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What are social insects telling us about aging?

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Abstract

Research on aging in social insects has progressed much more than has been generally acknowledged. Here I review what I think are the four greatest contributions of social insect work to the field of aging research with the hope of highlighting the truly exciting discoveries being made. These include the reversal of the fecundity / lifespan and size / lifespan trade-offs due to the evolution of sociality, that social environment can reverse the effects of aging, the contribution of social insect work to the overturning of the free radical theory of aging, and the discovery of vitellogenin as an important protein for longevity. All of these discoveries have important ramifications for human and mammalian aging.

Key words: Aging, ant, Formicidae, bee, free radical theory, longevity, social insects, vitellogenin, review.

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Introduction

Whenever I tell colleagues or others how the queen of one of the most common ants here in Britain (*Lasius niger*) can live nearly 30 years (KUTTER & STUMPER 1969), the response is always the same; they are amazed. Those who have more than a passing interest in aging research immediately realize that such a small organism with such a long lifespan must contain secrets about the evolution of aging. Clearly, organisms such as eusocial queens must have some way of reversing the usual fecundity / lifespan and size / lifespan trade-offs (see Box 1). Moreover, this reversal and extreme longevity correlates with the evolution of eusociality in termites, bees and ants (KELLER & GENOUD 1997).

Despite this initial appeal, there are many impediments to the study of aging in social insects. First and foremost is that aging research is directed at human health and welfare. This has created a hierarchy in funding and research focus with humans and translational research on top, followed by mouse, rat and other mammals as the main research systems, and the invertebrate models of *Drosophila melanogaster* and *Caenorhabditis elegans* of academic interest. Compared to the collective power of these model systems, long lived and challenging non-model systems like ants and bees might seem destined always to be several steps behind. However, the reality is that the results coming out of social insect work on aging are not just keeping up with the aging research field, but taking the lead as well. The challenge for those of us working in social insects is to get the biomedical research enterprise to notice our most transferable results.

There have been many comprehensive reviews of aging in social insects over the last several years (RUEPPELL & al. 2004, JEMIELITY & al. 2005, KELLER & JEMIELITY 2006, BOURKE 2007, REMOLINA & al. 2007, HEINZE & SCHREMPF 2008, MUNCH & al. 2008). Instead of just recovering this area, I wish to take a practical perspective and highlight the impact of social insects on biomedical aging research. The four most biomedically relevant results for aging from research in social insects are: 1) social evolution can reverse the assumed physiological trade-offs, 2) the social environment dictates and can reverse physiological changes associated with aging, 3) social insects' contribution to the overthrow of the free radical theory of aging, and 4) the multifaceted role of vitellogenin in aging.

Social evolution is associated with the reversal of fecundity / lifespan and size / lifespan trade-offs

The primary source for all research on social insect aging is the phylogenetic study of KELLER & GENOUD (1997). The authors used a phylogenetic analysis to test the hypothesis that the evolution of eusociality correlates with evolution of a long lifespan. The results were as statistically significant as they are visually impressive (Fig. 1). That sociality can increase lifespan can be argued for other social animals including humans (CAREY 2001). In general, sociality based on kin selection predicts a tendency to evolve longer lifespans (BOURKE 2007). In most cases of evolved increased lifespan, the evolutionary causes involved lowered extrinsic mortality for the reproductives in the social group. Social groups tend to protect the reproductives from predation while maintaining a more benign environment, thus reducing mortality from predators and stress.

Aging patterns and social structure are intimately connected in social organisms with social structure frequently determining the evolution of aging trajectories. This has been modeled and demonstrated for worker age polyethism in social insects (BESHERS & al. 2001, TOFILSKI 2002, TRIPET & NONACS 2004, TSUJI & TSUJI 2005). However, the evolution of extreme long life observed in ants and bees occurs primarily with female reproductives. In ants this creates an extreme difference in lifespan between queens (de-

cades), workers (years) and males (months) (SEELEY 1978, HÖLLDOBLER & WILSON 1990, HARTMANN & HEINZE 2003). Sometimes overlooked is the exceptional long life of workers (up to several years) (GODZINSKA & al. 1999) which is short compared to queens but is extremely long lived for their size. Interestingly, workers would seem to be under the same evolutionary pressures to evolve long life as queens, but only until they start to forage. This indicates that extrinsic mortality is once again the overriding factor.

