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REVIEW

Aging and immunosenescence in invertebrates

D Stanley

USDA/Agricultural Research Service, Biological Control of Insects Research Laboratory, 1503 South Providence Road, Columbia MO 65203, USA

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Abstract

Most contemporary research into aging is driven by interest in the human aging process and in interventions that attenuate the normal and pathophysiological effects of aging, or senescence. Operationally, senescence is the progressive, inevitable breakdown of the organism. Among the changes associated with senescence is the diminished capacity of the immune systems and reactions to challenge, known as immunosenescence. Senescence and age-related immunosenescence has been recorded in several invertebrates, including insects. Two invertebrates, the worm, *Caenorhabditis elegans,* and the fruit fly, *Drosophila melanogaster*, are model organisms for research into mechanisms of senescence and of prolonged life spans. In this essay, I will treat some of the available information on immunosenescence in invertebrates. The purpose is to move away from trying to understand human senescence and toward generating new ideas around the application of research into invertebrate immunosenescence to contemporary and emerging problems in aquatic and terrestrial agriculture. I cover mechanisms of senescence, beginning with the original idea of increasing oxidative damage and moving to more recent views. I provide a thumb-nail sketch of insect immunity as a model for the generality of complex invertebrates, then discuss selected examples of immunosenescence in invertebrates. In some instances, changes that look like immunosenescence may be physiological resource trade-offs and I highlight a few examples. Finally, I complete the essay with a few remarks on the potential practical significance of research to understand immunosenescence in invertebrates.

Key Words: invertebrates; immunity; aging; immunosenescence; *C. elegans*; *Drosophila*

Introduction

Aging and its many attendants have fascinated people since antiquity, at least since the Cato Maior De Senectute (Cato the Elder on Old Age) of Cicero (44 BCE). Most contemporary research into aging is driven by interest in the human aging process and in interventions that attenuate the normal and pathophysiological effects of aging and help achieve the controversial goal of 'successful aging' (Rowe and Kahn, 1987). Aging is the process of growing old. Some professionals in aging research prefer the term senescence (which first appeared in the English language in the late 1600s) to aging. Operationally, senescence is the progressive, inevitable breakdown of the organism. In humans, senescence is expressed in many ways, including loss of muscle and bone mass, reduced metabolic rates, impaired reaction times, diminished memory functions, reduced sexual activity and lessened hearing, olfaction, vision and organ functions. Among the changes associated with senescence is the diminished capacity of the immune systems and reactions to challenge, known as immunosenescence. Immunosenescence is associated with increased occurrences of cancers and sensitivities to infections. However, immunosenescence is complicated because some innate immune functions, particularly inflammation, increase with age. This is sometimes referred to as 'inflamm-aging' (Cannizzo *et al.*, 2011) and is thought to contribute to age- and inflammation-related health problems, including atherosclerosis and arthritis. While senescence, *per se*, is not a human

Corresponding author:
David Stanley
USDA/Agricultural Research Service
Biological Control of Insects Research Laboratory
1503 South Providence Road, Columbia MO 65203, USA
Email: stanleyd@missouri.edu

disease, a large research enterprise is deployed to understand the mechanisms of senescence and to ameliorate some of the conditions associated with it.

Senescence and death may be a universal feature of life, encapsulated in “No death, no life, no exceptions” by Fulgham (1986). Lanner and Conner (2001), however, raised the question of whether the long-lived bristlecone pine, *Pinus longaeva*, undergoes senescence. They analyzed several parameters of age-related declines, including tracheid diameter, annual shoot growth increments, pollen viability, seed weight, seed germinability and seedling biomass accumulation in bristlecone pines of widely varying ages, from 23 to 4,713 years. They found no significant differences within these and other parameters and concluded that this species does not senesce. There may be other such long-lived species in certain relatively unexplored habitats, such as deep ocean floors, however, in the common experience, the life histories of individuals end in senescence and death.

Senescence and age-related immunosenescence has been recorded in several invertebrates. Two invertebrates, the worm, *Caenorhabditis elegans,* and the fruit fly, *Drosophila melanogaster*, are model organisms for research into mechanisms of senescence and of prolonged life spans. Of course, the goal of research with these models is to discover new knowledge on fundamental aspects of senescence that will contribute to our understanding of human senescence processes. In this essay, I will treat some of the available information on immunosenescence in invertebrates. The purpose is to move away from trying to understand human senescence and toward generating new ideas around the application of research into invertebrate immunosenescence to contemporary and emerging problems in agriculture. I begin with a brief discussion of mechanisms of senescence, to which we now turn attention.

