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Ants as Naturally Long-lived Insect Models for Aging

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This chapter explains in what respects ants can be useful models in understanding the mechanisms of aging. It includes an introduction highlighting how ants fulfill a need for long-lived model systems in aging research. Three ant model systems are then described including their relevant natural history characteristics, collection and laboratory maintenance. Practical considerations are given for molecular studies and techniques. Finally, an overview is given of the available genomic resources and types of comparative studies that are possible.

Why Ants?

Humans owe their relatively long life span to living in societies that reduce the risk of extrinsic mortality. Other organisms in organized societies are also expected to exhibit a similar lengthening of life span over evolutionary time. One hundred million years before the first human stood up and walked, social insects existed in societies with cities, roads, division of labor, farming, slave making, and organized group defenses (Hölldobler and Wilson, 1990). Sociality has resulted in a 10- to 100-fold increase in the life span of queens in ants, bees, and termites; a trend that was rigorously demonstrated using phylogenetic methods to compare life span and social structure across the insects (Figure 24.1; Keller and Genoud, 1997).

The evolution of sociality and its associated increase in life span show a general trend that has independently evolved several times.

Ants represent an ideal system for studying how evolution effects the changes necessary for long life. In addition to the differences in life span among species, there are also order-of-magnitude differences in life span between different castes in a single species. For example, queens of the ant *Lasius niger* have been known to live nearly 29 years in a lab, while workers live for only a few years and males a few weeks (Kutter and Stumper, 1969; Hölldobler and Wilson, 1990). Given that the same egg can become either a queen or a worker, differential gene expression seems to be the key difference between those two castes.

The traditional model systems used to study aging (*Drosophila melanogaster, Caenborabditis elegans, Mus musculus and Saccharomycies cerivisiae*) all share a short generation time, making them ideal experimental systems in terms of laboratory rearing and experimental manipulations. Unfortunately, this has introduced a bias in the types of life histories that have been sampled in modern aging research as almost all the mechanisms proposed for aging have been limited to these systems. Some of these mechanisms are not thoroughly understood in an evolutionary context and may not truly represent adaptations necessary for long life. Hence, these mechanisms need verification in long-lived species like humans. In addition, studies of long-lived species such as ants may also reveal new and novel life span extending processes and mechanisms that have withstood the test of evolutionary time.

The Ant Model Systems

Ants as a group share certain important life history characteristics, such as living in family groups with overlapping generations. Ants are haploid–diploid; the queens and workers are diploid and female, whereas males are haploid and develop from unfertilized eggs. In monogynous colonies, there is one reproductive queen per colony, with sterile workers who do not contribute directly to the reproduction of the colony. Sexual winged queens and males are produced by the colony at specific times of the year. Queens of monogynous colonies remove their wings after mating and found a new colony independently (claustral founding). For polygynous species, where there is more than one reproductive queen per nest, newly mated queens are accepted back into their colonies. In these cases, new colonies are typically formed by budding.

For aging research, there are two ant species and one genus that are particularly well suited for development into model systems. The species *Lasius niger* and the genus *Pogonomyrmex* have considerable life spans and are easy to rear in laboratory settings. The third species, *Solenopsis invicta*, has a variety of modern genomic tools available such as a large microarray and a large cDNA

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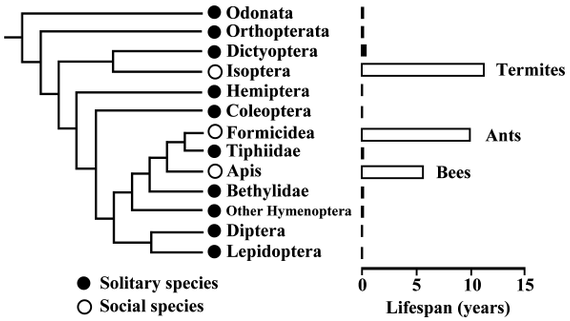

Figure 24.1. Sociality causes an increase in longevity. Redrawing of Figure 24.2 from Keller and Genoud 1997 showing how extreme life spans evolved multiple times in association with the evolution of sociality.

sequence database. These species are all very common in the Holartic, western North America, and the southern United States, respectively.

LASIUS NIGER

Queens of the common black ant, *Lasius niger*, have the longest recorded life span in the laboratory of 28 and 3/4 years (Kutter and Stumper, 1969). This monogynous species occurs in the Holartic region in forests and farm land. In central and northern European meadows, they occur in densities up to 1 mature colony per square meter. Colonies can be marked by placing a concrete paving slab over the colony entrance. The colony places brood underneath the slab for warming during the nonwinter months, which simplifies collection. The colonies do not seem to migrate, facilitating long-term monitoring and collection. Colonies can be huge with 10,000 or more workers, although smaller young colonies are easily maintained under laboratory conditions. *Lasius niger* has been extensively used in ecological studies as well as in genetic studies assessing colony relatedness, mating number, and sex ratio evolution (Fjerdingstad *et al.*, 2002; Fjerdingstad et al., 2003; Jemielity and Keller, 2003; Fjerdingstad and Keller, 2004). A recent search of the Web of Science Internet database yielded 141 publications on *L. niger*.

