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Chapter 4

Effects of sociality and extrinsic mortality on ageing

Title: Evolution of ageing along the spectrum of sociality in termites

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Abstract

The question of why organisms age is central in evolutionary biology. Social insects are promising model organisms for studying the causes of ageing as their lifespans vary enormously at both intra- and inter-specific levels. Recently, quantitative models aiming to understand ageing in social insects have been developed, but most of them focus on social Hymenoptera; models that are tailored to other social insects, such as Isoptera, are still lacking. Here, we developed the first quantitative model to investigate the effect of the degree of sociality on the evolution of ageing in termites. Using individual-based simulations, we aimed to understand (i) how patterns of ageing change depending on the degree of sociality (i.e., workers' reproductive potential); and (ii) what role caste-specific extrinsic mortality plays in shaping them. We show first that different degrees of sociality can lead to different levels of lifespan divergence between castes. The reproductive/worker lifespan ratio, an indicator of lifespan divergence, increased with increasing sociality. Second, reproductives evolved longer lifespans than workers in the low and high sociality scenarios, even in the absence of caste-specific extrinsic mortality. Surprisingly, reproductives only evolved a longer lifespan than workers when caste-specific extrinsic mortality was present in the intermediate sociality scenario. Our results suggest that the degree of sociality and caste-specific extrinsic mortality are both important factors that induce the lifespan divergence between reproductives and workers. We argue that

the varying ageing patterns across the spectrum of sociality in termites are a result of differential selection on workers' direct fitness, and depending on the social context, extrinsic mortality can modify the strength of selection on workers' direct fitness because it modifies the chance of nest inheritance.

4.1 Introduction

Ageing, the gradual deterioration in bodily functions over time, is associated with a decline in intrinsic survival probability with increasing age. It is a nearly omnipresent phenomenon that varies widely across the tree of life (Jones *et al.*, 2014a). The quest to understand why organisms age is central to evolutionary biology and various theories have been proposed to explain it. The basic principle underlying these theories is that ageing is a consequence of the negligible contribution of late-life reproduction to fitness. As organisms (even non-ageing ones) can rarely survive until very old age due to random extrinsic mortality, selection on late-life fitness is weak. Late-life selection may be insufficient to eliminate detrimental mutations, and these mutations can be passed on to the next generation and accumulated by genetic drift (the mutation accumulation theory of ageing, MA) (Medawar, 1952). Or, selection favours genes that are beneficial in early life but detrimental in late life (the antagonistic pleiotropy theory, AP) (Williams, 1957). A more mechanistic theory of ageing is the disposable soma theory, which explains ageing as a resource allocation trade-off between maintenance and reproduction (Kirkwood, 1977, 2017). Hamilton's model (Hamilton, 1966) formulates these verbal ideas mathematically and gives a clear picture of how the force of selection on age-specific vital rates declines with age in an age-structured population. He also predicts that the onset of a declining force of selection will be at the age of first reproduction. However, there has been a growing awareness that selection does not necessarily decline with age (Baudisch, 2005; Giaimo & Traulsen, 2022) and that ageing might not be inevitable (Jones & Vaupel, 2017). Indeed, the variable ageing patterns observed in nature suggest that it can be delayed.

Factors such as resource intake and social interactions between individuals in group-living animals have been shown to correlate with the rate of ageing (Flatt & Partridge, 2018). Reduced resource intake without malnutrition is known to delay ageing in a variety of organisms and this phenomenon has been intensively studied (Moatt *et al.*, 2016). However, the effect of sociality on ageing is still a muddy topic that deserves more research. Two of the most sought-after groups of model animals for studying the social effects on ageing are cooperatively breeding birds and some eusocial animals. In cooperative birds, breeders receive a load-lightening benefit from helpers and, therefore, gain an extended lifespan without a cost in reproduction (Downing *et al.*, 2021). Eusocial animals have overlapped generations and a reproductive division of labour that involves reproducing and helping individuals (Wilson, 1971). Typical eusocial organisms are social insects like termites, ants, some bees, and some wasps. Eusociality also exists in aphids, thrips, Ambrosia beetles, shrimps, and naked mole rats (Alexander *et al.*, 1991; Korb & Heinze, 2008). Eusociality in social insects is characterised by complex social organisations that vary greatly across taxa.

In social insects, individuals live in colonies consisting of from several hundred to up to millions of individuals, generally as a family. The royals are the queens, and for termites, also kings. They are characterised by a long lifespan and high fecundity, which seems to reverse the longevity-fecundity trade-off. The rest of the colony members forgo reproduction either permanently or facultatively and generally have a shorter lifespan. They all share a similar genetic background but differ enormously in lifespan according to the task they perform. This difference is a result of polyphenism.

In termites, the lifespan disparity between the royals and the rest is highly correlated with the degree of sociality (i.e., worker reproductive potential) of the species (Korb & Thorne, 2017). For species with sterile workers, the lifespan of reproductives can reach up to 20 years, while the workers have a lifespan of only a few months (e.g., *Macrotermes bellicosus*) (Elsner *et al.*, 2018). However, for species with totipotent workers, reproductives can live for up to 13 years and workers for 7 years (e.g., wood-dwelling termites)

(Monroy Kuhn *et al.*, 2019). So, why can reproducing individuals live so long, and what role, if any, does sociality play in this?

There are three main hypotheses for why reproducing individuals outlive non-reproducing individuals in social insects. First, the caste-specific extrinsic mortality hypothesis suggests that a longer lifespan for reproductives is selected for as they are often protected inside the nest and face lower extrinsic mortality than workers (Keller & Genoud, 1997). This hypothesis is based on a verbal argument by Williams (1957) that low extrinsic mortality selects for a long lifespan. Although Williams's argument might be an oversimplification of reality (Moorad *et al.*, 2019), it does point out correctly that extrinsic mortality could affect ageing, especially when it is age-dependent (Flatt & Partridge, 2018; Moorad *et al.*, 2019). Inspired by the caste-specific extrinsic mortality hypothesis, evolutionary models that are mainly tailored to social Hymenoptera tested the role of caste-specific and age-independent extrinsic mortality in shaping the lifespan disparity between queens and female workers (Kreider *et al.*, 2021; Kramer *et al.*, 2022). These models show that caste-specific extrinsic mortality is not necessary to let the lifespan difference between queens and workers (female workers) evolve. Instead, division of labour and worker reproductive potential have a more pronounced effect on the evolution of caste-specific ageing. These results are closely related to the remaining two hypotheses, which deal with the question of why division of labour may play a role. Hereafter, we refer to them as the trade-off hypothesis and the kin selection hypothesis. The trade-off hypothesis stems from the disposable soma theory of ageing, which describes ageing as a result of a resource allocation trade-off between maintenance and reproduction (Kirkwood, 1977, 2017). This trade-off seems to be absent for individuals in some social insects (Korb & Heinze, 2021), but may have been shifted to the colony-level as individuals share resources (Kramer & Schaible, 2013). The trade-off hypothesis is consistent with the superorganism perspective of insect societies, in which caste differentiation is analogous to soma-germline separation in a multicellular organism, and the degree of sociality (or degree of superorganismality) is dependent on the extent of germline-soma separation. Experimental studies show some support for this

hypothesis (Monroy Kuhn & Korb, 2016; Elsner *et al.*, 2018; Monroy Kuhn *et al.*, 2019). While the trade-off centred view provides an intuitive explanation of why the trade-off on individuals is absent, it does not answer the ultimate question of why such a shift to colony levels is favoured by selection in social insects. The kin selection hypothesis might provide an ultimate answer to it. Kin selection is the idea that one can propagate genes via relatives as related individuals share gene copies (Hamilton, 1964). It results in a change in the definition of fitness, expanding it to inclusive fitness (direct plus indirect fitness) (Korb & Heinze, 2016). The change in fitness definition implies corresponding changes in the force of selection (Bourke, 2007). Kramer *et al.* (2022) provide an analytical model that captures the reproductive value changes due to reproductive division of labour, resulting in the difference in the force of selection on queens and workers, and provides support for the kin selection effect on ageing in social Hymenoptera with sterile workers.

These three hypotheses are not mutually exclusive but rather complementary to each other and provide different perspectives on the same question. To our knowledge, quantitative models that explore these hypotheses are mainly based on social Hymenoptera, which have a particular relatedness asymmetry because of their haplodiploid sex-determination system (Alexander *et al.*, 1991). While empirical evidence suggests that ageing patterns vary greatly depending on species- and taxa-specific peculiarities, more models tailored to a diverse range of other social animals are required to test their generality and explain idiosyncratic ageing patterns (Korb & Heinze, 2021).

In light of this need, we developed quantitative models tailored to termites (Isoptera). Termites present excellent models for studying the interaction between sociality and ageing. Their social organisations vary enormously across the termite lineages, and they have evolved eusociality independently (Roisin & Korb, 2010). Phylogenetically distant from Hymenoptera, termites differ in many important aspects from ants and bees. They have a diplo-diploid sex-determination system, which in this regard is more similar to the naked mole-rat. The nest consists of both females and males, and the

reproductive caste consists of a queen and a king, and they mate continuously throughout their lifetime (Nalepa & Jones, 1991). In termites, males are as important as females, and most termites are monogamous, although polygyny and polyandry have also been reported (Korb & Thorne, 2017).