The reversals of trade-offs also extend to sexual conflict but for different reasons. Mating in insects frequently reduces female lifespan (CHAPMAN & al. 1995, PROMISLOW 2003), but the reverse was found in the ant *Cardiocondyla obscurior* where mating increases queen lifespan (SCHREMPF & al. 2005). Kin selection and the structure of eusocial colonies can favor workers to replace the queen when the queen's fertility begins to decline (LEE 2003, BOURKE 2007). This can happen when a queen's fecundity falls enough that the worker will pass on more of her own genes to the next generation by replacing the queen. Indeed, through kin selection, the queen herself may favor colony turn-over to a related sister or daughter after the queen's own fecundity has fallen to some level. The timing may also depend on how resources such as rank and territory may be inherited among kin. There may be a conflict in the timing of the colony turn-over if the worker's favored take-over time comes before the queen's favored hand-over time (BOURKE 2007). These sorts of intergenerational transfer situations provide a rich set of conditions and examples for studying senescence under varying social and kin selective forces.

The observed reversal of the fecundity / lifespan trade-off seen in many reproductive social insects is an unexpected and completely revolutionary finding from a molecular and physiological point of view. Until such reversals were documented and studied, the trade-offs were assumed more or less universal physiological constraints on longevity because they were observed across all of the short-lived model systems. Exceptions were noticed, but now they can be theoretically explained and it can be proven that many of these trade-offs are physiologically malleable to ecological and evolutionary forces.

These evolved intrinsic aging differences among castes offer an opportunity to test biochemical hypotheses and results from the short-lived model systems. The results have been surprising, sometimes supporting and sometimes contradicting the idea that aging will be correlated with the accumulated damage from free radicals. Comparing Superoxide Dismutase (SOD) across the sexes and castes found that the longest-lived castes did not have the most antioxidant protection contradicting the free radical theory of aging (PARKER & al. 2004a). This work also led to the discovery that insects possessed the extra-cellular form of SOD (PARKER & al. 2004b). Other molecular work comparing queens and workers showed the same trend with antioxidants in honey bees (CORONA & al. 2005, CORONA & ROBINSON 2006). Characterizing telomeres across sex and castes in the ant *Lasius niger* found both supportive and contradictory results for the free radical theory (JEMIELITY & al. 2007). More fundamental differences in caste metabolism were found with the TOR pathway (PAGE & AMDAM 2007, PATEL & al. 2007) which is a fundamental regulator of cell metabolism controlling many aspects of protein synthesis. The small workers and large queens were found to be associated with lower and higher TOR activity, respectively. This is the reverse of the expected TOR activities based on the lifespans (STANFEL & al. 2009). The free radical theory predicts that higher levels of TOR activity would lower lifespan by creating more metabolic activity and more free radical damage accumulation. Thus taken together, the biochemical nature of the difference in intrinsic aging among castes is raising doubts about the free radical theory just as model systems are.

One under-exploited aspect of caste-specific lifespans is the differences among polymorphic sterile worker castes in ants. CHAPUISAT & KELLER (2002) showed that large workers were shorter-lived than small workers in a weaver ant and argued that it was consistent with extrinsic mortality acting on the sterile individuals. In this case, there is reversal of the size / lifespan correlation for sterile workers. This hypothesis would depend upon some connection between the mortality rates of the large and small castes and the mortality rate of the colony which is the level selection must be acting. The relationship of these various levels acting across colony and different types of sterile workers is one place where programmed aging could occur. There is potential for future work to examine the biochem-

Box 1: Glossary.

Damage accumulation: the observed accumulation of molecular and physical damage to an organism with age

Extrinsic mortality: mortality caused by predation, disease, starvation, accidents or other environmental causes

Free radical theory of aging: the hypothesis that reactive oxygen species cause molecular and cellular damage to accumulate leading to aging

Immunity / longevity trade-off: the hypothesis that robustness of the immune response trades off against lifespan. One can only be increased at a cost to the other.

Intrinsic aging: increase with time in fragility or probability of dying due to internal causes

Intrinsic mortality: mortality caused by organ failure, cancer or other causes internal to the animal's body

Fecundity / lifespan trade-off: the hypothesis that lifespan and reproductive effort must trade-off. Increasing one requires decreasing the other. Also called the longevity / fecundity trade-off.

