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Ageing and somatic maintenance in social insects

Eric R Lucas and Laurent Keller

Social insects offer exciting prospects for ageing research due to the striking differences in lifespan among castes, with queens living up to an order of magnitude longer than workers. A popular theory is that senescence is primarily the result of an accumulation of somatic damage with age, balanced by investment into processes of somatic maintenance. Investigation of these predictions in social insects has produced mixed results: neither damage accumulation nor investment into somatic maintenance is consistently different between castes with different lifespans. We discuss some limitations of the studies conducted thus far and consider an alternative proximate theory of ageing that has been recently proposed.

Addresses
Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland

Corresponding author: Lucas, Eric R (eric.lucas@unil.ch)

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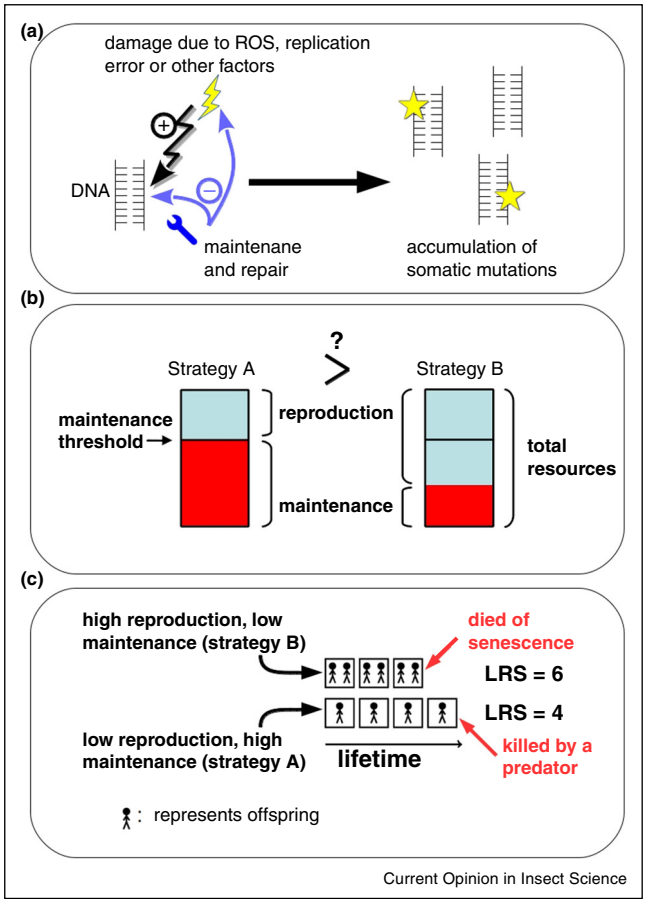
Introduction

Social insects capture the imagination because of their organisation, their division of labour and the fact that through force of numbers, small creatures can achieve impressive cooperative feats of engineering. Something that is under-appreciated is that they also provide an ideal system to study the mechanisms of ageing. The evolution of morphologically or behaviourally specialised castes is associated with great variation in lifespan, despite the fact that different castes can arise from the same genome [1]. Queens (the reproductive caste) have been recorded to live as long as 29 years [2], making them the longest-lived adult insects that we know of. Workers (which engage in nest maintenance and brood care) are substantially less long-lived [3] even in laboratory conditions where they are protected from extrinsic sources of mortality such as predation. Furthermore, variation in lifespan also exists among worker castes that specialise in different tasks [4,5]. Increasing efforts have therefore been devoted to studying these differences in lifespan, and many have focused on the possibility that they are due to differences in senescence linked to molecular damage accumulation and somatic maintenance.

