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Review article

Long live the queen: studying aging in social insects

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Abstract

Aging is a fascinating, albeit controversial, chapter in biology. Few other subjects have elicited more than a century of ever-increasing scientific interest. In this review, we discuss studies on aging in social insects, a group of species that includes ants and termites, as well as certain bee and wasp species. One striking feature of social insects is the lifespan of queens (reproductive females), which can reach nearly 30 years in some ant species. This is over 100 times the average lifespan of solitary insects. Moreover, there is a tremendous variation in lifespan among castes, with queens living up to 500 times longer than males and 10 times longer than workers (non-reproductive individuals). This lifespan polymorphism has allowed researchers to test the evolutionary theory of aging and – more recently – to investigate the proximate causes of aging. The originality of these studies lies in their use of naturally evolved systems to address questions related to aging and lifespan determination that cannot be answered using the conventional model organisms.

Introduction

The question of why organisms age and die is an old one. Much has been written about it by scientists, philosophers and poets, and throughout history humans have been fascinated by the perspective of a prolonged life. Despite this fundamental interest, our understanding of the forces that drive the evolution of a given lifespan and rate of aging is still limited, and we have only started to get a sense of the genes and metabolic pathways involved in these processes.

While there is no universally accepted definition, most biologists probably think of aging as a series of physiological and morphological changes that progressively increase an organism's risk of dying from non-environmental (intrinsic) causes and decrease its late-life fecundity. The fact that this process is so obviously detrimental to fitness and yet so widespread among sexually reproducing organisms raises some intriguing questions: Why and how has aging evolved? And what determines its rate?

According to evolutionary theory, aging evolves because natural selection has little power over what happens late in the life of an organism. The key to understanding this point is environmental (extrinsic) mortality. Since living in the wild is inextricably associated with the risk of dying of starvation, diseases or predation, young and intermediate age classes make up the greatest portion of the population and therefore generally contribute more to fitness than older age classes [reviews in Kirkwood and Rose (1991) and Stearns (1992)]. Under such conditions natural selection favors early reproduction, even if this happens at the cost of faster aging and a shorter lifespan (Kirkwood and Rose 1991; Williams 1957; Kirkwood 1977) and fails to prevent the accumulation of late-acting deleterious mutations, i.e., mutations that become deleterious only in old age classes (Medawar 1952).

The level of extrinsic mortality also helps explain why different species have different lifespans and aging rates (e.g., Stearns 1992). A high level of adult mortality is predicted to select for an increased

investment in early reproduction, thus diverting resources away from late survival and allowing late-acting deleterious mutations to accumulate. This results in a faster rate of aging and a shorter reproductive lifespan (i.e., lifespan after the onset of reproduction). Conversely, a low level of extrinsic mortality is predicted to select for a slower aging rate and a longer reproductive lifespan.

Although evolutionary theory explains why aging ultimately evolves, it does not make precise predictions on the proximate (physiological or mechanistic) causes of aging. This gap has been filled by numerous studies on model organisms, which have pinned down individual genes and molecular pathways that are likely to cause or regulate the loss-of-function spiral of the aging process [reviews in Medvedev (1990); Guarente and Kenyon (2000); Hekimi and Guarente (2003); Tatar et al. (2003)].

While studies on the classic model organisms yeast, mouse, *Drosophila melanogaster* fruitfly, and the nematode *Caenorhabditis elegans* form the backbone of our current knowledge on aging, they might not be telling us the whole story. All model organisms share a life history that places priority on early reproduction rather than a long lifespan, which might limit the extent to which model organisms are representative of other species. This bias is exacerbated by the fact that most experimental lines of model organisms have experienced hundreds of generations of artificial selection and inbreeding. We therefore argue that it is essential to complement our current image of aging and lifespan determination by studying a more diverse set of species, especially longer-lived organisms, in a variety of environmental conditions.