Programmed aging: the idea that organisms are genetically determined to grow old and die. Sometimes used as equivalent to intrinsic aging.

Rate-of-living theory of aging: the hypothesis that an organism's metabolic rate, sometimes scaled for size, determines the rate of aging. In the past, this was thought to happen through controlling the rate of free radical production and damage accumulation.

Size / lifespan trade-off: the hypothesis that size and lifespan trade-off. The original idea is that physics determines the relationship between size and metabolism and larger animals with lower metabolic rate experience less free radical damage.

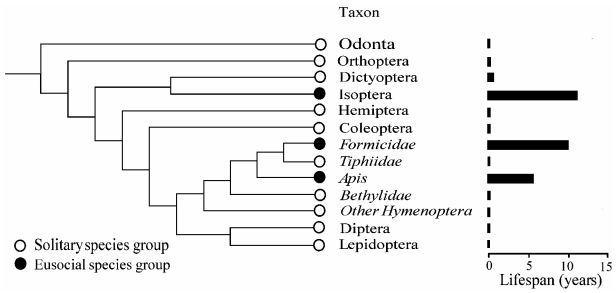

Fig. 1: The relative reproductive lifespan of various insect taxa showing the effect of eusociality. Details on the order Hymenoptera are given for several families because eusociality evolved independently in the genus *Apis* and in Formicidae (ants). Reprinted by permission from Macmillan Publishers Ltd: Nature (KELLER & GENOUD 1997), copyright 1997.

istry of the size / lifespan trade-off in such eusocial systems given that the ant with the most genomic resources available (*Solenopsis invicta*) also has an extreme size polymorphism among its sterile workers (WANG & al. 2007, 2008, WURM & al. 2009).

Social environment can change intrinsic aging patterns in social insects

Intrinsic aging rates can be dramatically altered by social environment. Honey bees in particular have provided many insights into the physiology of the plastic responses of intrinsic aging to social environment. For example, the size of the colony affects individual aging rates (RUEPPELL & al. 2009). In the trade-off between colony and individual, it is the individual situation that determines how the bees age (RUEPPELL & al. 2008). Environment has been shown to be more important in determining how honey bees age than the actual time the bee has been alive (RUEPPELL & al. 2007b). The key seems to be when workers make the transition to foraging and their mortality rates rise dramatically (RUEPPELL & al. 2007a, WOYCIECHOWSKI & MORON 2009).

In honeybees, workers that develop in the autumn are long-lived whereas workers developing in the spring are short-lived (MAURIZIO 1950, BECERRA-GUZMAN & al. 2005). These subcastes can be determined by diet during development and can live up to a year. These observations combined with the ability to revert foragers back to brood care by altering their environment (ROBINSON & al. 1992) provide manipulative tests for examining the changes associated with aging. This ability to revert aging phenotypes in this system has shown vitellogenin, Juvenile Hormone, and the insulin pathways as the regulators controlling and responding to the two subcastes (AMDAM & OMHOLT 2003, AMDAM & al. 2004, GUIDUGLI & al. 2005, AMDAM & al. 2007, CORONA & al. 2007, NELSON & al. 2007, MUNCH & al. 2008). The link to the insulin pathway is particularly intriguing as it is one of the main pathways associated with aging in model systems. Even immune system function and the ability to learn could be reversed in these types of experiments (AMDAM & al. 2004, AMDAM & PAGE 2005, BEHRENDS & al. 2007).

Proteomic studies have been done with the winter/summer bee system comparing the subcastes (SCHIPPERS & al. 2006, WOLSCHIN & AMDAM 2007a, b). Although confirming fundamental differences between the subcastes in many basic pathways, these studies demonstrate the classic limitation with proteomic and genomic aging studies. The typical experiment is to compare a long-lived and short-lived organism and the result is that the same basic metabolic pathways are usually affected. These include the insulin, TOR, and stress pathways. These are the three main biochemical processes that appear in many studies on aging (BROUGHTON & PARTRIDGE 2009, GREWAL 2009, PAPACONSTANTINOU 2009, STANFEL & al. 2009), but also so fundamental to regulating metabolism that any major change in the organism's metabolism will likely perturb them. The important result is that the subcastes are different at a basic metabolic level. This is a key lesson for anyone doing an "omics" experiment on aging. Thus far the results have been too general to be interpreted by themselves and have proven most valuable as preliminary work for future reductionistic studies (WHITFIELD & al. 2006, GRAFF & al. 2007). Most genomic studies require confirmatory work before meaningful results about specific genes and pathways can be interpreted.