Mechanisms of senescence

A basic mechanism of senescence is the accumulation of damage at several levels of biological organization, including damage to molecules, to cells, to tissues and to intact organisms. One form of damage is oxidative damage, which arises from formation of the superoxide radical in mitochondrial electron transport chains. This idea was put forth in the 1950’s (Harman, 1956) and continues to carry a great deal of weight (Cannizzo *et al.*, 2011; Cerullo *et al.*, 2012). The story is more complex, however, as Partridge (2009) discussed two reports that draw the theory of oxidative damage into question. Working with the worm, *C. elegans*, Doonan *et al.* (2008) and Van Raamsdonk and Hekimi (2009) report on the influence of silencing two genes encoding Mn superoxide dismutases on lifespan. The deletions led to increased sensitivity to a superoxide generator, paraquat, but either increased or did not reduce lifespan. Partridge (2009) discussed alternative explanations and concluded that superoxide damage may not attenuate lifespan in *C. elegans*. Gems and Doonan (2009) asked whether the oxidative damage theory of senescence is in error. They reviewed the outcomes of experiments designed to test the theory and concluded that many experimental results are not consistent with key predictions and suggested alternatives. One alternative is the molecular damage theory of senescence, which suggests that oxidative events are just one of several ways to induce life-shortening damage to molecules.

Several authors point out that senescence is not an adaptation. Many organisms undergo reproductive diapause, including some insect species and the worm *C. elegans* (Tatar and Yin, 2001). Diapause is a genetically- and neurophysiologically-mediated state of sharply reduced activity. Tatar and Yin (2001) found that mortality rates of post-diapause *Drosophila* are similar to the rates seen in newly emerged adults. They inferred that senescence is very much slower during diapause and, more importantly, the rates of senescence within a species exhibit considerable plasticity. The authors note that variable senescence rates are endocrine regulated. The point would be that plasticity in senescence rates is one mechanism of influencing immunosenescence and life spans, as seen in monarch butterflies (Herman and Tatar, 2001). Zhan *et al.* (2011) note that the long-distance migrations of monarchs depends on endocrine organization of several physiological processes, including the juvenile-hormone mediated increases in life span just cited. The idea of plastic rates of senescence may help understand how some insects, such as 13- and 17-year periodical cicadas, express very lengthy juvenile life spans.

Mis-regulation, or errors, of gene expression may be another senescence mechanism. MicroRNAs (miRNAs) are gaining recognition as an additional tier of regulating gene expression, particularly during development. They effectively silence gene expression by complementing the untranslated regions of some mRNAs, thereby blocking translation of the mRNA into protein. Liu *et al.* (2012) forged a miRNA based linkage between age and neurodegenerative diseases. miR-34 is one of the many miRNAs that regulate gene expression during development. Using the *Drosophila* model, they found that expression of miR-34 is up-regulated with increasing age. miR-34 mutants looked and acted normally in early adulthood, however, they suffered severely shortened life spans, reduced climbing ability and marked loss in brain integrity (indicated by appearance of many vacuoles in brains of older flies). miR-34 suppresses expression of a ecdysteriod-reglated gene, *E74A*. *E74A* is an antagonistically pleiotrophic gene; that is, it is beneficial in juvenile stages of *Drosophila* development, but quite harmful in adults. miR-34 expression functions to silence the expression of *E74A,* one of the genes that can exert negative effects on older flies and increase risks of age-associated diseases. Mis-regulation of miR-34 expression can contribute to the senescence process and to age-associated diseases in insects and other invertebrates.

A key point in understanding senescence is appreciation that the process is not an evolved trait,

in the sense that no genes responsible for senescence have been identified. Rather, senescence is more accurately understood as an unregulated side effect, or, to be a bit more precise, unregulated side effects of life processes taking place in different time courses and by different mechanisms in each of the many systems that make up animals (Partridge, 2010). At its basis, senescence is the outcome of many processes of increasing damage and decreasing functions. From a biomedical perspective, research into mechanisms of senescence has the goal of creating interventions that improve the health of older people and one outcome of improved health may be increased human life spans. In this essay I depart from the biomedical model with the proposal that some of the recent advances in invertebrate senescence may be applicable to some agricultural issues including aquaculture and insect pest management technologies. Before addressing these issues directly, let us consider a brief sketch of insect immunity as a model of invertebrates, and document cases of naturally-occurring immunosenescence in invertebrates.