Owing to their peculiar life history and social structure, social insects are particularly useful for this non-model-organism approach to aging. Ant, bee and wasp colonies generally consist of several phenotypically distinct female castes, which, with a few exceptions, arise through differential gene expression from a single genotype (Hölldobler and Wilson 1990; Wheeler 1986). These castes often differ in task specialization, body size, reproduction, and the level of extrinsic mortality they experience, thus providing numerous opportunities to test the direct effect of extrinsic mortality on the evolution of aging while controlling for confounding factors. Social insect colonies also provide fertile terrain for the study of proximate mechanisms of aging. Since they combine both a long lifespan and naturally occurring lifespan polymorphism, two characteristics that model organisms lack, social insects constitute an ideal testing ground for the candidate-aging and lifespan-regulation genes already found in model organisms. In addition, gene expression studies between genetically identical social insect castes with different lifespans might help identify new candidate genes involved in aging and lifespan determination.

Evolutionary theory of aging

Prior to discussing evolutionary studies on aging in social insects, we would like to provide a more detailed overview of the theoretical framework that underlies those studies. Currently, three hypotheses offer plausible explanations for why aging evolves in spite of its detrimental effects on fitness. All three hypothesis are based on the fact that extrinsic mortality, from which no species can entirely escape, leads to a decline in the force of natural selection with age (Hamilton 1966; Charlesworth 1980).

Medawar (1952) was the first to predict that under such conditions, mutations with late-acting deleterious effects could not be counter-selected and would accumulate in the genome, thereby causing the various physiological changes associated with aging. This hypothesis is known as the mutation accumulation hypothesis. A few years later Williams (1957) suggested a more active mechanism for how mutations that cause aging might accumulate. His hypothesis is known as the antagonistic pleiotropy hypothesis. It proposes that late-acting deleterious mutations might get fixed in the population because they confer a fitness advantage early in life (e.g., increased early fecundity or juvenile survival). Hence, there might be a trade-off between early reproduction and late survival, mediated by genes with multiple phenotypic effects (pleiotropic genes).

The third evolutionary hypothesis is the disposable soma theory of aging (Kirkwood and Rose 1991; Kirkwood 1977, 1981). It also postulates that early reproduction trades off against late survival, but based on optimal resource-allocation strategies rather than pleiotropic genes. Investing more resources in somatic maintenance and repair than needed to reach the typical extrinsic-mortality-determined lifespan would be counterselected by natural selection. In contrast, investing more resources in traits that

increase fitness early in life might be beneficial for an organism's fitness, even if this leads to the accumulation of various somatic defects that cause aging. The disposable soma theory provides a particularly simple and attractive way of explaining the variation in lifespan among groups of individuals that share the same genome, such as different phenotypic castes in social insects. For instance, it is easy to grasp how different hormone titers could steer queens and workers into different resource–investment strategies. Late-acting deleterious mutations, on the contrary, can explain different lifespan patterns between genetically identical castes only under rather restrictive assumptions. There would have to be a complex way of partially reversing the deleterious effects of late-acting mutations in the longer-lived castes. Alternatively, late-acting deleterious mutations would have to be restricted to genes that are expressed exclusively in the shorter-lived caste (Chapuisat and Keller 2002).

The evolutionary theory of aging yields several testable predictions. The most important one is that levels of extrinsic mortality should be negatively associated with intrinsic lifespan (i.e., lifespan unaffected by environmental factors).

Tests of the evolutionary theory of aging in social insects

A social insect colony is well described by E. O. Wilson's metaphor of 'a factory within a fortress' (Oster and Wilson 1978, p. 22). It is heavily defended by thousands of individuals armed with tough cuticles, powerful mandibles, chemical weapons or stings. In many species, the nest is also protected by a sheltered aerial position or by deep galleries, thick walls and small entrances. Once the first workers emerge and begin to forage, queens stay within the confines of the highly protected nests. They specialize in egg-laying and completely give up risky outdoor tasks such as foraging or territory defense. For these reasons, the evolutionary theory predicts that social insect queens should have a longer lifespan than solitary insects.