In termites, three degrees of sociality can be distinguished based on the reproductive potential of workers (Korb & Hartfelder, 2008; Roisin & Korb, 2010; Korb & Heinze, 2016) (**Fig. 4.1**). First, for all wood-dwelling species (i.e., all *Kalotermitidae* and *Archotermopsidae*), workers are totipotent immatures from which reproductives and soldiers develop (**Fig. 4.1a**). Thus, wood-dwellers can be assigned a low degree of sociality owing to the high reproductive potential of their workers. Reproductives and workers shelter in and consume the wood and probably face similarly low extrinsic mortality (e.g., mortality caused by predation or pathogens). Workers in these species are sometimes called “helpers”, are more like cooperative breeders in some birds (Downing *et al.*, 2021) or naked mole rats (Jarvis, 1981). They are “heirs to the throne” waiting to inherit the nests or to fly away and establish nests elsewhere. Secondly, termite species that belong to the intermediate sociality are typically some *Reticulitermes* (Noirot & Pasteels, 1987) and *Mastotermes*. For the foraging subterranean species from the *Mastotermitidae* and most *Rhinotermitidae*, there is a separation between the apterous line and the nymphal line at the early larvae stage (**Fig. 4.1b**). Workers have lost totipotency in the sense that they cannot moult into winged reproductives but only into wingless reproductives and they can inherit the nest. For this reason, they represent an intermediate degree of sociality. Similar to wood-dwellers, workers are heirs to the throne that can inherit the nest once they reach maturity. Different from wood-dwellers, workers perform risky foraging tasks and face high extrinsic mortality while reproductives are protected inside the nest. Hence, there is a large difference in extrinsic mortality between reproductives and workers. Lastly, termites of the family *Termitidae*, also known as higher termites, are classical examples of the high sociality group. The foraging species of *Termitidae* (higher termites) have sterile workers and also a bifurcated developmental pathway (**Fig. 4.1c**). Reproduction is completely monopolised by the sexual lines, which are all

dispersers. *Termitidae* represent a high degree of sociality. Similar to the intermediate species, there is a large difference in extrinsic mortality between castes.

Based on the three degrees of sociality, we developed individual-based models that capture the developmental pathways of termites and let ageing evolve based on a mutation accumulation mechanism (Medawar, 1952). We aimed to understand 1) how the degree of sociality changes ageing patterns in termites and 2) what role caste-specific extrinsic mortality plays in shaping them. The modelling results were complemented by some empirical data on termite longevity (**Table S4.1**).

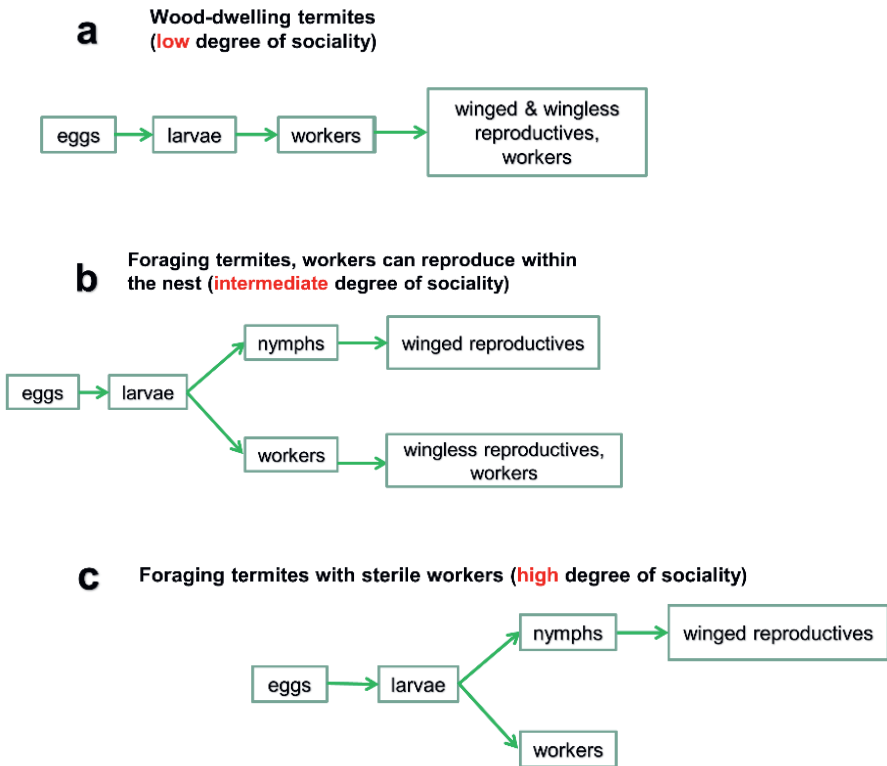


Figure 4.1. Termites can be classified into three degrees of sociality, depending on the workers' reproductive potential. **A)** termites with low sociality are characterised by the presence of totipotent workers that can develop into any caste and a wood-

dwelling lifestyle. Individuals follow a linear developmental pathway, all castes are derived from workers; **b**) termites with intermediate sociality have true workers (i.e., workers cannot develop eyes and wings and forage) but can still reproduce within the nest by developing into wingless replacement reproductives. Individuals follow a bifurcated developmental pathway where nymphal lines and worker (apterous) lines separate in the early larval stage (as early as the first instar), but workers still retain limited reproductive potential; **c**) termites with high sociality have sterile workers. Individuals follow a bifurcated developmental pathway similar to the intermediately social species, but workers have lost reproductive potential completely.

4.2 Methods

We constructed three individual-based simulation models with discrete timesteps (i.e., years) tailored to three species of termites that represent the three categories of sociality: *Cryptotermes secundus* (low sociality; totipotent workers), *Mastotermes darwiniensis* (intermediate sociality; workers lose totipotency) and *Macrotermes bellicosus* (high sociality; workers are sterile). All individuals were diploid and all colonies were monogynous. All parameter values are listed in **Table S4.2, S4.3, S4.4**.

Genes and mutations

For all three degrees of sociality, we started the model with a non-ageing population. Each individual in our model carries gene networks that encode real-valued numbers (hereafter survival values) that can be transformed into the caste- and age-specific probability of intrinsic survival via a logistic function. As all individuals are diploid, each individual carries two sets of gene networks, one inherited from the mother and the other from the father. The final survival value of the offspring is the mean of the maternal and paternal effects (i.e., additive gene action). x_a^C is the survival value of caste C at the age of a . The intrinsic survival probability that an individual of age a and caste C survives to the next year is

$$S_a^c = \frac{1}{1+e^{-x_a^c}} \quad (1)$$

In the initial population, the intrinsic survival value across all ages is x_{start} . The cause of ageing is mutations with Gaussian effect sizes that occur randomly with a per-locus probability of μ during gametogenesis and on average reduce survival value by b . As we have C castes and Ω age classes, a single set of a gene network is a vector containing $C \times \Omega$ survival values. The first Ω genes are the worker genes which are expressed when an individual is a worker. The remaining Ω genes are the reproductive genes that are expressed when an individual is a reproductive. This part of the design captures the fact that caste differentiation in social insects is a result of polyphenism. Reproductives carry the genes of workers but do not express them, and vice versa. Genes and mutations are implemented the same way in the three models. We do not assume sex-specific ageing; therefore, queens and kings are derived from the same reproductive caste whilst male and female workers are derived from the same worker caste. As a result, age-specific survival is the same in females and males.

General life events

For all three degrees of sociality, the life events of individuals share some common features among the three models but also bear some differences in developmental pathways (as shown in **Fig. 4.2**). Within each time step (i.e., year), the following life events take place in consecutive order:

Reproduction

This step only applies to the reproductives. Each of the reproductives produces a gamete. Then the two gametes from the parents combine into an egg. The expected number of eggs produced λ is a logistic function of colony size n (i.e., the number of individuals other than reproductives) (**Fig. S4.1, S4.2**):

$$\lambda = f + \frac{f_{\text{max}} - f}{1 + e^{-(l \times n)}} \quad (2)$$

Where f , together with f_{\max} which is the maximum fecundity of the queen, determines the baseline fecundity of the queen when no workers are around. l modifies the rate at which fitness benefits for the queen increase with the number of workers. The realized number of eggs produced is sampled from a Poisson distribution with the mean equalling to λ .

Maturation

An individual starts at the age of zero and increments its age every year if it survives to the next year. For the low and intermediate sociality models, an egg, worker, or nymph reaches maturity when it reaches the age of maturity a_m . For the high sociality model, this step doesn't exist as all workers are permanent immatures (sterile) and alates (i.e., future reproductives) disperse within a year after birth.