This species has large mating flights in mid to late summer when hundreds of newly mated deallate queens can be collected. New laboratory colonies can be started in glass test tubes filled halfway with water with a tight wad of cotton holding the water back. The founding queen is placed in the tube which is closed with a cotton plug (Figure 24.2).

The tubes are placed in the dark for approximately six weeks, until the first minims emerge. The cotton plugs are removed from the tubes which are placed inside plastic boxes with additional water tubes. The inside walls of the plastic boxes are painted with Fluon®, a fluoropolymer that most insects have difficulty climbing (available from Whitford Worldwide). Colonies will produce full-sized workers after approximately one year and can be transferred to progressively larger boxes as the colony grows. Best results are obtained when colonies are kept at 22°C with 60% constant humidity. *Lasius niger* prefers liquid food and our current food mix is 1:1:2 ground meal worms:eggs:honey plus 1% volume of liquid baby vitamins. To facilitate pipetting, the meal worms are flash frozen with liquid nitrogen and ground into a fine powder with a mortar and pestle. Aliquots of the mixture are stored frozen at −20°C and diluted 1:1 with water just before use. Colonies are fed three times a week and are given one or several drops of food, depending on their size.

TABLE 24.1
Internet resources

Description
Codehop program and degereneate PCR recommendations: http://bioinformatics.weizmann.ac.il/blocks/codehop.html
Fireant BAC library: https://www.genome.clemson.edu/cgi-bin/orders?page=productGroupandservice=bacrcandproductGroup=133
Fluon® manufacturer: http://www.whitfordww.com
Formis ant literature database: http://cmave.usda.ufl.edu/~formis/
Robert Johnson's *Pogonomyrmex* display nests: http://lsweb.la.asu.edu/sirg/pogonomyrmex/culturingPogonomymrexqueens.htm
Robert Johnson's North American Pogonomyrmex distribution maps and pictures: http://lsweb.la.asu.edu/sirg/pogonomyrmex/NORTHAMERICANPOGOS.htm
Texas A and M fire ant collection and maintenance: http://fireant.tamu.edu/materials/factsheets/FAPFS008.2002rev.pdf
Texas A and M fireant research and management project: http://fireant.tamu.edu
General ant information and images: http://www.antweb.org/index.jsp http://myrmecology.info/portal/news.php

AU: Kindly provide citation for this Table.

POGONOMYRMEX

The approximately 20 North American species of the seed harvester ant genus *Pogonomyrmex* are possibly the most studied genera of ants in the world with two books and a large body of primary literature dedicated to them (Cole, 1968; Taber, 1998; Johnson, 2000; 2001).

Figure 24.2. Founding *Lasius niger* queens. Queens being placed into water tubes after collection and stored in the dark until the first minims emerge.

Figure 24.3. *Pogonomyrmex rugosus* laboratory colony. The colonies are kept in plastic boxes normally used for rodent housing. There is an additional small box with water tubes for the area where the queen and brood live.

Pogonomyrmex contains the longest lived ant species recorded in the field, 30 years for *P. salinus* (Porter and Jorgensen, 1988). The best candidate model species within the genus *Pogonomyrmex* are *P. rugous*, *P. barbatus* and *P. occidentalis*.

These monogynous, multiple mating species are extremely common in the western deserts of North America, forming large conspicuous disc- or mound-shaped colonies. The colonies rarely move, facilitating permanent marking and long-term demographic studies. All of these species sting and can be very aggressive. The mating flights are usually rain triggered in the mid to late summer months. Queens can be collected after mating flights and colonies started in water tubes as for *L. niger*, transferring them to larger boxes with additional water tubes as needed (Figure 24.3).