A sketch of insect immunity

Some readers may not be fully aware of insect immunity and this simple sketch is designed to facilitate reading this essay without referring to external literature. Detailed treatments are available. Strand and Pech (1995) and Strand (2008) are excellent reviews of cellular immunity. Gillespie *et al.* (1997) review signal systems in insect immunity. Eicosanoid signaling has been treated by Stanley (2005; 2006). Humoral immunity is detailed in Lemaitre and Hoffmann (2007). Kanost and Gorman (2008) review prophenoloxidase activation, a central process in insect immunity. An *et al.* (2012) report on a novel aspect of innate immunity, neuropeptide-mediated prophylactic immunity in newly molted insects.

Invertebrates, including insects, express solely innate immunity, that is, non-specific immunity that does not depend on previous immune experiences. In insects and certain other invertebrates, the integument serves as an exoskeleton and also as a physical barrier to potential infectious agents (Davis and Engström, 2012). Many microbes enter insect bodies via the gastrointestinal tract, although it is not a completely open path for them. Insects express epithelial immunity, recorded in the salivary glands (Abdelsadik and Roeder, 2010), the gut (Cronin *et al.*, 2009), Malpighian tubules (Davies and Dow, 2009), and tracheal epithelia (Wagner *et al.*, 2008) of *Drosophila* and likely most other insects. Epithelial immunity is generally a humoral response, based on induced expression of genes encoding a wide range of anti-microbial peptides. Once infectious agents get into the open hemocel, insects detect and react to infection by launching humoral and cellular immune reactions. Humoral reactions, again, involve biosynthesis of anti-microbial peptides and the enzyme lysozyme, and release of prophenoloxidase (ProPO) from circulating oenocytoids, a type of hemocyte. These peptides and proteins appear in the hemolymph of infected insects 6 - 12 h post-infection. Again, far more detailed descriptions of humoral immunity, including non-self surveillance and the signaling pathways that lead to anti-microbial peptide expression are available (Lemaitre and Hoffmann, 2007).

The hemolymph of most insects has a standing population of approximately 4 - $6x10^6$ circulating hemocytes per ml hemolymph. The main immune effecter cells of Lepidoptera are plasmatocytes and granulocytes. These cells clear microbes from circulation by phagocytosis and a process called nodulation, a form of encapsulation. Nodulation begins with microaggregation of hemocytes with adhering microbes (Fig. 1), which grow into nodules. In the last phase of nodulation, plasmatocytes surround the nodules and activate a ProPO system to melanize them. Melanized nodules are finally attached to an internal organ or inner body wall and they can be a permanent record of previous infection. The darkened nodules remain in the body for the life of the insect and they are easily visible at 40x under a dissecting microscope (Fig. 1). Nodulation is responsible for clearing the majority of infecting microbes from circulation (Haines *et al.*, 2010). Invaders too large for phagocytosis, such as parasitoid eggs, are encapsulated in layers of hemocytes within resistant insect hosts. These also are melanized and attached to the inner body wall or an internal organ. The melanization process produces reactive oxygen forms that may chemically kill the invaders.

Immunosenescence in invertebrates

The worm *Caenorhabditis elegans* (Nematoda: Rhabditida: Rhabditidae) is a well-known model animal for studies of development, aging and immunity. Kurtz and Tan (2004) reviewed immunosenescence in *C. elegans*. While the molecular mechanisms are not clear, it is certain that younger worms are far more resistant to bacterial infection than older worms. *C. elegans* is generally maintained on a bacterial lawn of *Escherichia coli* (strain OP50) in laboratory cultures. Worms reared on living bacteria have shorter life spans than worms fed on killed bacteria, from which it is thought that *E. coli* can become an opportunistic pathogen in older worms. By extension, it seems immunocompetence declines as a worm ages. Work on *C. elegans* has produced new understandings of the aging process, some of which are conserved in the evolution of all metazoans.