Senescence can be regarded as a deterioration of function with age, typified by an increase in mortality and a decline in functions such as reproduction. The underlying causes of senescence are not fully understood, but one possibility is that it is due to an accumulation of physiological damage with age, for example as oxidative damage to macromolecules [6]. Somatic maintenance is the process of expending energy to avoid or repair damage, thus preserving the integrity of the organism (Figure 1a). The 'disposable soma' theory [7] predicts that investing sufficiently into somatic maintenance to avoid completely the accumulation of damage is not an optimal life-history strategy because organisms do better by allowing some amount of deterioration in order to invest extra energy into reproduction (Figure 1b and c); senescence is then the manifestation of this incomplete maintenance. The optimal allocation of energy to somatic maintenance will depend on the rate of mortality from extrinsic factors such as predation and accidental death [7] (Figure 1c). Social insect queens typically have low rates of extrinsic mortality because of the care that they receive from the workers and the protected environment provided by their nest. From an evolutionary perspective, the long lifespan of social insect queens may thus be explained by slow senescence due to their low extrinsic mortality. The physiological prediction of the disposable soma theory is that, all else being equal, long-lived phenotypes such as queens should invest more into processes of maintenance than short-lived phenotypes. The challenge is to identify the type of damage that underlies senescence and the processes that can mitigate this damage.

Most of the research on somatic maintenance has focused on damage to macromolecules because of the popular view that the most important form of damage for senescence is molecular oxidative stress [6,8]. Cellular processes such as mitochondrial respiration create Reactive Oxygen Species (ROS) [9] which can cause oxidative damage to a range of macromolecules, including DNA, proteins and lipids [8], and several processes have been identified that can remove ROS or deal with oxidative damage [9–11]. In this review, we summarise research into damage accumulation and somatic maintenance in social insects and discuss whether results so far support a role for these processes in explaining differences in lifespan between castes. We examine how the data support the following three predictions: that molecular damage

Figure 1



Somatic maintenance and the disposable soma theory. **(a)** Illustration of somatic maintenance at the level of DNA damage and repair. Mutations in DNA occur, for example due to replication errors or Reactive Oxygen Species (ROS). Maintenance can act to control the levels of ROS or repair damaged DNA. The balance between DNA damage and maintenance determines the rate of accumulation of somatic mutations with age. **(b)** Alternative investment strategies into maintenance and reproduction. Boxes represent total available resources, red is the investment into maintenance and blue is the investment into reproduction. The maintenance threshold is the investment that is necessary to avoid senescence. Even assuming an organism is able to invest sufficiently into processes of somatic maintenance to completely avoid senescence, this strategy will only evolve if it leads to higher fitness than an alternative in which some deterioration is allowed in order to increase reproduction. We therefore need to determine the optimal allocation of resources to maintenance. **(c)** This cartoon shows how investing into greater reproduction at the expense of some somatic maintenance can be a successful strategy (LRS = Lifetime Reproductive Success). This is because the risk of death from external factors means that for the most part, individuals will not live long enough to enjoy the benefits of their extended vitality (in the wild, few individuals reach the age at which senescence becomes marked). The disposable soma theory proposes that investing sufficiently into somatic maintenance to entirely avoid senescence is not an optimal strategy.

accumulates with age, that it does so more slowly in longer-lived castes, and that longer-lived castes show higher investment into processes of somatic maintenance such as antioxidant systems and DNA repair.

Accumulation of damage with age

When investigating the effect of age in social insects, it is important to consider that in many species workers undergo an age-related transition in behavioural caste, from a stage where they predominantly stay in the nest to care for the queen and brood, to foraging where they leave the nest to collect resources [1,12,13]. Care must therefore be taken not confuse the effects of age and behavioural caste on the trait of interest. Furthermore, if damage accumulates more quickly in one behavioural caste, then time since a caste transition may be more relevant to senescence than chronological age. The term 'foraging age' therefore refers to the time since an individual's transition from nursing to foraging.