A comparative study did indeed show that the evolution of social life in insects was associated with a 100-fold increase in adult lifespan (Keller and Genoud 1997). Social insect queens live for 10.1 years on average, and some reach the astonishing age of 28 years [for a detailed review on social insect lifespans, see Keller (1998)]. In solitary insects, the adult stage lasts only for 0.1 years on average. The sharp contrast in longevity between social insect queens and solitary insects is still statistically significant if controlled for the non-independence of species due to phylogenetic relationships (Keller and Genoud 1997). Hence, this study provides strong support for the hypothesis that the rate and timing of aging is primarily molded by the level of extrinsic mortality.

A further comparison showed that ant queens from species with a single queen per colony have longer lifespans than ant queens from species with multiple queens per colony. This difference is also consistent with the evolutionary theory of aging: because species with multiple queens per colony often live in short-lived and relatively unprotected nests (Keller and Genoud 1997; Hölldobler and Wilson 1977), their queens are believed to experience higher levels of extrinsic mortality. A high risk of queen loss is, incidentally, one of the major selective forces believed to select for having multiple queens per colony (Nonacs 1988; Reeve and Nonacs 1997).

A special difficulty in testing the evolutionary theory of aging is that differences in extrinsic mortality risk are often confounded with differences in body size and fecundity, which may also affect lifespan. By choosing the appropriate species, it is, however, possible to disentangle the effects of extrinsic mortality from the effects of body size or reproductive effort. One approach is to compare different worker castes that are specialized in different tasks and are therefore exposed to differential mortality risks. Interestingly, because workers are typically sterile and reproduce only indirectly (via the queen), the comparative study of lifespan between worker castes is not affected by confounding variables such as the cost and timing of reproduction.

To date, only one study has investigated whether differences in the level of extrinsic mortality have resulted in the evolution of variable intrinsic lifespan between morphologically distinct worker castes (Chapuisat and Keller 2002). In the weaver ant *Oecophylla smaragdina*, large workers belonging to the major caste perform all the risky tasks outside of the nest, including foraging, territorial defense, nest guarding and repairs. In contrast, small workers belonging to the minor caste stay within the highly protected arboreal nest, where they care for the

brood. This pronounced division of labor is associated with great differences in extrinsic mortality risks. The evolutionary theory of aging predicts that minor workers should have a longer intrinsic lifespan than major workers, despite their smaller size and probably higher metabolic rate. And indeed, in protected experimental nests, the minor workers lived significantly longer than the major workers (Chapuisat and Keller 2002). These results are consistent with the evolutionary theory of aging, and particularly with the 'disposable soma' concept (Kirkwood and Rose 1991; Kirkwood 1977, 1981). They suggest that investment in somatic maintenance is reduced when the risk of extrinsic mortality increases.

Another study compared individuals that do not differ in morphology, but vary in reproductive investment and extrinsic mortality (Hartmann and Heinze 2003). The ant *Platythyrea punctata* has a very unusual biology. Most often, colony members are genetic clones that are derived from unfertilized eggs. Reproductive and non-reproductive individuals are morphologically similar and have identical body sizes. However, reproductive individuals stay in the nests during their entire life, while non-reproductive workers forage outside of the nests when they become older. In protected laboratory conditions reproductive individuals lived significantly longer than non-reproductives, with lifespans three to 10 times longer (Hartmann and Heinze 2003). This great difference in lifespan between reproductive and non-reproductive individuals is most likely due to differences in extrinsic mortality, as the two types of individuals are indistinguishable in body size, morphology, mating status, diet and ontogeny (Hartmann and Heinze 2003). Similar results have also been found in another ant species in which reproductive and non-reproductive individuals had few morphological differences (Tsuji et al. 1996).

Together, these comparative studies in ants strongly suggest that the pattern of aging is molded by extrinsic mortality risks, rather than by differences in body size, reproductive investment or genotype. In both ants and termites, the type and degree of reproductive, behavioral and morphological specialization between castes is very diverse. This leaves room for many more intraspecific comparative studies that will help us to better distinguish the respective effects of behavioral and morphological differences on aging (Rueppell et al. 2004).