Age-related declines in invertebrate immune functions have been documented in several species, some of them outside the usual model animals. The cricket, *Gryllus assimilis*, is a simple example in which Park *et al.* (2011) recorded evidence for cellular immunosenescence. They used nodulation as a quantitative assessment of immune function, counting numbers of nodules formed in response to lipopolysaccharide (LPS; prepared from the bacterium *Serratia marcescens*) injections. Nodulation increased with time following treatment and with increasing LPS dosages. Males,

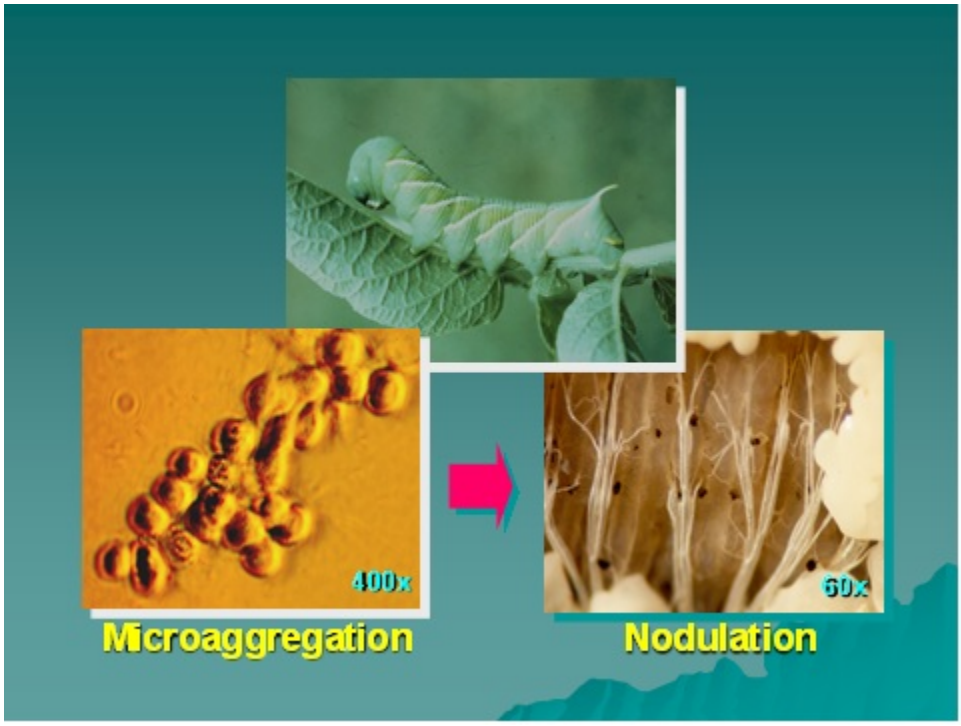

Fig. 1 Microaggregation and nodulation reactions to bacterial infection in fifth-instar tobacco hornworms, *M. sexta*. Hornworms were challenged by injecting the bacterium, *S. marcescens*, into the hemocel. For microaggregation, at 1 h post infection, hemolymph was withdrawn, diluted with buffer and placed on a microscope slide for observation and photography at 400x. The cells in these photographs range from 10 - 12 microns. For nodulation, at 4 h post infection the hemocel was exposed for photography at 40x. The nodules are 0.1 mm in diameter. Photo by JSM.

ages 0 to 3 weeks after adult emergence, produced about 12 - 15 nodules/male in response to experimental infection. Nodulation declined to about 9 nodules/male at 4 weeks and to about 3/male at 5 weeks. Total circulating hemocytes declined in parallel, from about $5x10^3$ at ages 1 and 2 weeks, to about $3x10^3$ at 3 weeks and to 1 to $2x10^3$ at weeks4 and 5. Declining hemocyte populations were attended by increased numbers of damaged hemocytes, from about 10 % to 60 % by week 5. The authors inferred that *G. assimilis* males undergo cellular immunosenescence.

