of neotenic queens are likely to be of fundamental importance in understanding the basic mechanisms for AQS.

Kin selection and sex ratio bias in AQS species

The AQS system in termites provides an ideal opportunity to test kin selection in diploid eusocial colonies (Kobayashi et al., 2013). Inclusive fitness theory predicts that an individual should bias resource investment towards certain close relatives so as to maximize the transmission rate of its own genes to the next generation (Hamilton, 1964). Sex-biased investment in haplodiploid eusocial insects is the most successful demonstration of this prediction, where workers are related more to their sisters than to their brothers (Trivers and Hare, 1976; Boomsma and Grafen, 1991; Sundström, 1994; Chapuisat and Keller, 1999). However, such an approach is impossible in diploid social insects, including termites, because they lack relatedness asymmetry.

In AQS termites, father–daughter inbreeding never occurs because founding queens are genetically immortal until the colony dies. In contrast, mother–son inbreeding can occur when the founder king dies and a secondary king – the son of the founder queen and king – differentiates in the colony. Mother–son inbreeding results in sex-asymmetric genetic contributions, where F2 individuals inherit 0.75 maternal genes and 0.25 paternal genes (Fig. 7). This sex-asymmetric inbreeding in AQS species can generate asymmetric reproductive values (gene transmission rates) between male and female alates. The mathematical model predicts interspecific variation in sex ratio patterns in diploid termites with and without AQS (Kobayashi et al., 2013) just as the Trivers and Hare (1976) hypothesis did for the eusocial Hymenoptera.

Sex allocation is indeed female biased in the AQS species *R. speratus*, *R. virginicus* (Kobayashi et al., 2013) and *R. lucifugus* (Luchetti et al., 2013), whereas non-AQS species show no bias (Kobayashi et al., 2013). The proportional investment in females was greater in *R. virginicus* (0.655) than in *R. speratus* (0.587). This