Survival

Every year, death events are sampled from a Bernoulli distribution according to caste- and age-specific intrinsic survival S_a^C and extrinsic mortality m_w for workers, and m_R for reproductives. This event applies to all eggs, workers, nymphs, and reproductives. For dispersers, death events are sampled additionally during dispersal, according to m_d .

Dispersal

This step differs among the different degrees of sociality. After reaching maturity, individuals that have reproductive potential have the option of becoming reproductives. For the totipotent workers in the low sociality model (**Fig. 4.2a**), dispersal events are sampled from a Bernoulli distribution according to the probability d . Those who become dispersers will fly away from the nest to found a new nest. Others who stay in the nest can develop into replacement reproductives if one of the same-gender reproductives dies. Otherwise, they just stay in the colony as workers. In the intermediate sociality model, workers lose totipotency, they cannot disperse, but the matured nymphs (nymphal lines) will all disperse and go through death events

caused by dispersal according to a probability of m_d (**Fig. 4.2b**). For the high sociality model, all nymphs disperse in the first year after birth and then go through death events according to a probability of m_d (**Fig. 4.2c**).

Replacement

The replacement events (or inheritance events) take place when at least one reproductive has died. A matured worker of the same sex as the dead reproductive will be picked randomly to become the replacement reproductive. For the high sociality model, this step does not exist as workers are sterile and cannot inherit the colony.

Sociality-specific life cycles

We start the model with N_{start} monogynous colonies, and each colony consists of a queen and a king, but some life events only apply to specific degrees of sociality.

For the low sociality model, reproductives produce totipotent worker eggs that follow a linear development towards reproductives (as shown in **Fig. 4.2a**). Individuals (eggs + workers + nymphs) then mature and their survival is checked. Individuals who survive continue along the life cycle. Individuals who become dispersers will fly away from the colony and attempt to establish a new colony. Individuals who stay as workers will have the opportunity to become replacement reproductive. Others who do not disperse or become replacement reproductives will stay in the colony as workers. As all reproductives develop from matured workers, the reproductives are at least three years old, and the queen part of the gene network is only expressed after the age of three.

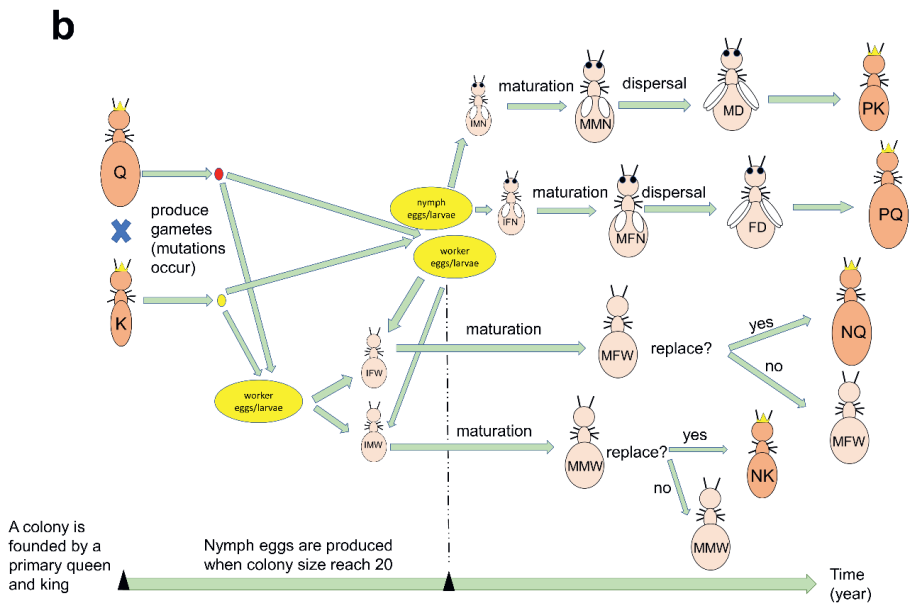
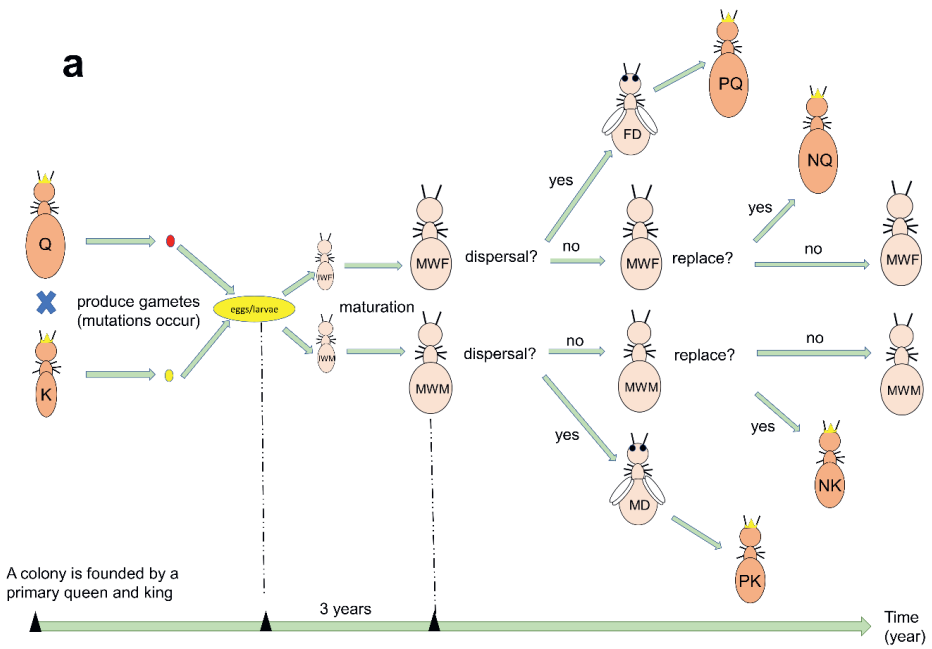
For the intermediate sociality model, individuals' fates are determined at the early instar stage. Individuals follow a bifurcated developmental pathway, but workers still retain reproductive potential (**Fig. 4.2b**). Individuals developed from worker-destined eggs cannot disperse, but they still retain the reproductive potential to become replacement reproductives. Individuals

developed from nymph-destined eggs will all disperse after reaching the age of maturity a_m (**Table S4.3**). During the early period (i.e., when colony size is less than 20), reproductives only produce worker-destined eggs. After reaching a sufficient colony size, reproductives produce both worker- and nymph-destined eggs and the probability that the egg is nymphal is d . Similar to the low sociality model, a colony then goes through maturation and survival steps. Matured nymphs that survive will disperse. Matured workers that survive can become replacement reproductives if a same-gender reproductive dies. Otherwise, they just stay as workers and proceed to the next time step.

For both low and intermediate sociality models, a colony is dead if the replacement event is not successful, meaning there is no matured worker of the same gender as the dead reproductive. The life events involved in these two models are reproduction, maturation, survival, dispersal, and replacement.

For the high sociality model, individuals also follow a bifurcated developmental pathway, but workers are sterile, and nymphs all disperse within the same year they are born (**Fig. 4.2c**). Similar to the intermediate sociality model, reproductives only produce nymph-destined eggs when there is sufficient number of workers around (**Fig. 4.2c**). Then survival is checked for all individuals. Nymphs that survive will all disperse. Different from the low and intermediate sociality models, a colony is dead as soon as one of the reproductives is dead. Life events involved in this model are reproduction, survival, and dispersal.

For all three models, at the end of each time step, dead individuals and colonies are removed, and new colonies are recruited from the dispersers produced by all the colonies from the previous time step. A female disperser and a male disperser are randomly chosen to found a new colony. New colonies are recruited until the population size reaches a limit of N_{\max} .



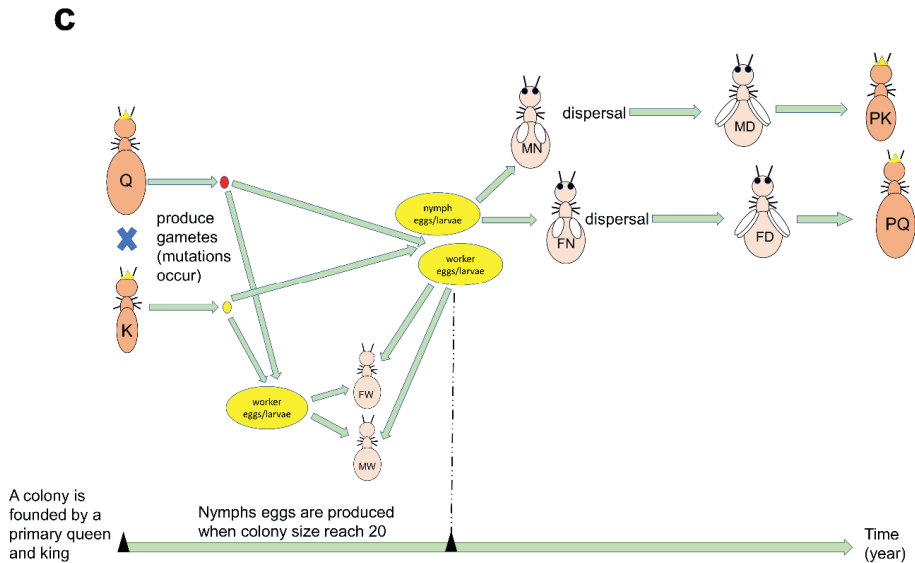


Figure 4.2. Model designs are based on the developmental pathways of termites with varying degrees of sociality. **A)** The developmental pathway of termites with low degree of sociality, such as *Cryptotermes secundus*; **b)** the developmental pathway of termites with intermediate sociality, such as *Mastotermes darwiniensis*; and **c)** the developmental pathway of termites with high degree of sociality, such as *Macrotermes bellicosus*. Abbreviations: Q=queen; K=king; IWF=immature female worker; IWM=immature male worker; MWF=mature female worker; MWM=mature male worker; NQ=neotenic queen; NK=neotenic king; FD=female disperser; MD=male disperser; MW=male worker; FW=female worker; MN=male nymph; FN=female nymph. Definitions of terminologies: nymphs are individuals with wing pads and can develop into winged sexuals (dispersers). Neotenics: replacement reproductives molted from workers. In the simulation, we did not distinguish between eggs and larvae.