Cellular immunosenescence has also been reported for the model fly, *D. melanogaster* (Mackenzie *et al.*, 2011). The authors injected fluorescently labeled *Escherichia coli* into adult male flies, and then recorded the proportion of hemocytes able to phagocytose the bacterial cells. About 25 % of the hemocytes in flies age 1 week phagocytosed the microbes, which was reduced to about 16 % in flies age 4 weeks. They also considered the circulating hemocyte populations as a function of age, showing that hemocyte numbers declined in females, but not in males, as the flies aged from 1- to 4-weeks old. This work documented cellular immunosenescence in fruit flies with the suggestion that the decline in cellular immune functions may be one mechanism of increased susceptibility to disease in older flies. Kurtz (2002) took a different approach to a similar conclusion. His research goal was to understand sources of variation in hemocyte phagocytosis in scorpionflies, *Panorpa vulgaris*. He collected flies in the fall and allowed their offspring to overwinter in outdoor cages. The adults were collected the following spring as they emerged and males were used for experiments. He collected very small hemolymph samples (1 μl) for his phagocytosis assays. The author considered a wide range of variation sources; to list a few, these included genetic variation, development, gender and environment. He also studied variation associated with age, from which he determined that the ability of *P. vulgaris* hemocytes to perform phagocytosis declined sharply with age. I conclude that at least some insects, maybe most of them, undergo substantial declines in cellular immune functions as they age.

Aside from cellular immunity, aspects of insect humoral immunity may also decline with age. This is a controversial idea because several studies report increased transcripts of immune response genes in *D. melanogaster* as the flies age (Pletcher *et al.*, 2002). Zerofsky *et al.* (2005) noted that while such transcripts increase with age, it remains unknown whether the increases are due to increased (and possibly chronic) exposure to microbes or due to age-related changes in immune system elements. For their work, Zerofsky et al. used the expression of a gene encoding the anti-microbial protein (AMP) diptericin (*dpt*) as a

measure of immune function. They found older females had higher *dpt* expression than younger ones in untreated, control flies. When challenged with infection with living bacteria, *dpt* levels increased at about the same rates for younger and older females, however, younger females reached highest *dpt* expression at 12 h post-infection, then rapidly declined. Results with older flies differed, however, because *dpt* induction continued throughout the 48 h test period. The authors inferred that persistent induction of genes encoding AMPs in older flies accounts for higher levels of their indicator gene, *dpt*. They then challenged flies with killed bacteria. The killed bacteria induced more *dpt* expression in younger flies than in older ones. They understood from this observation that the ability to induce expression of AMP genes declines with age. If this holds up, it might be concluded that insects undergo age-related senescence of cellular and humoral immune functions.

A study of hemocyte populations and ProPO activity in newly emerged and sexually mature field-captured adults of the damselfly, *Lestes viridis*, complicates the common view of immunosenescence. Rolff (2001) reported lower circulating hemocyte populations in newly emerged males and females than in older, sexually mature adults. He also registered higher ProPO activity in sexually mature females compared to younger ones and compared to older males. The ProPO data are consistent with recent work on honeybees, as noted in the next section. The hemocyte data run contrary to other findings that hemocyte populations decline with advancing age. This may follow from experiments with damselflies captured in the wild, with no information on the immune history of the individuals.

Individual organs can undergo immunosenescence. The mammalian brain includes a cell type known as microglia that makes up the brain's innate immune system (Streit and Xue, 2010). These cells comprise about 12 % of the cells in the human brain and they serve important positive roles, including recognizing and responding to infections via pattern recognition receptors (Block *et al.*, 2007). Microglia can also become over-activated by stimulations such as infection, environmental toxins or neuronal damage. In their over-activated states, microglia become important players in destructive neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Beyond the influence of over-activation, Streit and Xue (2010) report that microglia undergo senescence, characterized by several features, especially fragmenting the cytoplasm of microglia, and thereby lose their ability to protect neurons. They believe that this is a process of immunosenescence that leads to age-related neurodegeneration. Invertebrates may also undergo CNS-level immunosenescence, perhaps similar to the effects of mis-regulation of miR-34 in *Drosophila*, mentioned earlier.

In some instances physiological trade-offs, the subject of the next section, rather than senescence, may account for what looks like immunosenescence. Let us move on to a few examples.

Ecological immunity: trade-offs between immune functions and other physiological systems

Apparent age-related losses of immune functions have been attributed to trade-offs among physiological systems. Adamo *et al.* (2001) reported on immunocompetence in adults of the cricket *Gryllus texensis*. They found approximately similar resistance to bacterial infections in nymphs and adults at ages 3 days and 14 days. Adults over 4 weeks old, however, were very susceptible to bacterial infection, with less than 5 % survival. Circulating hemocyte populations did not decline with age and phenoloxidase activity progressively declined in aging males but not in females. The authors interpreted their results in terms of physiological a trade off: as males enter the reproductive phase of adulthood, they trade immunocompetence for reproductive potential. It might be thought that the findings with *G. texensis* are at odds with outcomes of the experiments reported just above on the related cricket, *G. assimilis*. The differences in the two reports are probably due to experimental designs. Park *et al.* (2011) used a direct measure of an immune function, that is, the ability to form nodules in response to infection in their assessment of immunocompetence. Adamo *et al.* (2001) used an broader measure, namely, resistance to infection. Also, Park *et al*. (2011) removed the cost of reproduction by not allowing *G. assimilis* males to court and mate with females. I conclude that crickets are able to trade off immunocompetence for reproduction.