A range of types of molecular damage has been investigated. Oxidative damage to proteins and lipids results in the formation of protein carbonyls and lipid peroxidation, which can be detected *in vitro* [14–16]. The incomplete degradation of lipids and proteins due to oxidative damage can result in the accumulation of lipofuscins, which can be detected under laser-scanning microscopy [17]. Furthermore, the accumulation of misfolded or damaged proteins tagged with ubiquitin for degradation can be measured by performing a Western Blot for ubiquitin [18••]. Damage can also occur to DNA, either through mutations or physical breaks in the chromatin [10].

Overall, the evidence for damage accumulation in social insects is equivocal, with some results supporting the notion that damage accumulates with age while others have found no increase, or even evidence of a decrease with age. In the honeybee, analyses of whole worker heads and thoraces revealed no increase with age in the levels of protein carbonyls [15]. Furthermore, the abundance of ubiquitinated proteins or lipid peroxidation does not increase with foraging age in the brain [18••]. In fact, when foragers were allowed unrestricted flight, the lipid peroxidation marker was lower in old foragers than in young foragers. Similarly, mitochondrial DNA damage does not seem to increase with foraging age in honeybee worker brains. Of two measures of damage that were used, one revealed no change with age, while the other decreased with age [19•].

In contrast, studies in specific tissues revealed age-linked accumulation of damage. In the hypopharyngeal glands of honeybee foragers, lipofuscin was found to accumulate with age [16]. Similarly, in the abdominal fat cells and trophocytes of honeybee queens, the levels of lipofuscin, protein carbonyls and lipid peroxidation also increased with age [20]. Similar results were obtained for workers [21], but age was confounded with behavioural caste in this study.

The discrepancy between the results of these different studies suggests that damage accumulates at different

rates in different tissues. It is likely that the impact of damage on fitness varies with the type of damage and the tissue in which it occurs. It would therefore be valuable to conduct studies comparing the accumulation of damage in different tissues and determine whether damage accumulates faster in tissues that are less important for an organism's performance and survival.

Difference in the rate of accumulation of somatic damage between castes with different lifespans

The greatest disparity in lifespan in social insects is the one between queens and workers. This contrast would therefore offer the strongest test of whether differential damage accumulation accompanies lifespan differences between castes. Unfortunately, we know of no statistical comparisons of the age-related accumulation of molecular damage between queens and workers, although the low levels of polyunsaturated fatty acids in queens should make them less susceptible to lipid peroxidation [22].

Among workers, comparisons of damage have been performed in honeybees, where three behavioural castes exist. During the brood-rearing season, workers may be nurses or foragers, but during the winter months, when brood-rearing does not occur, workers become so-called 'winter bees'. These bees engage in neither foraging nor brood care, but instead work to maintain colony temperature during the winter [23]. They have the longest life expectancy of the three worker behavioural castes, while foragers have the shortest [4,24•].

Among these behavioural castes, there is tentative evidence that short life expectancy is associated with a greater molecular damage load. However, whether this is due to a difference in the rate of accumulation of damage with age or simply represents a stable difference in damage levels between behavioural castes remains an open question and further studies are needed to resolve this. In localised areas of the honeybee head, damaged proteins and lipids tend to be higher in foragers than in the nurses and winter bees. Lipofuscin levels in the hypopharyngeal glands and pars intercerebralis were found to be higher in foragers than in winter bees of similar age, while no difference was found between these castes in the calyx [24•]. Seehuus *et al.* [14] used staining and microscopy to investigate protein carbonyls and nitrotyrosine (an indicator of nitration damage to proteins) in three areas of the honeybee brain. While in general they found little evidence of either type of damage, there was a detectable amount of carbonylated proteins in the optic lobe, which was significantly greater in foragers than nurses and winter bees.

Levels of DNA damage between castes have been little-investigated. A pilot study using only one pool of DNA per behavioural caste found higher levels of DNA mutations (measured as the similarity of sequences with the honeybee mitochondrial genome) in the wing muscles of foragers compared to winter bees of at least the same age [25]. Further work is needed to confirm this.