Simulations and statistics

The model was implemented in C++ and compiled on a BwForCluster using g++ 10.2. Each simulation model was supported by 20 replications and ran for sufficient timesteps to reach a quasi-equilibrium. At the end of each

simulation, we output the genomes of all individual queens to record age-specific intrinsic survival for all castes. The evolved intrinsic life expectancy (hereafter life expectancy) was calculated as the area under the age-specific intrinsic survival curve (i.e., the sum of age-specific intrinsic survival). Life expectancies were divided by the maximum lifespan (i.e., 19) to be transformed into proportions. This proportion data was used to perform beta regressions, which compared the differences in life expectancy between castes. Beta regression was performed using the function “betareg” from the R-package betareg (Cribari-Neto & Zeileis 2010). We also recorded average age, intrinsic survival, colony size, and maximum age (hereafter evolutionary variables) every 100 timesteps to show the evolution of the simulated populations (**Fig. S4.3, S4.4, S4.5, S4.6**). All data were visualised in R (version 4.1.2) using ggplot2 (Wickham 2016).

4.3 Results

We explored the effect of the degree of sociality on ageing when controlling for caste-specific extrinsic mortality. First, extrinsic mortality was set the same for both reproductives and workers (i.e., $m_R = m_W = 0$) to estimate a pure effect of sociality. Then, extrinsic mortality was raised to a higher level (i.e., $m_R = m_W = 0.2$) to test whether the sociality effect still persists. Lastly, caste-specific extrinsic mortality, that resembles reality the most, was introduced to test how it affected the sociality effect (low sociality: $m_R = 0, m_W = 0.2$; intermediate sociality: $m_R = 0, m_W = 0.8$; high sociality: $m_R = 0, m_W = 0.8$). Workers in intermediate and high sociality species perform high-risk tasks (e.g., foraging), whereas workers in low sociality species face similar low extrinsic mortality as reproductives. As a result, workers from low sociality species had much lower extrinsic mortality than those from intermediate or high sociality species.

In the low sociality model, reproductives evolved a slightly higher life expectancy than workers regardless of extrinsic mortality (**Fig. 4.3a, 4.3b**,

4.3c). In the intermediate sociality model, when workers faced the same level of extrinsic mortality as reproductives, they evolved a higher life expectancy than reproductives (**Fig. 4.3d, 4.3f**). However, when workers faced higher extrinsic mortality than reproductives, they evolved a lower life expectancy (**Fig. 4.3e**). In the high sociality model, reproductives evolved a much higher life expectancy than workers regardless of extrinsic mortality (**Fig. 4.3g, 4.3h, 4.3i**). Statistical details are listed in **Table S4.5**.

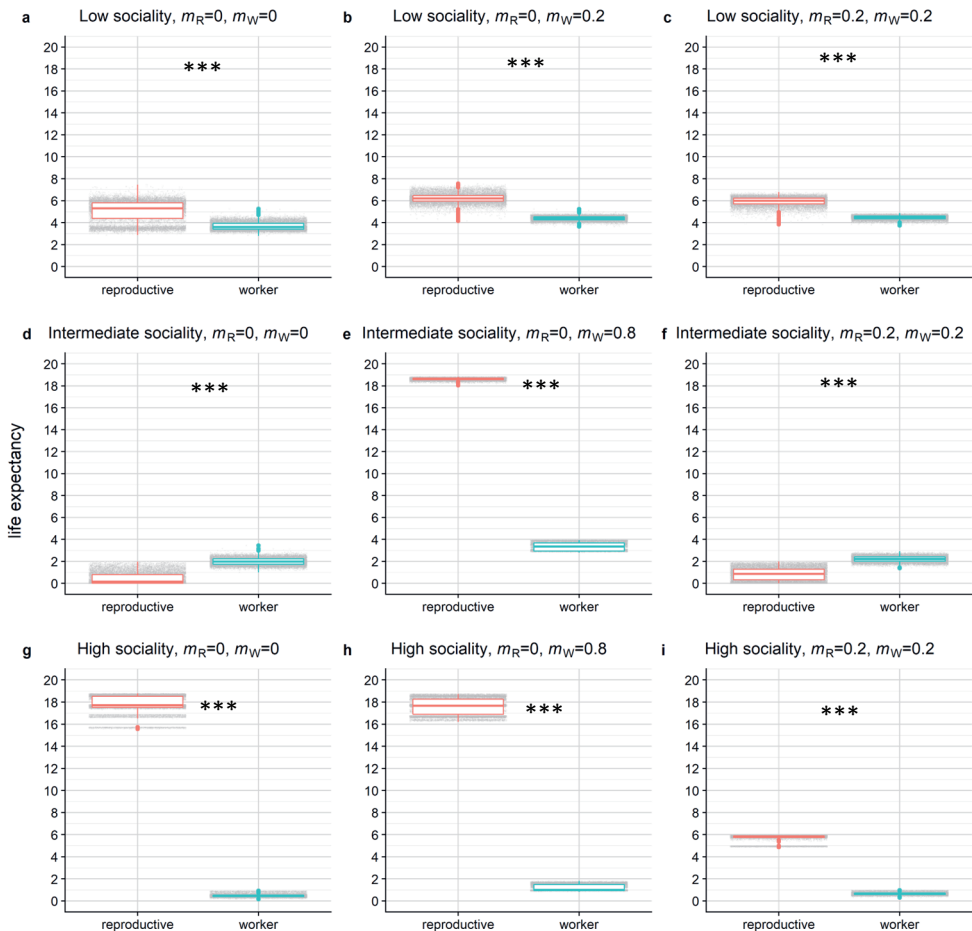


Figure 4.3. Boxplots of the evolved life expectancy in **a)** the low sociality model without the effect of extrinsic mortality; **b)** the low sociality model with caste-specific extrinsic mortality; **c)** the low sociality model without caste-specific

extrinsic mortality, but equally high extrinsic mortality in both castes; **d**) the intermediate sociality model without the effect of extrinsic mortality; **e**) the intermediate sociality model with caste-specific extrinsic mortality; **f**) the intermediate sociality model without caste-specific extrinsic mortality but equally high extrinsic mortality in both castes; **g**) the high sociality model without the effect of extrinsic mortality; **h**) the high sociality model with caste-specific extrinsic mortality; **i**) the high sociality model without caste-specific extrinsic mortality but equally high extrinsic mortality in both castes. Each grey dot represents the calculated life expectancy based on an individual queen's genome ($n=20000$). Red represents the reproductive, and blue represents the worker. Stars *** indicate p value < 0.001 .

The reproductive/worker lifespan ratio

According to the models with caste-specific extrinsic mortality (which most closely resemble reality), the reproductive/worker lifespan ratio increased with increasing sociality (**Fig. 4.4**).

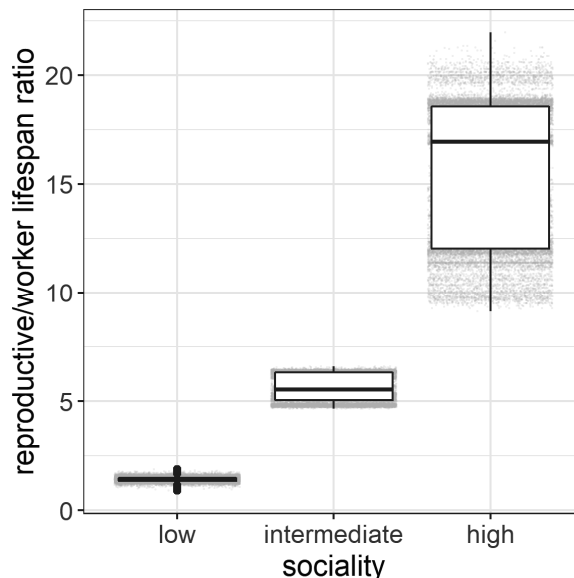


Figure 4.4. Boxplot of the estimated reproductive/worker lifespan ratio based on life expectancy. Each grey dot represents the lifespan ratio calculated based on an individual queen's genome ($n=20000$).