Honeybees, *Apis mellifera*, trade off immunocompetence for the energy-demanding foraging phase of adult life. In their work on biochemical signaling in insect cellular immunity, Bedick *et al.* (2001) tested the hypothesis that prostaglandins and other eicosanoids mediate nodulation reactions to infection in adult honeybees. The authors challenged newly-emerged adults with freeze-dried bacteria, *Serratia marcescens*, and later determined numbers of nodules formed in response to the challenges. The young adults were competent to produce over 100 nodules/ bee at 4 h post-challenge. Similar experiments with foraging bees revealed that bacteria-challenged foragers did not form nodules. On closer study, the foragers had virtually no circulating hemolymph and very few, if any, circulating hemocytes. The authors concluded that immunosenescence may occur in honeybees, but they could not judge whether it was linked to the age of the bees or to the ontology of tasks.

The influence of age and task can be separated using the single cohort colony procedure. Schmid *et al.* (2008) created precocious foragers and over-aged nurses, then assessed hemocyte numbers. They found that age-right nurse bees had far more circulating hemocytes than over-aged nurses and precocious foragers similarly had far more hemocytes than age-right foragers. These data indicate the changes seen in honey bee hemocyte populations are related to age and not related to task. This would be consistent with a physiological trade-off of cellular immunocompetence for the

energetic costs associated with foraging flights. However, age-related declines in honeybee hemocyte populations differ from the findings of Amdam *et al.* (2005). They reported the outcome of another reversion protocol that led to production of new hemocytes and what they took to be a reversal of immunosenescence in worker honeybees. The story is still more interesting, however, because Schmid *et al.* (2008) also assessed hemocyte numbers in queens and drones, finding that all three phenotypes, workers, queens and drones undergo steep declines in circulating hemocytes with advancing age. Queens live most of their lives within their colonies, which is not congruent with a direct trade-off of hemocytic immunocompetence for the costs of foraging. Schmid *et al.* (2008) suggested the possibility that a more subtle trade-off may take place at the colony level, where all energy-withdrawing activities, both within and outside the hives, should be taken into account.

The loss of hemocytes with increasing honeybee age is offset by maintaining phenoloxidase (PO)-based immune functions (Schmid *et al.*, 2008). In workers, PO activities increased over the first week of adult life, then leveled off. Queens were different because PO activity increased steadily with age, attaining twice the activities found in workers. PO activity levels declined slightly with age in drones. Maintaining PO-based immune functions demands much less investment than maintaining standing populations of millions of hemocytes. It appears that honeybees may reduce the risks associated with trading off hemocyte-based immunity by retaining PO-based immunity, as also seen in social leaf cutting ants, *Acromyrmex octospinosus* (Armitage and Boomsma, 2010). Here, changes in honeybee immunity illustrate the complexity of assessing immunocompetence over the life span of insects.

Bumble bee workers also undergo immunosenescence. In their research with two bumble bee species, *Bombus terrestris* and *B. lucorum*, Doums *et al.* (2002) used the ability to encapsulate a nylon filament as a measure of immunocompetence, a fairly direct measure of cellular immunity. In the range of 30-day aging periods, they recorded reduced encapsulation and slightly increased fat body sizes as workers aged. Senescence did not influence hemocyte population sizes in these experiments. Later work with *B. terrestris*, however, revealed a more complex picture (Moret and Schmid-Hempel, 2009). These authors recorded reduced immunocompetence, measured by zone of inhibition assays, reduced hemocyte populations and reduced PO activities in immune challenged workers as they aged. They also recorded increases in hemocyte populations and PO activities in workers taken from older colonies. They interpreted their findings with respect to colony fitness, suggesting that older colonies are more prone to infection due to increased numbers of individual bumble bees and other factors. Older colonies also produce sexuals, which are essential to the reproductive fitness of the colonies. In this view, the expectation is the older colonies would produce workers with enhanced immunocompetence. Such pliable adjustments in individual immunity may optimize colony-level fitness. This view raises important questions about the mechanisms involved and also suggests that immunosenescence at the individual level may be altered somehow. As with honeybees, it is difficult to make absolute judgments about immunosenescence in eusocial contexts.