One particularly relevant result for biomedicine is the observed restoration of an age related decline in the immune system (AMDAM & al. 2004, 2005). It is well established that immune responses change over life time and are dependent on the behaviors (foraging or inside workers) of the worker bees and ants (DOUMS & al. 2002, BOCHER & al. 2007, MORET & SCHMID-HEMPEL 2009). Immune function declines with age in bumble bees (DOUMS & al. 2002) but stays the same or even increases in an ant (BOCHER & al. 2007). In honey bees, it drops when bees begin to forage but can be restored when these bees are switched back to hive tasks (AMDAM & al. 2005). Thus, the decline in immune function with age is reversible and not the result of permanent damage accumulation. Indeed, the prospect of rejuvenating the human immune system is one serious idea being considered to combat human aging (DORSHKIND & al. 2009).

The idea that social interactions are important in aging has recently been tested in a *Drosophila* system which produced a genuinely baffling result (RUAN & WU 2008). The authors cited the above honey bee work as the justification and inspiration for these experiments. Short-lived fruit flies with a cytoplasmic Cu-Zn SOD mutation were placed in an environment with long-lived flies and with younger short-lived flies. Living with younger flies caused the mutant lines to recover a significant portion of the wild type fly lifespan compared to controls. The authors then went through a series of carefully controlled experiments (controlling food, decapitation, clipping wings, making the SOD mutants deaf, co-housing in the dark) to show that the effect was mediated by behavior. How this works is yet to be explored, but the conclusion is that social environment effects individual aging rates, even in what are usually thought of as solitary organisms.

This idea that organisms age depending on their social interactions has the potential to be one of the most clinically relevant transferable findings to human aging. Humans are social animals and almost certainly under similar selective pressures for increased longevity as social insects. Indeed social environment has been shown to have an effect on aging in humans although the effect is mediated by

the type of interactions (ROHR & LANG 2009). Clinical research has tended to separate positive and negative social interactions with subsequent positive and negative effects on lifespan. While there are ethical barriers to the most obvious replication of the honey bee results in humans, we can test whether persons with child-care responsibilities later in life are aging differently (mimicking the shift from forager to nurse bee). Early human studies suggest mixed results depending on various external stressors (BURNETTE 2000). It may be that honey bees are an ideal case where there are no negative effects on lifespan by virtue of evolutionary pressures on the sterile castes. Coming from the human clinical direction, the effect of quality of the interactions in humans might be tested in less socially evolved insects like wasps. How does the rate of aging change with respect to position in a dominance hierarchy (TSUJI & TSUJI 2005), amount of violent interactions, and just as importantly, what molecular mechanisms might underpin any change?

Social insects and the death of the free radical theory of aging

The old free radical theory, that accumulation of oxidative damage causes aging (HARMAN 1956), is in the midst of being overthrown. There is now overwhelming evidence that aging is not caused by a simple accumulation of free radical damage on macromolecules (BLAGOSKLONNY 2008, BRINK & al. 2009, GEMS & DOONAN 2009, PEREZ & al. 2009). Instead, free radicals appear to be signaling molecules related to stress as well as participants in fundamental metabolic processes (BLAGOSKLONNY 2008, JUAREZ & al. 2008, MAGLIARO & SALDANHA 2009). Part of this is the regulation of damaged molecule turn-over (CHEN & al. 2009). Hence, the accumulation of damage and the increased mortality rate that defines aging appear to be caused by fundamental changes in regulatory and developmental pathways (BUDOVSKAYA & al. 2008). These in turn are likely effecting the accumulation of oxidative damage, autophagy and cellular repair mechanisms. What is happening to the free radical theory of aging is a reversal of cause and effect with damage looking more like the effect of aging processes as opposed to the cause of aging (BLAGOSKLONNY 2008).