4.4 Discussion

How does sociality change ageing?

The importance of worker reproductive potential

Molecular studies have demonstrated that termite reproductives and workers age differently depending on their degree of sociality. For example, *M. bellicosus* (high sociality) reproductives experience negligible ageing. Their gene expression patterns have few changes when growing old, and they are also protected against one of the major causes of ageing: transposon activity (Elsner *et al.*, 2018). On the other hand, *M. bellicosus* workers have upregulation of transposon activities when growing old and are short-lived (Elsner *et al.*, 2018). Reproductives can live up to twenty times longer than workers (see **Table S4.1** for longevity data). By contrast, *C. secundus* (low sociality) reproductives do experience ageing whereas workers invest in anti-ageing mechanisms (Monroy Kuhn *et al.*, 2019) and are more protected against oxidative stress than reproductives (Rau & Korb, 2021). Their lifespan divergency between castes is less pronounced than those in the high sociality group. These sociality-specific ageing patterns have been hypothesized to be caused by varying worker reproductive potential (Monroy Kuhn *et al.*, 2019; Korb & Heinze, 2021).

To investigate how and why worker reproductive potential affects ageing, we classified the degree of sociality solely based on workers' reproductive potential. Our findings show that the reproductive/worker lifespan ratio, an indicator of lifespan divergence between reproductives and workers, increased with increasing sociality (i.e., decreasing worker reproductive potential). The link between worker reproductive potential and lifespan divergence between castes is consistent with previous theoretical research (Kramer *et al.*, 2022), though their definition of sociality is not solely based on worker reproductive potential but also on the breeding system (e.g., polygyny or monogyny). These findings imply that worker reproductive

potential plays an important role in shaping ageing in social insects, perhaps because it modifies the reproductive values of workers.

As shown in **Fig. 4.5**, workers with low sociality have full reproductive potential (i.e., they can develop into winged and wingless sexuals) and engage less in behaviours that increase indirect fitness. For example, workers of *C. secundus*, do not care for eggs or young larvae (Korb, 2007b). They contribute to the future gene pool directly through inheritance or dispersal. As a result, their reproductive values may be as high as those of the reproductives and they are subjected to relatively strong selection. Our models predict that lifespan divergency between castes is small in this case.

Workers in the intermediate scenario retain partial reproductive potential (i.e., they can only develop into wingless sexuals). Workers forage and care for the brood (Korb & Thorne, 2017). These activities expose workers to high extrinsic mortality. As a result, they have a slim chance of gaining direct fitness by inheriting the nest. Our model predicts that workers in this case contribute to the future gene pool primarily through indirect fitness and, as a result, have lower reproductive values than reproductives (**Fig. 4.5**). Therefore, lifespan divergence between castes increases compared to the low sociality group (**Fig. 4.4**).

Workers are sterile in the high sociality scenario and can only gain indirect fitness. They have zero reproductive value. Reproductives, on the other hand, contribute to the future gene pool via direct fitness. They have high reproductive values and are under relatively strong selection against mortality. Our model predicts that the lifespan divergence will be the most extreme in this case.

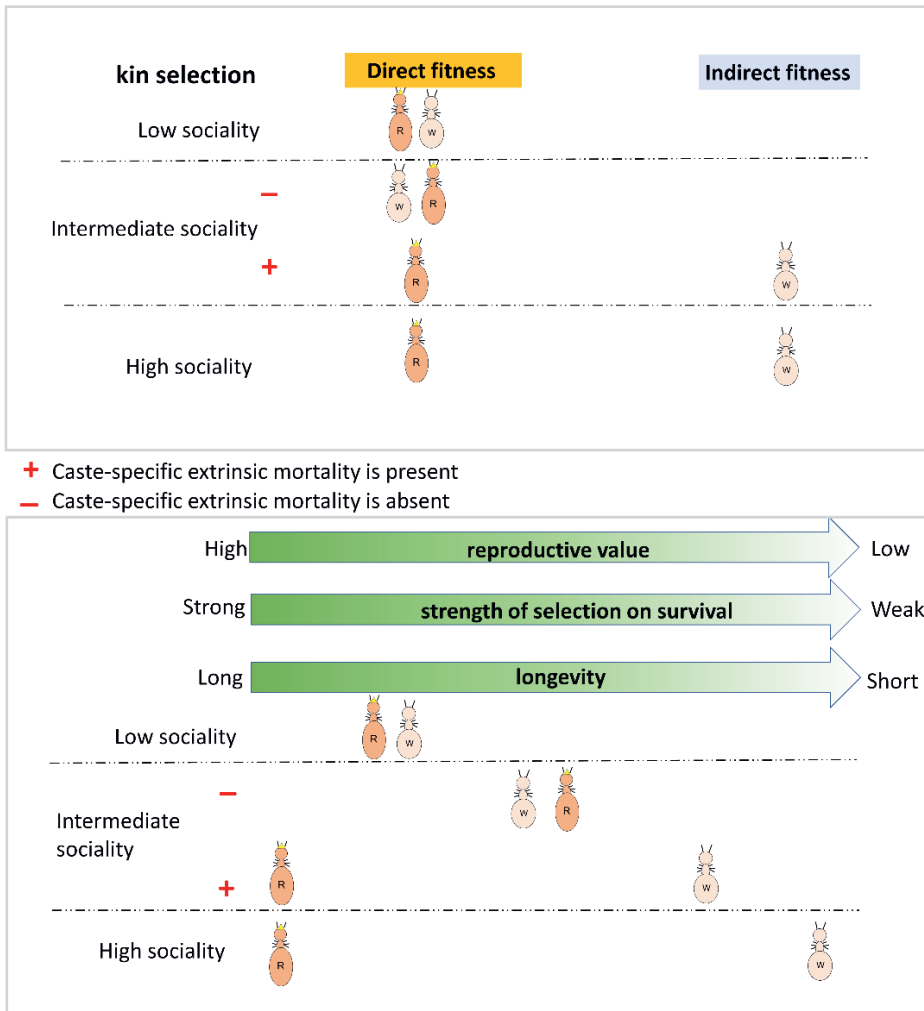


Figure 4.5. Cartoon depicting the impact of sociality on lifespan divergence between castes. Selection strength on survival is determined by the reproductive and worker contributions to the future gene pool. Depending on their reproductive potential and extrinsic mortality, workers can contribute directly or indirectly to the future gene pool. Workers can become reproductives in species with low sociality. As a result, they obtain direct fitness and have high reproductive value, as well as being long-lived. Extrinsic mortality can affect the probability of workers attaining direct fitness for species with intermediate sociality. Workers are more likely to obtain indirect fitness and have low reproductive values when extrinsic mortality is high, whereas

they are more likely to gain direct fitness and have high reproductive values when extrinsic mortality is low. Workers can only gain indirect fitness in species with high sociality; therefore, they have zero reproductive value and are short-lived. Abbreviations: R = reproductive; W = worker.

The effect of extrinsic mortality is dependent on sociality

Theoretical models have demonstrated that caste-specific extrinsic mortality is not required to explain why queens outlive workers (Kreider *et al.*, 2021; Kramer *et al.*, 2022). This conclusion is supported by our findings from the low and high sociality models. Our results in the intermediate model, however, show that caste-specific extrinsic mortality is required to induce a longer lifespan of reproductives than workers.

In a hypothetical scenario for the intermediate sociality model, workers faced the same extrinsic mortality as reproductives, and they evolved a higher life expectancy than reproductives (**Fig. 4.3d, 4.3f**). This could be because workers have higher reproductive values than alates. In this hypothetical situation, workers' reproductive values are realised by inheriting the nest, while alates' reproductive values are realised by dispersal. Therefore, stronger selection on colony inheritance than on colony dispersal might result in higher reproductive values of workers than alates. In the hypothetical scenario, colony inheritance might have been favoured by selection for two possible reasons.

First, alates face additional dispersal mortality when establishing new colonies. Workers, on the other hand, face no additional risk of inheriting the nest. Therefore, colony inheritance might be a more secure way than dispersal to pass genes to future generations. Second, newly inherited colonies might be more successful than newly founded colonies because the former start reproduction with more resources (e.g., workers and nests) than the latter.

In a more realistic scenario for the intermediate sociality model, workers facing higher extrinsic mortality than reproductives evolved shorter life expectancies than reproductives (**Fig. 4.3e**). This reversal is most likely due

to workers' reduced reproductive opportunities. When extrinsic mortality is high, the chance that workers will inherit the nest is reduced, resulting in lower reproductive values for workers and a higher reproductive value for reproductives.