To the best of our knowledge, only two studies have investigated age-related accumulation of damage in different castes. In one of these studies, lipofuscin was found to accumulate with age in the hypopharyngeal glands of honeybee foragers but not nurses [16], despite foragers and nurses in this study being matched for age. However, a study of protein carbonyl content from whole-tissue extracts of honeybee heads and thoraces found no evidence that either nurses or foragers show an accumulation of this form of damage with age [15]. These contrasting results may be due to the fact that the second study investigated whole brains rather than more localised tissues. As differences in protein damage levels between foragers and other behavioural castes vary locally [14], whole tissue analyses may be too coarse to detect important differences.

Is there a difference in investment into somatic maintenance between castes?

A slower accumulation of oxidative damage in longer-lived phenotypes could be due to a lower rate of production of ROS. There is some support for this possibility in vertebrates, where interspecific comparisons have revealed that longer-lived organisms have lower rates of hydrogen peroxide production [26] (but see Ref. [27]). To the best of our knowledge, this has not been investigated in social insects. However, evidence that queens are more resistant to induced oxidative stress than workers [28•] suggests that queens have some mechanism which make them better able to deal with ROS. The most important prediction of the disposable soma theory is that the higher rate of damage accumulation in short-lived organisms stems from reduced energetic investment into processes of maintenance. Longer-lived castes should therefore be those that have the highest expression of antioxidant and molecular repair genes. Studies in two ant species and in the honeybee did not find support for this hypothesis.

In ants, antioxidant activity has been compared between queens and workers. Contrary to predictions, workers and males were found to have higher superoxide dismutase activity than queens in the ant *Lasius niger* [29]. In *Harpegnathos saltator*, some workers can become reproductive workers (gamergates) after the queen's death. This transition has been shown to be associated with increased lifespan and higher resistance to stress. However, the longer-lived gamergates do not have higher activity of superoxide dismutase and glutathione peroxidase, and have even less catalase activity, than the short-lived workers [28•]. A shortcoming of both studies is that they did not control for age.

In honeybees, comparisons have been made both among worker behavioural castes and between queens and workers. Among workers, two non age-controlled studies found no support for the prediction that behavioural castes with shorter life expectancies under-express antioxidants. Expression of antioxidant genes was found to be higher in foragers than nurses [30], while a study of whole-body protein composition found that, out of five detected antioxidant proteins, two were upregulated in foragers and one was upregulated in nurses [31]. An age-controlled comparison between workers and queens revealed that young queens had higher levels of overall antioxidant gene expression than young workers, while the opposite was true in older individuals [30]. This age effect may be due to the older workers being foragers rather than nurses; the increased expression of antioxidant genes may therefore be due to the energetic demands of flight activity.

Finally, there is also only limited support for the hypothesis that longer-lived castes invest more into repairing damage after it occurs. While a whole-transcriptome RNA sequencing study in honeybees revealed that winter bees have a higher expression of DNA repair genes than do nurses and foragers [32], a comparison between queens and workers revealed that only one out of nine DNA repair genes was significantly differentially expressed between the two castes at all ages and that this gene was upregulated in workers relative to queens [25].

What next?

Overall, the available data do not provide much support for the view that lifespan differences among social insect castes are primarily driven by differential somatic maintenance of oxidative damage. However, as the data are still limited it would be premature to conclude that the disposable soma theory and variation in somatic maintenance between individuals with contrasting lifespan are irrelevant to patterns of social insect lifespan. Few studies have so far investigated the accumulation of DNA damage and mutations, while studies of damage accumulation have compared different worker behavioural castes, but not queens and workers, and have focused primarily on the honeybee.

