Social modulation of ageing in termites

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Chapter 1

Introduction

1.1 Why do organisms age?

Ageing, or senescence, defined as the physiological decline of biological functions over time, is associated with declining survival and fertility (Kirkwood & Rose, 1991). Cellular senescence was first described by Hayflick and Moorhead (1961) when they discovered that fibroblast cells of humans have a limited lifespan in vitro (Pawlikowski *et al.*, 2013). Since then, ageing has been recognized as a nearly ubiquitous phenomenon that is spread out across a wide range of taxa (Comfort, 1979; Jones & Vaupel, 2017). In humans, ageing is associated with many age-related diseases, such as cancer and diabetes (López-Otín *et al.*, 2013). Understanding the causes of ageing is of vital importance to controlling health and disease.

Mechanisms of ageing

Ageing is proximately caused by damage to molecules and tissues on a molecular level (Partridge & Gems, 2002). For example, during aerobic metabolism, the human body generates reactive oxygen species (ROS), which can damage lipids, proteins, and DNA (Schieber & Chandel, 2014). Heat shock proteins can repair damage, particularly DNA damage, and antioxidants can detoxify reactive oxygen species (ROS). Superoxide dismutase, glutathione peroxidase, and catalase are antioxidant enzymes that can catalyse ROS detoxification (Speakman *et al.*, 2002). However, oxidative stress causes ageing when cells produce too many ROS or are insufficient in detoxifying ROS and repairing the damage (Tasaki *et al.*, 2021). Other mechanisms, such as telomere attrition and loss of proteostasis, can also cause DNA and protein damage, and have been extensively reviewed in López-Otn *et al*. (2013). As a result, they are not addressed here.

The rate of ageing is determined by the body's ability to repair damage. The conserved nutrient-sensing pathways: insulin/insulin-like growth factor-1 signalling (IIS) and the target of rapamycin (TOR) are the most well-studied genetic pathways that modulate ageing (Partridge *et al.*, 2011; López-Otín *et al.*, 2013; Flatt & Partridge, 2018). The IIS/TOR pathways detect nutrient availability, such as glucose and amino acids, and are involved in key life

events like growth and reproduction in organisms (Flatt & Partridge, 2018). Surprisingly, reduced IIS/TOR signalling can activate cellular detoxification pathways such as autophagy and protein synthesis, extending longevity in a variety of model organisms such as the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* (Partridge *et al.*, 2011).

Understanding the mechanisms of ageing is important for finding ways to delay ageing, but it is not sufficient because these mechanisms are frequently interconnected with other life history traits, and changes in them can have unintended consequences. For example, dietary restriction that downregulates the IIS pathway increases lifespan but decreases reproduction (Moatt *et al.*, 2016). To find ways to safely extend lifespans with the fewest side effects, it is also necessary to understand the evolutionary causes of ageing and how they are linked to other life history traits.

Evolution theories of ageing

Why ageing evolves is a fascinating yet complex question. Ageing is an evolutionary paradox because it reduces an individual's reproductive success and, as a result, should not be favoured by natural selection. The search for an evolutionary explanation for why ageing can persist in the face of natural selection began in the nineteenth century. August Weismann was a pioneer thinker in the evolution of ageing. He suggested that ageing evolved as a result of renunciation of resources from soma maintenance to germ reproduction (reviewed in Kirkwood & Cremer 1982). Other early explanations for why ageing evolves are frequently caught in circular arguments (Kirkwood, 1977). Ageing, for example, is necessary to rid the world of the elderly and free up resources for the young. The argument only makes sense assuming that the elderly experience reproductive cessation, which is a phenomenon of ageing itself (Kirkwood, 1977).

Modern evolutionary theories of ageing are established based on two important observations by J. B. S. Haldane (Partridge & Gems, 2002) and Peter Medawar. Haldane (1941) observed that dominant lethal mutations can be maintained in the population if their effects only occur after reproduction.

Based on this idea, he further proposed that weak selection in late life is the cause of ageing (Partridge & Gems, 2002), although he did not explain why selection should be weak in late life. This question was addressed by Medawar (1952), who observed that the chance that individuals survive until an advanced age is slim in the wild due to random extrinsic hazards. Therefore, the force of selection should be strong in early life and decrease with age. This arguably creates a shadow of selection against genes that are detrimental in late life. These abstract ideas were first formulated mathematically by Hamilton (1966) and later developed by Charlesworth (2000). They described how the force of natural selection operating on the survival rate and fecundity declines with progressive age, which provides the framework for evolutionary theories of ageing.