Outside of social insects, several comparative analyses have found an association between high extrinsic mortality risks and shorter intrinsic lifespan (Austad and Fischer 1991; Promislow 1991; Rose 1991; Tatar et al. 1997; Ricklefs 1998; Dudycha 2001). However, a recent comparative study in fishes found the opposite pattern. Guppies derived from natural populations with high predation risks had a longer lifespan than guppies from predator-free populations (Reznick et al. 2004). This last study challenges the conventional view that high mortality always selects for early aging. It provides support for more complex models that take into account the effects of mortality risk on population-density regulation and competition, as well as the effect that somatic investment can have in reducing extrinsic mortality. These more complex models show that, depending on ecological conditions and demography, extrinsic mortality can either decrease or increase intrinsic lifespan (Charlesworth 1980; Abrams 1993). Whether these alternative models need to be applied only in exceptional cases or as a rule remains to be established by future studies.

The evolutionary theory of aging has also been tested by selection experiments in the fruitfly *Drosophila*. These tests globally support the prediction that extrinsic mortality risks lead to the evolution of higher intrinsic mortality and shorter lifespans (Luckinbill et al. 1984; Rose 1984; Hillesheim and Stearns 1992; Partridge and Fowler 1992; Zwaan et al. 1995a, b; Partridge et al. 1999; Stearns et al. 2000). Experimental evolution studies from species other than *Drosophila* are clearly needed. However, this powerful approach is not easily applied in social insects because they are often difficult to breed and have long generation times.

Proximate causes of aging

The evolutionary theory of aging makes no precise predictions regarding the proximate (physiological) causes of aging and lifespan determination. Aging might therefore be caused by very diverse and idiosyncratic molecular pathways. Alternatively, it is possible that a number of conserved genes play a key role in aging across taxa. The fact that species with near-centenarian lifespans and a slow rate of aging have evolved repeatedly and independently in bivalves, fish, reptiles, and mammals (Stearns 1992,

p. 184) suggests that the first scenario might be more likely. On the other hand, some single-gene mutations have been shown to consistently increase lifespan in diverse model organisms, suggesting that the second scenario cannot be ruled out (Hekimi and Guarente 2003). In order to better understand how conserved the aging process is, we need to look at a range of species with contrasting life-history strategies. Studying the proximate mechanisms of aging only in model organisms is not sufficient in that respect, because all model organisms share similar life-histories: they are short-lived, they start reproducing early, and they produce many offspring.

Dozens of molecular pathways and processes have been suggested as being involved in aging and lifespan determination [reviews in Medvedev (1990); Guarente and Kenyon (2000); Hekimi and Guarente (2003); Tatar et al. (2003)]. These include the insulin/insulin-like growth factor signaling (IIS) pathway, oxidative damage to the cell and its macromolecules, age-dependent changes to DNA (e.g., physical damage or loss of methylation), changes to protein structure, the accumulation of dysfunctional mitochondria, and the loss of irreplaceable cells. In the following two paragraphs we will briefly discuss two important candidates from this non-exhaustive list: oxidative damage and the IIS pathway.

The idea of a causal link between oxidative damage and aging is known as the free radical hypothesis of aging. This hypothesis states that organisms senesce because their cells and macromolecules are damaged by reactive oxygen species (ROS), highly reactive molecules that are produced as metabolic byproducts (Harman 1956). Several lines of evidence suggest that oxidative damage does indeed contribute to aging [reviews in Sohal et al. (2002); Finkel and Holbrook (2000)]. It has, for instance, been shown that ROS production is inversely correlated with lifespan across several mammalian species (Ku et al. 1993). In addition, numerous studies have demonstrated that oxidative damage accumulates as cells and organisms age. Finally, several studies have shown that the transgenic over-expression of anti-oxidative enzymes can extend lifespan in the fruitfly. For instance the over-expression of cytoplasmic Cu/Zn superoxide dismutase (SOD1), an enzyme involved in the chemical detoxification of the highly reactive superoxide molecules, is able to increase the lifespan of fruitflies by up to 50% when over-expressed [review in Aigaki et al. (2002)].