Much support for the paradigm shift is coming from studies in social insects. The already stated reversal of the fecundity / lifespan trade-off in queens supports the new interpretation. Levels of the antioxidant Superoxide Dismutase were higher in short-lived ant castes contrary to predictions of the old free radical theory (PARKER & al. 2004b) while much more extensive genomic work found the same trends in honey bee (CORONA & al. 2005, CORONA & ROBINSON 2006). Additionally, free radical damage was found not to be associated with age in honey bee (SEEHUUS & al. 2006a) despite the antioxidant vitellogenin (SEEHUUS & al. 2006b). Heatshock-gene expression does not agree with the simple free radical theory as some important heatshock-gene expression levels were observed to decline with age in worker honey bees (AAMODT 2008). Finally, brain development and complexity increase with age in both bees (FARRIS & al. 2001) and ants (SEID & al. 2005, SEID & al. 2008) while "wear and tear" would predict the opposite. There are some correlations in the other direction such as the observed lifespan correlation with peroxidation-resistant lipids (HADDAD & al. 2007) and requeening of wood ant nests as predicted by the old free radical theory. The evidence does point in both directions, even in single studies as seen in ant telomeres where males have shorter telomeres than workers and queens as predicted by the old free radical theory, but workers and queens have telomeres of about the same length contradicting the theory (JEMIELITY & al. 2007). The simple free radical theory and damage accumulation can not explain the contradicting cases and a new theory is needed.

The idea that aging is not caused by accumulated damage can be very difficult to accept. There is a common everyday example that explains how an organism can age without accumulating physical damage. Consider how many computer failures you have had in your lifetime. By failure, consider any time your machine locked up (needing a restart) which would be the equivalent of death in a biological system. Now consider how many have been due to hardware failures and how many due to software problems. In almost all cases computers are far more likely to lose function due to their internal state than due to the physical breakdown of a component. Thus, computers and other complex systems like living organisms can fail globally because they arrive in an inappropriate state, without any specific subsystem failure (all the parts can be functional, but the whole is not). Such bad states in organisms and cells would involve changes to fundamental metabolic states or developmental programs and might be expected to cause stress and damage leading to the observed correlations of damage and aging.

How then can an organism age when the cause is not accumulated damage? There are at least three recently proposed and one older relatively unknown theory for how organisms can age without accumulating damage being the cause. Demetrius (DEMETRIUS 2004, BRINK & al. 2009) proposes a metabolic instability hypothesis that the rate of living coupled with demographic factors leads to metabolic instability causing aging. BLAGOSKLONNY (2008) describes what he calls "quasi-programmed aging" based on TOR-related pathways being switched on by mistake over time. BUDOVSKAYA & al. (2008) suggest that aging is caused by developmental programs becoming inappropriately activated with age and labels it the developmental drift theory of aging. In 1978, Robert Rosen proposed the feedforward theory of aging arguing that all complex systems with feedforward pathways (predictive models, not self-correcting) will inevitably fail at the global level without the need for any specific subsystem failure (ROSEN 1978). Basically, his idea is that every complex system will inevitably get into a state incompatible with function as in the computer example above. Although these theories have not been fully developed nor synthesized, they all share the characteristics of being systems level explanations and can explain the results that the free radical theory and damage accumulation can not.

The important point is that the experimental evidence currently supports the notion that higher system level effects are more important drivers of aging than physical damage accumulation. Social insect aging results are supporting this emerging paradigm. Social-insect researchers are already used to thinking in terms of emergent properties and systems level phenomena. Hence, we should be in an intellectually strong position to contribute to this new emerging paradigm.

The role of vitellogenin in aging

The significance of the discovery of vitellogenin as a major player in aging (AMDAM & OMHOLT 2003, OMHOLT & AMDAM 2004, NELSON & al. 2007, AMDAM & al. 2009) has yet to be recognized for its true potential significance. This is an excellent example of how a discovery in social insects is slow to penetrate into the more mainstream aging field. Vitellogenin is thought to be related to mammalian low density lipoproteins (LDL) (SAPPINGTON & RAIKHEL 1998, SMOLENAARS & al. 2007). LDL reactions in the vascular endothelium of humans involve oxidative stress, inflammation, and programmed cell death and are thought to be major players in human cardiovascular disease (SIMA & al. 2009, WILENSKY & MACPHEE 2009). Understanding the dynamics of vitellogenin might yield insights into human disease.