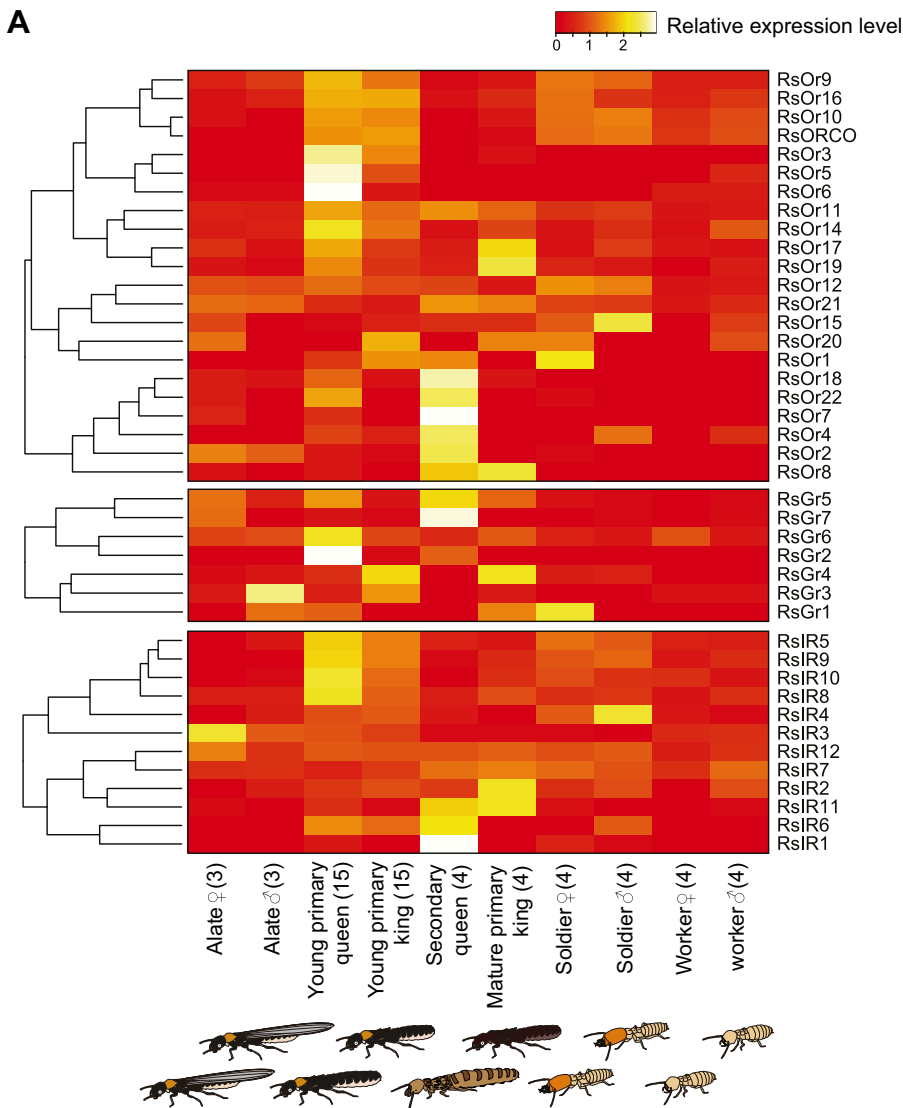


Fig. 6. Differential expression of chemoreceptor genes among castes in *R. speratus*. (A) The heat maps exhibit the differential expression of 22 odorant receptor (Or), 7 gustatory receptor (Gr) and 12 ionotropic receptor (IR) genes among termite castes. Relative expression level indicates the mean normalized counts per million (cpm), ranging from red (scaled expression of 0) to white (scaled expression of 2.5). The tree on the left corresponds to hierarchical clustering of cluster-averaged expression. Numbers in parentheses after caste types refer to the number of biological replications. (B) Caste-specific expression patterns of chemoreceptors RsOr10 and RsOr3. Comparison of the expression levels of six receptor transcripts among castes and between the sexes of each caste. Error bars denote s.e.m. Results of statistical analyses for each gene expression are shown for each graph: n.s., not significant, *false discovery rate (FDR)<0.05, ***FDR<0.001. Red arrows indicate parthenogenetically produced neotenic queens. Modified from Mitaka et al. (2016).



difference may be explained by the higher proportion of secondary kings in *R. virginicus* (60%) than in *R. speratus* (7%). Future comparative studies of alate sex ratios in higher termites between species with and without AQS may provide more robust support for kin selection in termites.

Conclusion

Starting with the identification of the unusual reproductive system of AQS in a single termite species, an interdisciplinary body of

research has been developed spanning questions on sexual conflict, genetic conflict, kin selection, pheromone communication and extended longevity. The convergent evolution of AQS systems in lower and higher termites clearly shows that these reproductive systems illustrate important evolutionary forces acting on termites. AQS is a so-called ‘cryptic parthenogenesis’ because it cannot be identified without collecting and genotyping kings and queens, which are often in the hidden royal chambers. The possibility of AQS has been checked in a very small proportion of the

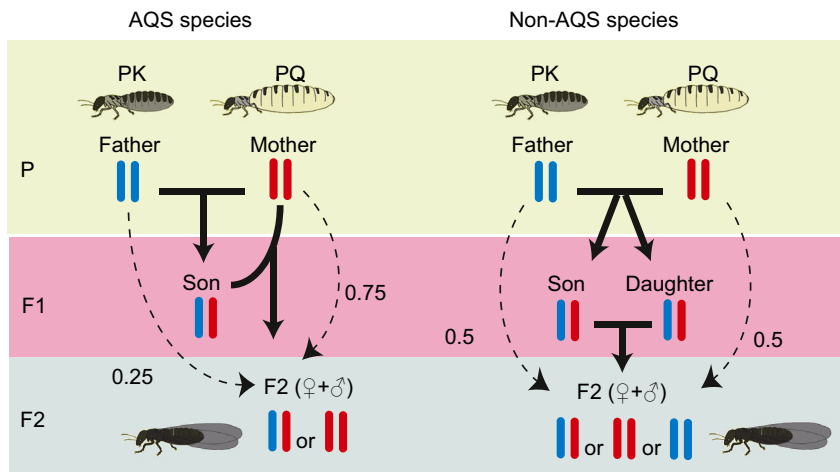


Fig. 7. AQS and sex-asymmetric inbreeding. In non-AQS species, mother–son, father–daughter and full-sib inbreeding can occur because both primary kings (PK) and primary queens (PQ) die at a certain point in colony life. In contrast, in AQS species, only mother–son inbreeding can occur, not father–daughter inbreeding, because primary queens are replaced by their parthenogens. Modified from Kobayashi et al. (2013).

approximately 3000 termite species (Engel et al., 2009). Undoubtedly, future world-wide studies will identify AQS in many more species. For further studies of termite breeding systems, it should be noted that AQS species share the following characteristics, which could be reasonable clues to finding new AQS species. (i) Mature colonies of AQS species usually have a single primary king and multiple neotenic queens. (ii) AQS species have a large colony size in comparison with closely related non-AQS species. (iii) Kin selection favors female-biased alate sex ratio in AQS termites. However, it must be noted that other life-history traits also affect the sex allocation into dispersers. For example, the occasional presence of sexually produced neotenic females and the production of dispersers before asexual queen replacement may mask this effect. (iv) In AQS species, female alates that fail to find males after flight are able to found colonies by parthenogenesis. (v) AQS species show fewer recessive deleterious genes in comparison with closely related non-AQS species as a result of purging. Comparison of the insertion of transposable elements (Luchetti and Mantovani, 2009) may be helpful in predicting the presence of an effective purging system.

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Competing interests

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