Overall, these findings imply that the effect of caste-specific extrinsic mortality on caste-specific ageing is dependent on sociality (i.e., worker reproductive potential). Extrinsic mortality can modify workers' reproductive values in the intermediate sociality group but not in the low and high sociality groups. This is because workers in the low sociality group always have high reproductive values, as all reproductives develop from workers. Workers in the high sociality group always have zero reproductive values because they are sterile. However, workers' reproductive values are highly sensitive to extrinsic mortality in the intermediate sociality group. Because workers' direct fitness is achieved by colony inheritance, and extrinsic mortality can modify the chance of colony inheritance.

It has been argued that extrinsic mortality can only affect the pattern of ageing if it is age-dependent (Moorad *et al.*, 2019). However, this argument assumes no social interactions. We incorporated different degrees of social interaction in our models and showed that extrinsic mortality, even age-independent, can affect ageing. Our finding also provides a novel explanation for how caste-specific extrinsic mortality affects caste-specific ageing. It was proposed that caste-specific extrinsic mortality causes caste-specific ageing because high extrinsic mortality selects for a short lifespan while low extrinsic mortality selects for a long lifespan (Keller & Genoud, 1997). In this study, we propose a new explanation: caste-specific extrinsic mortality causes caste-specific ageing through modifying selection on workers' direct fitness (i.e., the chance of inheritance).

In general, we show that both sociality (i.e., worker reproductive potential) and caste-specific extrinsic mortality are important factors in explaining the ageing pattern in termites. In particular, the impact of extrinsic mortality needs to be examined based on social context. We reveal a striking link

between the evolution of sociality, ageing, and extrinsic mortality. Such a link might also exist in other social animals whose social behaviours are shaped or partially shaped by ecological conditions.

4.5 Author Contributions

All authors contributed to designing the model. S.L and I.P implemented the model. S.L wrote the first draft, J.K and I.P edited.

4.6 Acknowledgements

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4.7 Data Accessibility

C++ codes and R scripts for the analysis are available at https://github.com/silulinlulu/termite_ageing_model.

4.8 Supplementary materials

Table S4.1. Termite longevity data.

Sociality	Species/family	Longevity	Reference
Low	<i>Cryptotermes secundus</i>	Reproductives: mean lifespan 11.1y (SE: ± 0.7), maximum lifespan 13y in the lab	Monroy Kuhn <i>et al.</i> , 2021
		Reproductives: max lifespan 13y; workers: >7y	Monroy Kuhn <i>et al.</i> , 2019
Intermediate	<i>Reticulitermes spp.</i>	Workers: 9-10y in the wild	Buchli, 1958
	<i>Reticulitermes spp.</i>	Primary reproductives: up to 18y in the field	Lainé & Wright, 2003
	<i>Rhinotermitidae</i>	Reproductives: 25y in the lab	Snyder, 1926
	<i>Mastotermes darwiniensis</i>	Reproductives: up to 17y	Watson & Abbey, 1989
High	<i>Macrotermes bellicosus</i>	Reproductives: median lifespan 10y (including subterranean phase); max lifespan 20y in the field	Elsner <i>et al.</i> , 2018
		Reproductives: up to 20-30y (based on the abdomen size); workers: a few months in the field	Estimated by Judith Korb

Table S4.2. Model parameters used for the low sociality model.

Category	Parameter	Definition	Values
mutation related	μ	mutation probability per gene	0.001
	b	mean of mutational effect size	-0.2
	σ	standard deviation of mutational effect size	0.4
individual design	C	number of castes: reproductives and workers	2
	Ω	number of age classes	20
	a_{\max}	maximum age an individual can get	19
	p	all individuals are diploid	2
	m_W	extrinsic mortality for workers	0, 0.2
	m_R	extrinsic mortality for reproductives	0, 0.2
	x_{start}	initial survival values at all ages	3.2
dispersal related	a_m	age of maturity	3
	d	probability that an individual will disperse	0.1
	m_d	probability of dying during dispersal	0.8
reproduction related	f	determinant of initial fecundity of the queen without workers	5
	f_{\max}	max number of eggs that a queen can lay	50
	l	regulator of fitness benefits for the queen per worker	0.2
colony related	N_{start}	number of colonies at the beginning	1000
	N_{\max}	max number of colonies allowed in a population	1000
	t	number of timesteps to run	1000000

Table S4.3. Model parameters used for the intermediate sociality model.

Category	Parameter	Definition	Values
mutation related	μ	mutation probability per gene	0.001
	b	mean of mutational effect size	-0.2
	σ	standard deviation of mutational effect size	0.4
individual design	C	number of castes: reproductives and workers	2
	Ω	number of age classes	20
	a_{\max}	maximum age an individual can get	19
	p	all individuals are diploid	2
	m_W	extrinsic mortality for workers	0, 0.2, 0.8
	m_R	extrinsic mortality for reproductives	0, 0.2
	x_{start}	initial survival values at all ages	3.2
dispersal related	a_m	age of maturity	1
	d	probability that an individual will disperse	0.1
	m_d	probability of dying during dispersal	0.8
reproduction related	f	determinant initial fecundity of the queen without workers	5
	f_{\max}	max number of eggs that a queen can lay	50
	l	regulator of fitness benefits for the queen per worker	0.2
colony related	N_{start}	number of colonies at the beginning	1000
	N_{\max}	max number of colonies allowed in a population	1000
	t	number of timesteps to run	1000000

Table S4.4. Model parameters used for the high sociality model.

Category	Parameter	Definition	Values
mutation related	μ	mutation probability per gene	0.001
	b	mean of mutational effect size	-0.2
	σ	standard deviation of mutational effect size	0.4
individual design	C	number of castes: reproductives and workers	2
	Ω	number of age classes	20
	a_{\max}	maximum age an individual can get	19
	p	all individuals are diploid	2
	m_W	extrinsic mortality for workers	0, 0.2, 0.8
	m_R	extrinsic mortality for reproductives	0, 0.2
	x_{start}	initial survival values at all ages	3.2
dispersal related	d	probability that an egg is a nymph egg	0.1
	m_d	probability of dying during dispersal	0.8
reproduction related	f	determinant of initial fecundity of the queen without workers	20
	f_{\max}	max number of eggs that a queen can lay	100
	l	regulator of fitness benefits for the queen per worker	0.2
colony related	N_{start}	number of colonies at the beginning	1000
	N_{\max}	max number of colonies allowed in a population	1000
	t	number of timesteps to run	1000000

Table S4.5. Beta regression results for different scenarios.

Scenario	Factor	Estimate	SE	z value	P-value
Low sociality $m_R = m_W = 0$	(Intercept)	-1.03	0.001	-714.7	<0.001
	Caste.worker	-0.39	0.002	-182.0	<0.001
Low sociality $m_R = 0, m_W = 0.2$	(Intercept)	-0.72	0.001	-1286.1	<0.001
	Caste.worker	-0.48	0.001	-570.6	<0.001
Low sociality $m_R = m_W = 0.2$	(Intercept)	-0.79	0.001	-1529.4	<0.001
	Caste.worker	-0.39	0.001	-514.7	<0.001
Intermediate sociality $m_R = m_W = 0$	(Intercept)	-4.61	0.009	-518.6	<0.001
	Caste.worker	2.58	0.009	283.3	<0.001
Intermediate sociality $m_R = 0, m_W = 0.8$	(Intercept)	3.90	0.002	1717	<0.001
	Caste.worker	-5.44	0.002	-2243	<0.001
Intermediate sociality $m_R = m_W = 0.2$	(Intercept)	3.34	0.005	-619.2	<0.001
	Caste.worker	1.39	0.006	228.8	<0.001
High sociality $m_R = m_W = 0$	(Intercept)	2.79	0.004	780.7	<0.001
	Caste.worker	-6.24	0.006	-1019.6	<0.001
High sociality $m_R = 0, m_W = 0.8$	(Intercept)	2.62	0.003	825.3	<0.001
	Caste.worker	-5.25	0.005	1143.9	<0.001
High sociality $m_R = m_W = 0.2$	(Intercept)	-0.85	0.001	-1534	<0.001
	Caste.worker	-2.48	0.001	-1673	<0.001

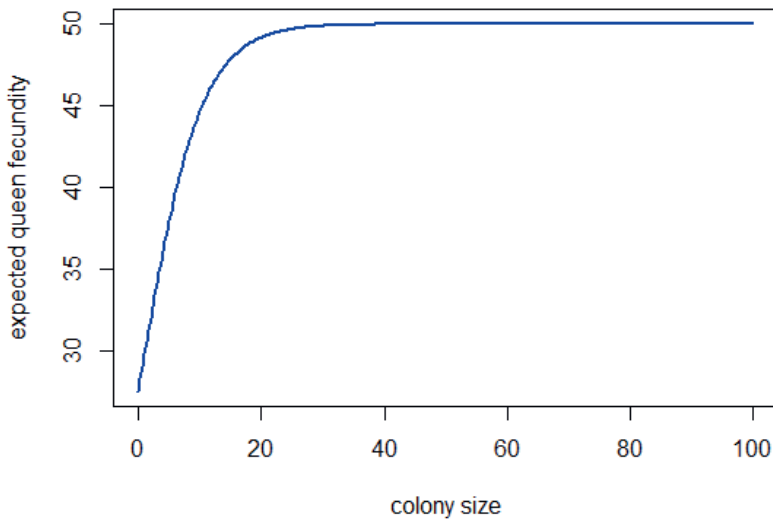


Figure S4.1. Relationship between colony size and queen fecundity for the low and intermediate sociality models.