Social insects appear unique in that the adult workers can be expressing high amounts of vitellogenin in a complex regulatory network with juvenile hormone (JH) (GUIDUGLI & al. 2005, AMDAM & al. 2007). In sterile workers, JH can suppress vitellogenin and vitellogenin in turn can suppress JH in what is called the double repressor hypothesis (AMDAM & OMHOLT 2003, OMHOLT & AMDAM 2004). The switch-over from nurse bee (high vitellogenin) to forager (low vitellogenin) is then associated with a shift to more rapid aging (FLURI & al. 1981, 1982).

Vitellogenin acts as an antioxidant in honey bees by becoming irreversibly carbonylated (SEEHUUS & al. 2006b). Vitellogenin scavenges oxidants by essentially offering itself as a noncatalytic target for the damage. On the surface, this seemed a perfect prediction for the damage accumulation version of the free radical theory, but the measured accumulation of damage was better explained by social role than age.

The most novel finding is that vitellogenin is a zinc carrier and this function might be associated with the immunity / longevity trade-off (AMDAM & al. 2004). The significance of this discovery urgently needs further exploration, especially as it does not appear that zinc is associated with the antioxidant effect of vitellogenin. The highly expressed zinc containing enzymes Cu-Zn SODs are major enzymes implicated in aging. Also, a well known age-related neurodegenerative disease, familial amyotrophic lateral sclerosis (or motor neuron disease) and other neurological damage can be caused by changes in Cu-Zn SOD that destabilize the zinc-binding ability of the enzyme (ESTEVEZ & al. 1999, ROBERTS & al. 2007, SMITH & LEE 2007, DING & DOKHOLYAN 2008). Furthermore, zinc is required in important gene regulatory pathways (zinc fingers) and interacts with the NMDA receptor which is important for learning (KLUG 2005, KANDEL 2009, PAOLETTI & al. 2009). The vitellogenin results are pointing to the hypothesis that the movement and correct localization of zinc may be important to aging.

Thus, vitellogenin is one of the most exciting molecular leads for mainstream model system research in aging coming from social insects today. I predict that we will see this discovery being explored in flies and mammalian systems in the next several years.

The future

One of the main goals of those working in aging research in social insects has to be transferring our results to the mainstream biomedical aging-research community. As I see it, the key points of entry are 1) the evolution of reversals of physiological constraints on lifespan, 2) socially reversible aging phenotypes, 3) the paradigm shift in the free radical theory, and 4) the role of vitellogenin in aging. We have the advantage of natural and easily manipulated systems that by definition far outstrip the capabilities of any model system to explore these areas. Indeed the weaknesses of our social experimental systems can be overcome by testing in mainstream model systems through closer collaboration with biomedical researchers. Only when these results are replicated in established laboratory mammalian models will our results finally be transferable to human health.

Social behavior is the ultimate determinate of longevity in humans, and social insects offer the only real option for understanding the evolutionary forces underpinning sociality and lifespan. Making this connection, and always looking towards the general principles at both the proximate and ultimate level can not help but raise the profile of aging research in social insects.

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Zusammenfassung

Die Erforschung der Alterung bei sozialen Insekten hat viel größere Fortschritte gemacht als landläufig bekannt. Hier biete ich einen Überblick über die vier meiner Ansicht nach größten Beiträge der Forschung an sozialen Insekten zum Gebiet der Alterungsforschung, in der Hoffnung, die wahrlich fesselnden Entdeckungen, die gemacht wurden, herauszustellen. Dies sind die Umkehr der Trade-offs von Fruchtbarkeit und Lebensspanne und von Größe und Lebensspanne in Folge der Evolution von Sozialität, die Erkenntnis, dass die soziale Umwelt die Effekte des Alterns umkehren kann, der Beitrag der Forschung an sozialen Insekten zum Kippen der Freie-Radikale-Theorie des Alterungsprozesses sowie die Entdeckung von Vitellogenin als ein für die Langlebigkeit wichtiges Protein. Alle diese Entdeckungen sind ausgesprochen relevant in Hinblick auf das Altern beim Menschen und bei anderen Säugetieren.

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