The link between the IIS pathway and aging was discovered by studying long-lived mutant model organisms. Several genes involved in the IIS pathway in fruitflies, nematodes and mice have been shown to increase lifespan by up to 100% if their expression is changed to decrease insulin/insulin-like growth factor signaling [reviews in Guarente and Kenyon (2000); Tatar et al. (2003); Aigaki et al. (2002)]. This lifespan extension mechanism probably also involves secondary hormonal signals downstream from IIS. In insects, candidates for such secondary hormonal signals are juvenile hormone (JH) and the steroid hormone ecdysone [review in Tatar et al. (2003)], both of which seem to increase the rate of aging. In addition, the IIS pathway is also involved in controlling reproduction. It has therefore been suggested that the regulation of lifespan via the IIS pathway might be adaptive, allowing organisms to temporarily switch to a more survival-oriented life history when environmental conditions are adverse and do not allow for reproduction (Tatar et al. 2003).

Although the finding that single-gene mutations can increase lifespan is exciting, it leaves us with an open question: Are the genes that have been found to extend the lifespan of model organisms in the laboratory the same as those that are responsible for the intra- and inter-specific variations in lifespan observed in nature? This is highly uncertain. A candidate gene that increases the lifespan of a fruitfly in the laboratory when over-expressed might not have the same effect in a natural population, where organisms face competition and other ‘real-world’ challenges. In line with this caveat, two recent studies showed that under nutritional stress mimicking natural conditions, *age*-1 and *daf*-2 long-lived mutant worms were out-competed by wild-type worms in only a few generations (Walker et al. 2000; Jenkins et al. 2004). Previously these mutants had been thought to be as healthy and fecund as the wild type. A similar fitness cost under stressful conditions has also been discovered in *Indy* long-lived mutant flies (Marden et al. 2003). Thus, if we want to determine which genes are truly responsible for intra- and inter-species differences in lifespan and rate of aging, testing candidate genes in natural populations is an essential step. This could be done by testing whether candidate genes and proteins show the predicted expression and activity patterns in social insect castes with different, naturally evolved intrinsic lifespans.

Studies of the proximate mechanisms of aging in social insects

Social insects are an ideal system for studying aging because of their exceptional variation in lifespan among different castes and species. However, the proximate mechanisms underlying lifespan determination and aging in this group of species remain largely unknown. Recently published work in this area has focused on two species: the honey bee (*Apis mellifera*) and the black garden ant (*Lasius niger*). In the honey bee, researchers have investigated the proximate causes of the lifespan differences between worker bees specializing in different tasks. In ants, specific candidate genes and proteins (identified by aging research in model organisms) are being tested for differential activity patterns between long-lived and short-lived castes. Both these approaches have been productive, opening up an original domain in aging research.

Honey bee workers can be divided into two behavioral groups with different aging trajectories. The first group, consisting of young bees, does the work inside the hive, whereas the second group, consisting of older bees, specializes in foraging for pollen and honey (Neukirch 1982). The foragers age and die rapidly after switching to foraging (within two weeks), whereas hive bees that are prevented from switching to foraging can live over eight times longer [see Amdam et al. (2004) and the references therein].

In a recent paper, Amdam et al. (2004) propose that the proximate mechanism for this difference in lifespan is a juvenile hormone (JH) and vitellogenin-mediated shutdown of the forager immune system. Switching to foraging is associated with an increase in juvenile hormone (JH) (Fluri et al. 1982), which in turn leads to the cessation of vitellogenin synthesis (Pinto et al. 2000). Amdam et al. have now shown that the lower vitellogenin titers in foragers are associated with lower zinc titers and a smaller percentage of functional haemocytes, which play an important role in insect immune defense. They also show that low zinc titers cause a decrease in the number and percentage of normal haemocytes, at least *in vitro*. From an evolutionary perspective, the shutdown of the immune system in foragers has been interpreted as a colony-level energy-saving strategy (Amdam et al. 2004). Because foragers experience a higher risk of external mortality than hive bees, less energy should be invested in the maintenance of the forager soma than that of hive bees.