In model organisms such as flies, nematodes and mice, there have also been mixed results concerning the relevance of molecular damage to senescence [11,33,34] (but see Refs. [35,36]). This has led to the proposition of a new proximate theory of ageing [37], which could have implications for social insects [38]. The hyperfunction theory proposes that processes necessary for growth or reproduction, typically regulated by nutrient-sensitive pathways including Target of Rapamycin (TOR), fail to become downregulated in older individuals and lead to over-accumulation of biogenic products and deleterious effects [39•,40]. Senescence is therefore due to an excess of products that played important roles in a previous life stage, such as lipids and yolk [41]. Lifespan should therefore be linked to the rate at which development progresses. For example, it has been suggested that dietary restriction prolongs lifespan by slowing the rate of growth and maturation [42]. Under this scenario, the short lifespan of workers should be associated with a faster progression of age-linked profiles of physiological traits. This could be tested by comparing proteomic or transcriptomic changes that accompany ageing in both workers and queens, and asking whether these changes occur at different rates. However, a difficulty in applying the hyperfunction theory to social insects comes from the behavioural caste transitions which many species undergo. Hyperfunction is typically applied to processes of growth and development which result in the accumulation of products causing senescence in adulthood [40]. However, senescence in at least one feature (learning ability) is slower in honeybee nurses than in foragers [16,43] and appears to be reversed if foragers revert to nursing [44]. How can hyperfunction theory explain this? Senescence in foragers might be the result of hyperfunction of processes involved in the behavioural caste transition, rather than in development to adulthood. The changes in the levels of proteins and hormones that occur during this transition may continue, deleteriously, after transition is complete. The reverse transition from foraging to nursing may then act to reverse these trajectories and the senescence which accompanied them. Nutrient signalling has been linked to the nurse-forager transition [45], thus providing a parallel with many of the processes that have been proposed to be involved in hyperfunction.

This possibility has two implications. First, while senescence is minimal in the nurse stage, it can be detected [46]. If this senescence is due to the accumulation of products that will subsequently be decreased during the transition to foraging, then this transition should also be associated with a transient reversal of senescence. Second, pre-foraging and post-foraging nurses should develop different senescent pathologies. This is because the physiological trajectory from foraging to nursing is likely to be different to that from larval development to nursing, leading to the accumulation of different products in the two groups.

In conclusion, social insects are increasingly recognised as an interesting system to study patterns of ageing. An important limitation of many of the studies that have been performed is the lack of genetic tool to manipulate patterns of gene expression between individuals with contrasting lifespan. Progress in social insect genomics and transcriptomics, as well as gene manipulation technologies such as RNA interference (RNAi), now offer opportunities to use these insects to study how genetic and environmental contributions interact to control ageing.

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References

1. Wilson EO: *The insect societies*. Cambridge, MA: Bellknap Press of Harvard University Press; 1971, .

2. Hölldobler B, Wilson EO: *The ants*. Berlin: Springer-Verlag; 1990, .

3. Kramer BH, Schaible R: **Colony size explains the lifespan differences between queens and workers in eusocial Hymenoptera**. *Biol J Linn Soc* 2013, **109(3)**:710-724.

4. Fluri P: **How long do worker honeybees live?** *Schweizerische Bienen Zeitung* 1990, **113(11)**:620-625.

5. Chapuisat M, Keller L: **Division of labour influences the rate of ageing in weaver ant workers**. *Proc R Soc B-Biol Sci* 2002, **269(1494)**:909-913.

6. Harman D: **Free-radical theory of aging**. *Mutat Res-DNA Aging* 1992, **275(3–6)**:257-266.

7. Kirkwood TBL, Holliday R: **Evolution of ageing and longevity**. *Proc R Soc London Ser B-Biol Sci* 1979, **205(1161)**:531-546.

8. Shringarpure R, Davies KJA: **Free radicals and oxidative stress in aging**. In *Handbook of theories of aging*. Edited by Bengston VL, Gans D, Putney NM, Silverstein M. New York: Springer; 2009: 229-243.

9. Finkel T, Holbrook NJ: **Oxidants, oxidative stress and the biology of ageing**. *Nature* 2000, **408(6809)**:239-247.