In a nutshell, ageing is a result of natural selection postponing the onset of deleterious effects of genes. A developed evolutionary theory of ageing mainly contains three components that explain the mechanisms that enable the deleterious effects. The mutation accumulation theory (MA) states that the deleterious effects result from the accumulation of deleterious mutations of small effects in late life (Medawar, 1952). The antagonistic pleiotropy theory (AP) states that the deleterious effects result from genes that are beneficial in early life but harmful in late life (Williams, 1957). The disposable soma theory (DS) states that the deleterious effects result from insufficient energy allocation to self-maintenance/soma due to the trade-off with reproduction (Kirkwood, 1977, 2017). These mechanisms are not mutually exclusive but rather complementary to each other and help us understand the complexity of ageing in nature.

Although evolutionary theories of ageing appear to have described the principles of why, ultimately, ageing evolves, the key question remains: how can we delay or even reverse ageing? To answer this question, we need to:

1) identify factors that affect the rate of ageing,
2) investigate the mechanisms underpinning slower rates of ageing, and
3) understand the evolutionary forces that drive a slower rate of ageing.

1.2 How to not age?

Remarkably, the pace and trajectory of ageing differ tremendously across taxa (Jones *et al.*, 2014a), suggesting that ageing is not inevitable (Jones & Vaupel, 2017). First, ecological factors such as nutrition intake and extrinsic hazards (e.g., predation and disease) can modify the rate of ageing. For example, dietary restriction, defined as reduced food intake without malnutrition, has extended the lifespan of many organisms (Savola *et al.*, 2021). And in humans, the average lifespan has greatly increased, accompanied by a radical decrease in the extrinsic hazards we are exposed to (Vaupel *et al.*, 2021). Second, accumulative evidence suggests that ageing is not programmed (Flatt & Partridge, 2018). Mechanistically, ageing can be delayed by preventing or repairing damage caused by various biological processes (Kirkwood, 2005). With advancements in molecular technology, a large number of genes regulating longevity have been uncovered in model organisms like the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* (Kirkwood, 2005). By manipulating the expression of these genes, the rate of ageing can be controlled.

However, according to the DS theory, the extension of the lifespan inevitably involves some side-effects on reproduction. The price of a long lifespan might be a slow rate of growth and reproduction. To overcome this trade-off, attention needs to be drawn to the long-lived animals in nature that are able to escape ageing but also reproduce successfully. In freshwater polyps of the genus *Hydra*, survival and reproductive rate do not decline with age and they are believed to be immortal due to their remarkable ability of regenerating new cells from stem cells (Martínez, 1998; Martínez & Bridge, 2012). Other well-known non-senescent animals show striking links to sociality. For example, naked mole rats live a social life that is similar to that of social insects (i.e., ants, bees, and termites) and show no decline in survival over time (Ruby *et al.*, 2018). Queens can produce up to 900 pups throughout their lifetime while enjoying a long lifespan, indicating an uncoupling of the longevity-fecundity trade-off (Flatt & Partridge, 2018). This uncoupling is further amplified in social insects. Insect queens (or kings in termites) are

characterised by exceptional longevity (up to several decades) and high fecundity (up to 20000 eggs per day in termites), defying the almost universal longevity-fecundity trade-off in the animal kingdom.

Social life seems to contribute to achieving a long lifespan without reproductive cost, but how and why it does so remains elusive. Recently, genomic studies in honeybees and naked mole-rats have begun to unravel the genetic pathways that link nutrition and social stimuli to pro-longevity mechanisms (Korb & Heinze, 2021). However, much less is known about other social organisms, particularly termites.

Plagued by bad press, termites often impress people as pests that destroy properties and cause economic damage. However, their bad reputation is only attributed to a few destructive species. The majority of termite species occupy an important ecological niche as decomposers. In the African savanna, the thermo-regulated termite mounds also offer shelter for some ants and small reptiles (Korb, 2007a). Termites are therefore both ecologically and economically important species to study.

Termites are believed to be the oldest social insects that evolved a social life 130 million years ago (Korb, 2007a). Sharing ancestry with cockroaches, termites are also known as “social cockroaches”, belonging to the infraorder Isoptera (Korb, 2007a). They are hemimetabolous insects that have evolved sociality independently from social Hymenoptera and have a diplo-diploid sex determination system. Due to the presence of sterile soldiers, all termites are eusocial, but they differ in the degree of social complexity and lifestyles. In **Fig. 1.1**, I show termites with different social complexities along the three steps of major evolutionary transitions: group formation, group maintenance, and group transformation (Korb, 2010; Korb & Heinze, 2016; Bernadou *et al.*, 2021).