Practical significance of immunosenescence in invertebrates

I finish this essay with a few comments on potential role of immunosenescence in two areas of applied invertebrate biology. These are untested speculations meant to stimulate discussion rather than suggest specific actions. Although invertebrate immunosenescence is documented, it remains an open field with a great deal of new knowledge awaiting discovery. Aside from tremendous contributions to understanding of human senescence and immunosenescence, knowledge of invertebrate immunosenescence may be applied to existing problems in invertebrate aquaculture and in insect pest management.

Aquaculture is a global-scale agricultural industry that produces large harvests of a wide range of edible invertebrates, including abalone, crab, clam, crayfish, oyster, mussel, scallop and shrimp for human consumption. The industry produces millions of metric tons of products with first sale values in billions of dollars (Stentiford, 2011). Aquaculture systems are subject to a wide range of disease agents, including bacterial, protistan, fungal, and viral afflictions. Some lead to epizootics with very high mortalities and huge financial losses. Others can lead to serious opportunity costs. In the case of lobster, for example, diseases have inhibited development of aquaculture systems (Shields, 2011).

A question, then, is whether the expanding knowledge of invertebrate immunity and immunosenescence can be applied to the goal of sustaining the health of commercial aquaculture systems. Immunosenescence has not received sufficient attention in cultured invertebrate species; however, if the situation in insects is similar to other invertebrates, it is reasonable to expect reduced immune capacity in older invertebrates. The corollary, of course, is older invertebrates are more susceptible to disease than younger ones. Aside from this sort of generalization, research into invertebrate immunity and immunosenescence may contribute to understanding and possibly mitigating the influences of global climate change on farmed and wild invertebrate species. Matozzo *et al.* (2012), for example, investigated the influence of acidification and temperature, two parameters of global climate that potentially affect the health of seafood species. They worked with two bivalve species, the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*. The authors maintained the animals for 7 days at three pH values, two temperatures and three salinity values. Salinity of seawater, as practical salinity units (PSU, a dimensionless quantity) is approximately 35. Exposing *C. gallina*, to artificial seawaters with lower (28 PSU) and higher (40 PSU) salinities led to increases in circulating hemocytes at 22 ^{o}C. The

authors considered several other immune parameters, as well, and concluded that global climate changes can exert powerful effects on bivalve immunity by influencing hemocytes.

We turn to the significance of insect immunosenescence in developing biological control technologies to manage insect pest populations. Tunaz and Stanley (2009) carried out a field study of infections in pest insects captured in agrarian fields, and reported that virtually all of the hundreds of examined pest insects had internal, melanized nodules, sure signs the insects had been infected in their pasts and had recovered from the infections to continue consuming crop plants. One of our conclusions from this work is insect immunity may impose limits on the efficacy of some microbial biocontrol agents or even naturally occurring microbes. Taken in the context of immunosenescence, there is considerable interest in disabling insect immunity or greatly increasing the rate of immunosenescence.

Shrestha *et al.* (2010) reported on using gene silencing to disable insect immunity. They identified five genes that act in signaling and coordinating insect cellular immunity in the red flour beetle, *Tribolium castaneum*. The five genes encode slightly different phospholipases A_2 (PLA_2s), enzymes responsible for releasing arachidonic acid from cellular phospholipids, the first step in biosynthesis of prostaglandins. Prostaglandins are key signal moieties in cellular immune reactions. Silencing these genes by injecting dsRNA constructs designed to separately complement mRNAs for each PLA_2 led to reduced immunocompetence in experimental beetles. Immunocompetence was determined by counting nodules, the quantitatively predominant cellular immune reaction to bacterial infections. This work serves as a proof of the concept that insect immunity can be disabled by silencing target genes. It also opens possibilities to silence genes encoding other key proteins in insect immune systems, such as genes acting in surveillance. Discovery of genes that underlie mechanisms of immunosenescence also may lead to increasing the senescence rates in pest populations.

Aside from contributing to understanding of aging in humans, research into immunosenescence in invertebrates has enormous possibilities for sustaining aquatic animals and for inventing new pest control technologies.

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