10. Lombard DB, Chua KF, Mostoslavsky R, Franco S, Gostissa M, Alt FW: **DNA repair, genome stability, and aging**. *Cell* 2005, **120(4)**:497-512.

11. Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H: **Trends in oxidative aging theories**. *Free Radic Biol Med* 2007, **43(4)**:477-503.

12. Robinson GE: **Regulation of division of labor in insect societies**. *Ann Rev Entomol* 1992, **37(1)**:637-665.

13. Mersch DP, Crespi A, Keller L: **Tracking individuals shows spatial fidelity is a key regulator of ant social organization**. *Science* 2013, **340(6136)**:1090-1093.

14. Seehuus S, Krekling T, Amdam GV: **Cellular senescence in honey bee brain is largely independent of chronological age**. *Exp Gerontol* 2006, **41(11)**:1117-1125.

15. Williams JB, Roberts SP, Elekonich MM: **Age and natural metabolically-intensive behavior affect oxidative stress and antioxidant mechanisms**. *Exp Gerontol* 2008, **43(6)**:538-549.

16. Münch D, Baker N, Rasmussen EMK, Shah AK, Kreibich CD, Heidem LE, Amdam GV: **Obtaining specimens with slowed, accelerated and reversed aging in the honey bee model**. *JoVE* 2013, **78**:e50550.

17. Terman A, Brunk UT, Lipofuscin: **Int J Biochem Cell Biol**2004, **36(8)**:1400-1404.

18. •• Tolfsen CC, Baker N, Kreibich C, Amdam G: **Flight restriction prevents associative learning deficits but not changes in brain protein-adduct formation during honeybee ageing**. *J Exp Biol* 2011, **214(8)**:1322-1332.
An elegant experiment restricting flight opportunities finds evidence that flight activity, rather than age, is the primary cause of senescence in learning ability in honeybees. However, flight activity was associated with lower levels of lipid peroxidation.

19. • Hystad EM, Amdam GV, Eide L: **Mitochondrial D.N.A. integrity changes with age but does not correlate with learning performance in honey bees**. *Exp Gerontol* 2014, **49**:12-18.
Investigates levels of DNA integrity in honeybee foragers and finds that DNA damage decreases with foraging age.

20. Hsieh Y, Hsu C: **The changes of age-related molecules in the trophocytes and fat cells of queen honeybees (*Apis mellifera*)**. *Apidologie* 2011, **42(6)**:728-739.

21. Hsieh Y, Hsu C: **Honeybee trophocytes and fat cells as target cells for cellular senescence studies**. *Exp Gerontol* 2011, **46(4)**:233-240.

22. Haddad LS, Kelbert L, Hulbert AJ: **Extended longevity of queen honey bees compared to workers is associated with peroxidation-resistant membranes**. *Exp Gerontol* 2007, **42(7)**:601-609.

23. Stabentheiner A, Pressl H, Papst T, Hrassnigg N, Crailsheim K: **Endothermic heat production in honeybee winter clusters**. *J Exp Biol* 2003, **206(2)**:353-358.

24. • Münch D, Kreibich CD, Amdam GV: **Aging and its modulation in a long-lived worker caste of the honey bee**. *J Exp Biol* 2013, **216(9)**:1638-1649.
Diutinus bees do not show signs of senescent decline in learning despite their age, but begin to decline after becoming foragers. Patterns of variation in lipofuscin levels among castes and ages differ between tissue types.

25. Aamodt RM: **Age- and caste-dependent decrease in expression of genes maintaining DNA and RNA quality and mitochondrial integrity in the honeybee wing muscle**. *Exp Gerontol* 2009, **44(9)**:586-593.

26. Lambert AJ, Boysen HM, Buckingham JA, Yang T, Podlutsky A, Austad SN, Kunz TH, Buffenstein R, Brand MD: **Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms**. *Aging Cell* 2007, **6(5)**:607-618.