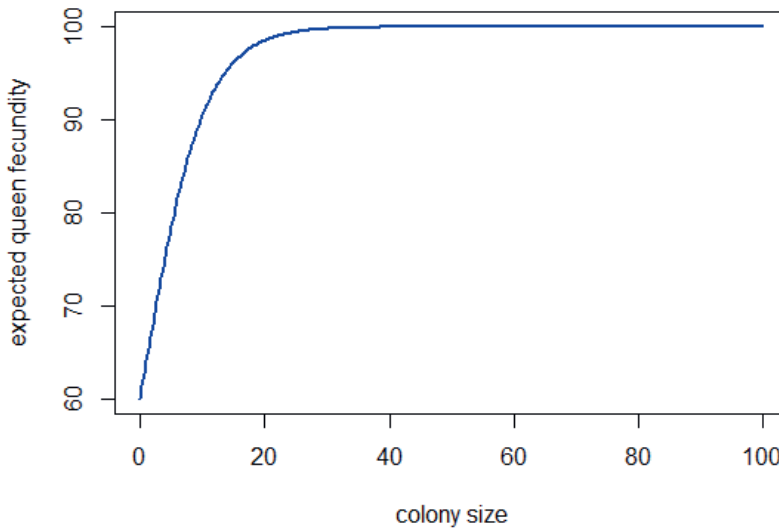


Figure S4.2. Relationship between colony size and queen fecundity for the high sociality model. Note that the high sociality simulation died quickly with the same initial and maximum fecundity settings as the low and intermediate sociality models. This could imply that higher fecundity is needed for high sociality species to evolve.

to workers' reduced reproductive opportunities. When extrinsic mortality is high, the chance that workers will inherit the nest is reduced, resulting in lower reproductive values for workers and a higher reproductive value for reproductives.

Overall, these findings imply that the effect of caste-specific extrinsic mortality on caste-specific ageing is dependent on sociality (i.e., worker reproductive potential). Extrinsic mortality can modify workers' reproductive values in the intermediate sociality group but not in the low and high sociality groups. This is because workers in the low sociality group always have high reproductive values, as all reproductives develop from workers. Workers in the high sociality group always have zero reproductive values because they are sterile. However, workers' reproductive values are highly sensitive to extrinsic mortality in the intermediate sociality group. Because workers' direct fitness is achieved by colony inheritance, and extrinsic mortality can modify the chance of colony inheritance.

It has been argued that extrinsic mortality can only affect the pattern of ageing if it is age-dependent (Moorad *et al.*, 2019). However, this argument assumes no social interactions. We incorporated different degrees of social interaction in our models and showed that extrinsic mortality, even age-independent, can affect ageing. Our finding also provides a novel explanation for how caste-specific extrinsic mortality affects caste-specific ageing. It was proposed that caste-specific extrinsic mortality causes caste-specific ageing because high extrinsic mortality selects for a short lifespan while low extrinsic mortality selects for a long lifespan (Keller & Genoud, 1997). In this study, we propose a new explanation: caste-specific extrinsic mortality causes caste-specific ageing through modifying selection on workers' direct fitness (i.e., the chance of inheritance).

In general, we show that both sociality (i.e., worker reproductive potential) and caste-specific extrinsic mortality are important factors in explaining the ageing pattern in termites. In particular, the impact of extrinsic mortality needs to be examined based on social context. We reveal a striking link

sociality model without the effect of extrinsic mortality; **h**) the high sociality model with caste-specific extrinsic mortality; **i**) the intermediate sociality model without caste-specific extrinsic mortality but equally high extrinsic mortality in both castes. Curves represent mean values. Shaded areas represent 95% confidence intervals. Red represents the reproductive and blue represents the worker.

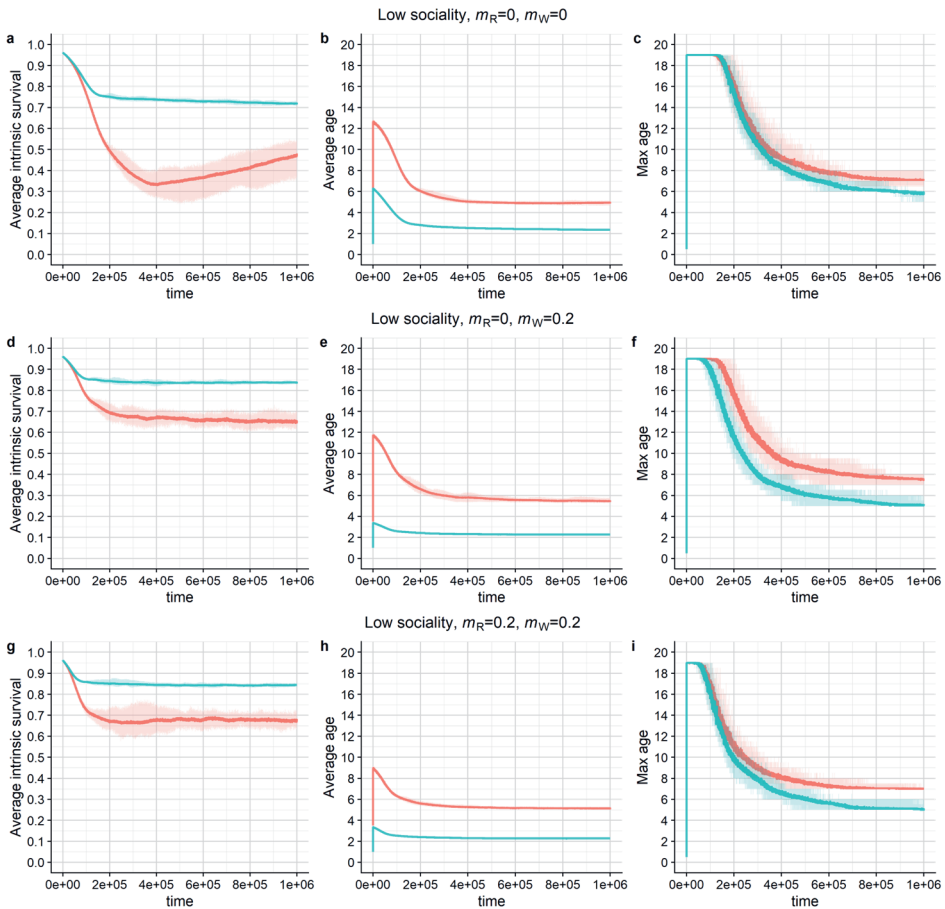


Figure S4.4. Evolution of the simulated populations in the low sociality model with different extrinsic mortality settings. Curves represent the mean values of all the individuals of the corresponding caste that are present in the population at a certain timestep. Shaded areas represent 95% confidence intervals based on 20 replicates. The first row shows the scenario without the effect of extrinsic mortality: **a**) the evolution of the average intrinsic survival of reproductives (red) and workers (blue); **b**) the evolution of the average age of reproductives (red) and workers (blue); **c**) the

evolution of the maximum age of reproductives (red) and workers (blue). The second row shows the evolution of the populations when caste-specific extrinsic mortality is present: **d**) the evolution of the average intrinsic survival of reproductives (red) and workers (blue); **e**) the evolution of the average age of reproductives (red) and workers (blue); **f**) the evolution of the maximum age of reproductives (red) and workers (blue). The third row shows the scenario without caste-specific extrinsic mortality but equally high extrinsic mortality in both castes: **g**) the evolution of the average intrinsic survival of reproductives (red) and workers (blue); **h**) the evolution of the average age of reproductives (red) and workers (blue); **i**) the evolution of the maximum age of reproductives (red) and workers (blue).