Although a causal relationship among JH, vitellogenin and the immune system has yet to be established, this study constitutes an interesting starting point for future investigations. It would be particularly useful to know whether there are signals upstream of JH, and what other downstream effects JH has in workers besides decreasing vitellogenin levels.

In the ant *L. niger*, Parker et al. (2004a) tested whether the candidate gene *SOD1* (cytoplasmic Cu Zn superoxide dismutase) might play a role in the extreme longevity of queens. This gene had previously been identified as important in the context of the free radical hypothesis of aging in model organisms. SOD enzymes are an important primary defense against oxygen free radicals generated by leakage from the electron transport chain. Increased levels of SOD1 were found in several *Drosophila* lines selected for long lifespan (Arking et al. 2002; Dudas and Arking 1995). In addition, transgenic expression of SOD1 in *Drosophila* has been shown to increase lifespan against some genetic backgrounds, including wild type (Orr and Sohal 1994; Spencer et al. 2003; Sohal et al. 1995; Sun and Tower 1999; Tower 2000).

In the ant *L. niger*, queen ants live for 20 to 30 years, workers for 1 to 3 years and males for only a few weeks. Thus, if SOD1 plays a role in these differences in lifespan, queens should have much higher cytoplasmic SOD1 levels than workers, which should in turn have higher SOD1 levels than males. Looking at expression levels of cytoplasmic SOD1 and protein activity assays, however, revealed that the queens always have the lowest or tie for the lowest activity and expression in all comparisons (Parker et al. 2004a). This finding is consistent with previous cross-species comparisons showing lower levels of antioxidants in longer-lived species (Perez-Campo et al. 1998) and suggests that increased expression and activity of SOD1 is not required for the evolution of a long lifespan. Instead, lifespan extension might evolve through decreased ROS generation from the mitochondria.

During this study an additional Cu Zn SOD was cloned from *L. niger*. This gene was shown to be the ortholog of mammalian and nematode extra-cellular Cu Zn SOD and was previously unknown in insects (Parker et al. 2004b). The discovery that insects possess the full set of *SOD* genes may have important implications in light of previous work on lifespan determination and SOD in *Drosophila* cited above.

Future investigations will show whether expression and activity patterns of other candidate genes and proteins confirm the results obtained for SOD1. Genomic tools, such as DNA microarrays that can detect expression differences for thousands of genes simultaneously, will be particularly helpful in teasing out what genes make social insect queens so much longer-lived than workers and males. The challenge for such studies will be to distinguish genes causally involved in lifespan determination from those differentially expressed as a consequence of other physiological and behavioral differences between castes. This problem can be partly overcome by doing multiple comparisons: comparing queens to workers and long-lived workers to short-lived workers in various species should help narrow down the number of candidate genes. Ultimately, however, functional analysis of the remaining candidate genes by RNA interference or transfer of genes to *Drosophila* will be essential in establishing causality (Rueppell et al. 2004).

Conclusion

Together with comparative and experimental evidence in other organisms, studies on aging in social insects provide overwhelming support for the evolutionary theory of aging. As predicted, the intrinsic lifespan of social insects seems to be primarily determined by the level of extrinsic mortality. Regarding the proximate causes of aging the situation seems to be less clear. Although aging research in model organisms has made quantum leaps in the last few years, many pieces of the puzzle are still missing. There is, for instance, a tremendous discrepancy between the frequently observed 10- to 100-fold variations in lifespan within and between species and the generally less-than-one-fold increases in lifespan that can be explained by altered IIS signaling or reduced oxidative damage. Expanding aging research to non-model organisms with naturally evolved lifespan differences might help us fill in some of these gaps.

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