27. Montgomery MK, Hulbert AJ, Buttemer WA: **Does the oxidative stress theory of aging explain longevity differences in birds? I. Mitochondrial ROS production**. *Exp Gerontol* 2012, **47(3)**:203-210.

28. • Schneider SA, Schrader C, Wagner AE, Boesch-Saadatmandi C, Liebig J, Rimbach G, Roeder T: **Stress resistance and longevity are not directly linked to levels of enzymatic antioxidants in the ponerine ant *Harpegnathos saltator***. *PLoS ONE* 2011, **6(1)**:e14601.
An investigation into differences in stress resistance and antioxidant systems in callows, workers, gamergates and isolated workers in a species in which workers can replace the queen and become reproductive.

29. Parker JD, Parker KM, Sohal BH, Sohal RS, Keller L: **Decreased expression of Cu–Zn superoxide dismutase 1 in ants with extreme lifespan**. *Proc Natl Acad Sci U S A* 2004, **101(10)**:3486-3489.

30. Corona M, Hughes KA, Weaver DB, Robinson GE: **Gene expression patterns associated with queen honey bee longevity**. *Mech Ageing Dev* 2005, **126(11)**:1230-1238.

31. Wolschin F, Amdam GV: **Comparative proteomics reveal characteristics of life-history transitions in a social insect**. *Proteome Sci* 2007, **5(10)**.

32. Seehuus S, Taylor S, Petersen K, Aamodt RM: **Somatic maintenance resources in the honeybee worker fat body are distributed to withstand the most life-threatening challenges at each life stage**. *PLOS ONE* 2013, **8(8)**:e69870.

33. Gems D, Doonan R: **Antioxidant defense and aging in *C. elegans***. *Cell Cycle* 2009, **8(11)**:1681-1687.

34. Edrey YH, Salmon AB: **Revisiting an age-old question regarding oxidative stress**. *Free Radic Biol Med* 2014, **71**:368-378.

35. Liochev SI: **Reactive oxygen species and the free radical theory of aging**. *Free Radic Biol Med* 2013, **60**:1-4.

36. Zimniak P: **What is the proximal cause of aging?** *Front Genet* 2012, **3** Article 189.

37. Blagosklonny MV: **Answering the ultimate question "what is the proximal cause of aging?"**. *Aging* 2012, **4(12)**:861.

38. Parker JD: **What are social insects telling us about aging?** *Myrmecol News* 2010, **13**:103-110.

39. • Gems D, Partridge L: **Genetics of longevity in model organisms: debates and paradigm shifts**. *Annu Rev Physiol* 2013, **75**:621-644.
A consideration of the damage accumulation and hyperfunction theories of aging.

40. Gems D, de la Guardia Y: **Alternative perspectives on aging in *Caenorhabditis elegans*: reactive oxygen species or hyperfunction?** *Antioxid Redox Signal* 2013, **19(3)**:321-329.

41. Ackerman D, Gems D: **The mystery of *C. elegans* aging: an emerging role for fat**. *BioEssays* 2012, **34(6)**:466-471.

42. Blagosklonny MV: **MTOR-driven quasi-programmed aging as a disposable soma theory**. *Cell Cycle* 2013, **12(12)**:1842-1847.

43. Behrends A, Scheiner R, Baker N, Amdam GV: **Cognitive aging is linked to social role in honey bees (*Apis mellifera*)**. *Exp Gerontol* 2007, **42(12)**:1146-1153.

44. Baker N, Wolschin F, Amdam GV: **Age-related learning deficits can be reversible in honeybees *Apis mellifera***. *Exp Gerontol* 2012, **47(10)**:764-772.

45. Ament SA, Corona M, Pollock HS, Robinson GE: **Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies**. *Proc Natl Acad Sci U S A* 2008, **105(11)**:4226-4231.

46. Remolina SC, Hafez DM, Robinson GE, Hughes KA: **Senescence in the worker honey bee *Apis mellifera***. *J Insect Physiol* 2007, **53(10)**:1027-1033.