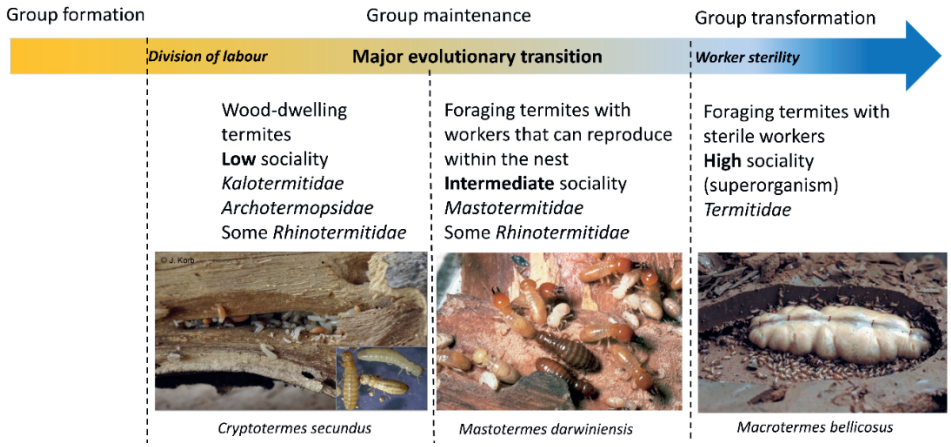

Figure 1.1 The sociality of termites along the steps of major evolutionary transitions. The *M. darwiniensis* photo is attributed to Dr. Barbara L. Thorne and adapted from Lewis (2009). The *C. secundus* and *M. bellicosus* photos are attributed to Dr. Judith Korb.

Termite societies are characterised by a reproductive division of labour. Within a colony, the queen and the king monopolise reproduction while their nestmates forgo reproduction either temporarily (e.g., workers in lower termites) or permanently (i.e., soldiers and workers in higher termites), depending on their lifestyles and social complexity. Although all individuals share a similar genetic background, the reproductives in colonies outlive the non-reproductives, leading to the apparent reversal of the longevity-fecundity trade-off (Korb & Heinze, 2021). This reversal also varies across species with different degrees of social complexity (Monroy Kuhn *et al.*, 2019). For these reasons, termites represent excellent model organisms for studying the evolutionary causes of ageing, particularly the effect of sociality on ageing.

1.3 Outstanding questions

The aim of this thesis is to understand how and why sociality changes the longevity-fecundity trade-off in termites. To this end, I asked myself three questions and tried to provide answers to them in the respective chapters.

1) What is the molecular basis underlying the long life and high fecundity of termite queens?
2) Is there a trade-off at the colony level if we cannot see it at the individual level?
3) Why do termite queens and kings live longer than workers?

1.4 Outline of this thesis

This thesis contains three main results chapters.

In **Chapter 2**, using weighted gene co-expression network analysis, I uncover a gene network (the queen central module, QCM) that characterises the queen. QCM encompasses genes involved in nutrient-sensing, fecundity, juvenile hormone (JH) signalling, and chemical communication. It contains many genes that are known to regulate longevity and fecundity, as well as genes that are involved in chemical communications. A further experiment that reduced JH in queens yielded negative effects on genes involved in chemical communication and fecundity, suggesting that the hypothesis of re-wiring along the nutrient-sensing/endocrine/fecundity axis cannot explain why social termite queens are long-lived. However, these results revealed striking links between social communication and the longevity-fecundity trade-off in termite queens.

In **Chapter 3**, I restricted food availability in a termite to investigate how it affects the maintenance-reproduction trade-off for individuals and colonies. I detected a shift from maintenance to reproduction at the colony level as workers gave up cooperation and developed into winged sexuals. These

findings show how sociality interacts with resource availability and modulates central life history trade-offs. They also reveal striking analogies between social insects with low social complexity and multicellular organisms with low biological complexity, like *Hydra*. These results indicate that the longevity-fecundity trade-off might have shifted to a colony level in social insects.

In **Chapter 4**, to disentangle the ultimate causes of caste-specific ageing in termites according to the mutation accumulation theory of ageing, I used individual-based simulations and let ageing evolve in different scenarios of sociality. The results demonstrate a critical and causal role of worker reproductive potential and caste-specific extrinsic mortality in explaining the ageing divergency between reproductives and workers. Moreover, for the first time, it generates testable predictions of ageing patterns in termites based on the degree of sociality.

Chapter 2

Live fast, die old: what makes a queen?

Title: Transcriptomic analyses of the termite, *Cryptotermes secundus*, reveal a gene network underlying a long lifespan and high fecundity

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Abstract

Organisms are typically characterized by a trade-off between fecundity and longevity. Notable exceptions are social insects. In insect colonies, the reproducing caste (queens) outlive their non-reproducing nestmate workers by orders of magnitude and realize fecundities and lifespans unparalleled among insects. How this is achieved is not understood. Here, we identified a single module of co-expressed genes that characterized queens in the termite species *Cryptotermes secundus*. It encompassed genes from all essential pathways known to be involved in life-history regulation in solitary model organisms. By manipulating its endocrine component, we tested the recent hypothesis that re-wiring along the nutrient-sensing / endocrine / fecundity axis can account for the reversal of the fecundity / longevity trade-off in social insect queens. Our data from termites do not support this hypothesis. However, they revealed striking links to social communication that offer new avenues to understand the re-modelling of the fecundity / longevity trade-off in social insects.