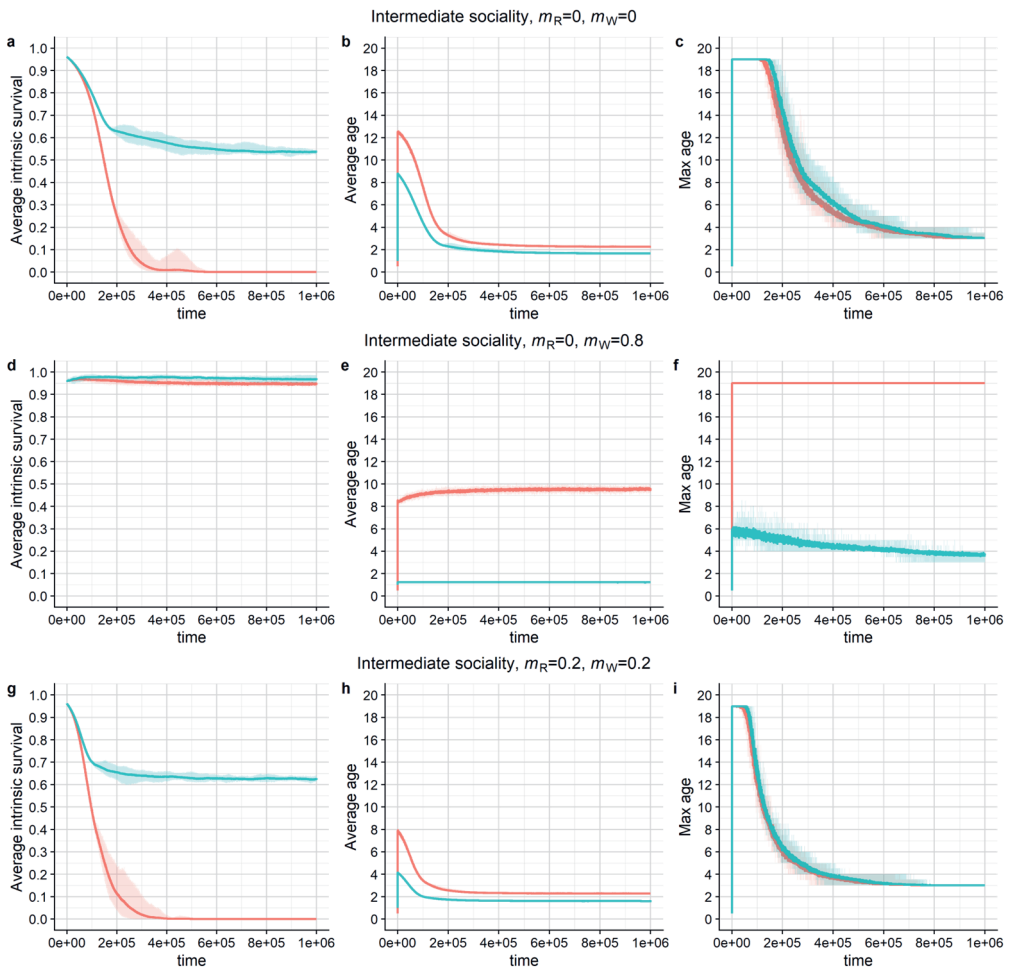


Figure S4.5. Evolution of the simulated populations in the intermediate sociality model. For more information, see **Fig. S4.4**.

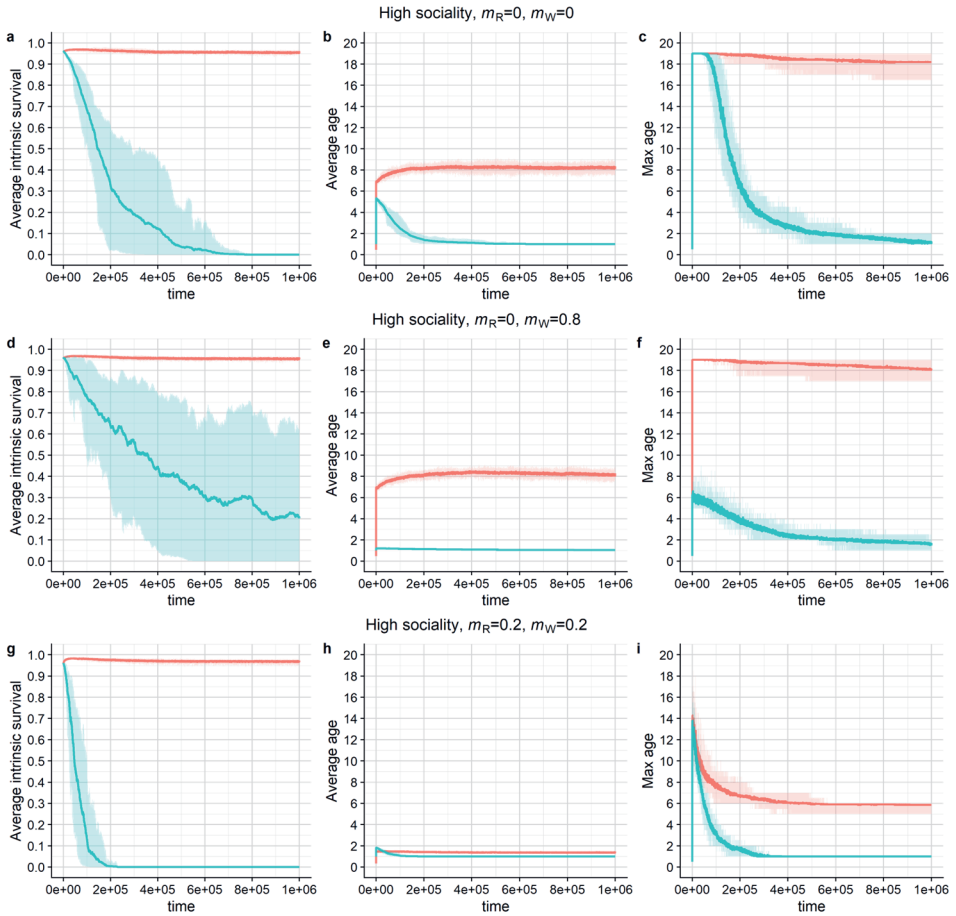


Figure S4.6. Evolution of the simulated populations in the high sociality model. For more information, see **Fig. S4.4**.

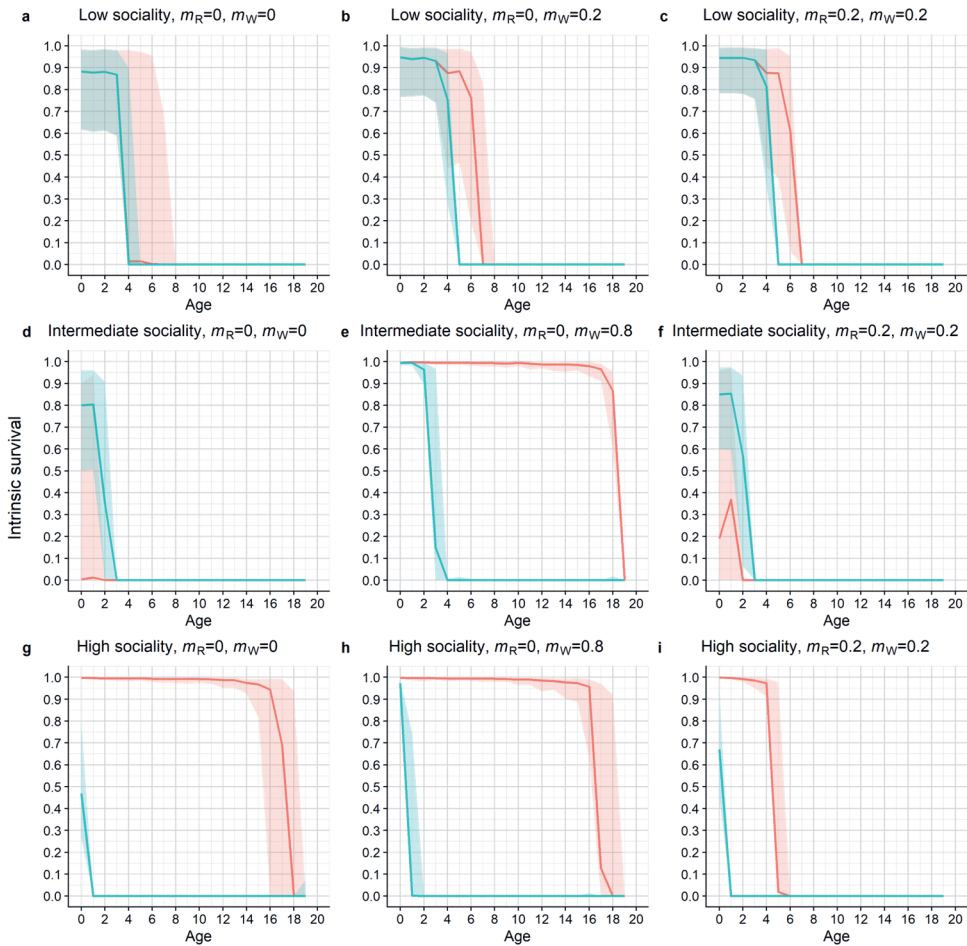


Figure S4.7. The evolved age-specific intrinsic survival (i.e., the probability of surviving to the next year) in **a**) the low sociality model without the effect of extrinsic mortality; **b**) the low sociality model with caste-specific extrinsic mortality; **c**) the low sociality model without caste-specific extrinsic mortality but equally high extrinsic mortality in both castes; **d**) the intermediate sociality model without the effect of extrinsic mortality; **e**) the intermediate sociality model with caste-specific extrinsic mortality; **f**) the intermediate sociality model without caste-specific extrinsic mortality but equally high extrinsic mortality in both castes; **g**) the high sociality model without the effect of extrinsic mortality; **h**) the high sociality model with caste-specific extrinsic mortality; **i**) the high sociality model without caste-specific extrinsic mortality but equally high extrinsic mortality in both castes. Curves

represent mean values. Shaded areas represent 95% confidence intervals. Red represents the reproductive and blue represents the worker.