Being desert ants, they should be kept at 30 to 35°C. Seed harvesters are poor climbers, but Fluon® is

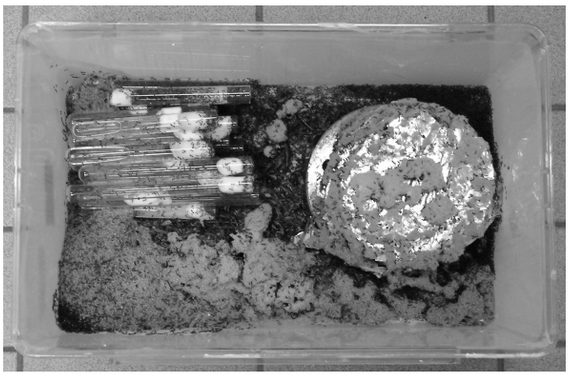

Figure 24.4. *Solenopsis invicta* laboratory colony. The colonies are kept in plastic boxes used for small rodent housing.

recommended to help contain them within their plastic boxes. A more elegant design is a sandwich style nest with either soil or plaster (see Johnson website for pictures and details). *Pogonomyrmex* should be fed pesticide-free grass seed or small bird seed, dead insects such as frozen crickets or meal worms, and a 1:1 mixture of honey and water. Colonies can be raised to large numbers and, in many cases, can produce sexuals in 2 to 3 years.

SOLENOPSIS INVICTA

From a molecular biology viewpoint, the most attractive model ant species is the red imported fire ant, *Solenopsis invicta*. Although queens live only 2 to 5 years, they have the advantage of existing in polygynous and monogynous colonies. This ant has the most extensively developed genomic tools available with a large cDNA EST database and a microarray chip nearing completion (J. Wang and L. Keller, pers. comm.) A BAC library is also available for purchase (see website list). Primary tissue culture and gene expression studies have been successfully employed using *S. invicta* (Chen, 2004). In addition, the first gene directly affecting social structure (i.e. queen number) was discovered and cloned from *S. invicta* (Krieger and Ross, 2002).

Solenopsis invicta are common where introduced and easily cultured in the laboratory. Their transient, shallow nests makes it easy, if not sometimes painful, to collect mature colonies with queens. An entire nest can be shoveled into a Fluon® -coated bucket and water added slowly. *Solenopsis invicta* form living rafts in response to flooding and the floating colony raft can be scooped from the water and placed into plastic boxes treated with Fluon® (Figure 24.4).

Newly mated queens can also be collected after mating flights in the early summer and colonies started in water tubes as for *L. niger* and *Pogonomyrmex* . Their optimal laboratory temperature is 25 to 30°C. Their dietary requirements are more demanding, requiring freshly

killed insects and a constant source of water. Specific method descriptions and food recipes can be found online (see Internet section of the appendices).

There are two problems that must be considered when working with *S. invicta*. First, it is impossible to avoid being stung on a regular basis when working with these small, aggressive ants. Some people develop sensitivity to the stings and can experience life threatening anaphylactic shock (Solley *et al*., 2002). Secondly, this is a highly destructive and invasive species that should never be transported to, or kept in, warm moist regions of the world where they have not already been established.

Sampling at the colony level

Mature queens of single queen colonies such as *Pogonomyrmex* and *L. niger* are almost impossible to collect due to the depth and size of the colonies. Fortunately, the genotype of a queen can be determined by genotyping male allates from the colony. As males are the product of unfertilized eggs, a sample size of 6 will cover 99% of the queen's genome. The genotype of the father(s) of the colony, as well as the queen's, can also be reconstructed by sampling workers. In both cases, such sampling will not negatively impact large colonies and represents a benign way to monitor the genetic structure of extant, wild long-lived populations.

Social insects should be sampled at the level of colony or group of colonies for molecular studies. When the queen has been fertilized by only one male, all of her daughters are full sisters and share the same haploid father and diploid mother. Thus, workers from a monogynous colony headed by a singly mated queen are identical for 75% of their genome. This can lead to pseudo-replication, as colonies represent closely related families with related genetic backgrounds. Measurements of a group of 25 workers consisting of 5 workers from 5 different colonies can not be considered 25 independent samples because of within-colony relatedness. Instead, this example contains measurements of 5 independent colonies, each consisting of the average from 5 workers. Thus, in most cases, worker colony samples should be averaged and one value taken per colony for statistical tests.

Molecular Methods

There are some practical considerations one should keep in mind when isolating DNA, RNA and protein from ants. Queens tend to be very high in fat content before their mating flight as well as in their physogastric form. Supplementary extractions with ether to remove this fat can be helpful for DNA and RNA isolation. Ants also tend to have much harder exoskeleton and higher surface-to-mass ratio than *Drosophila* and complete homogenization of tissue can be difficult without a bead type shaker homogenizer.

Some experiments are better conducted on dissected tissues or body sections. Physogastric queens lay large quantities of eggs, and the mRNA profiles of the queens can become dominated by that of the eggs. Males contain a large amount of sperm and subsequently large amounts of DNA for their body weight. Beginning a DNA extraction with dissected vas deferens containing sperm represents an immediate 10-fold purification. The formic acid in Formicine ants can also be problematic as was found in protein preparations from *L. niger* workers (Parker *et al*., 2004a). The acidity can effect isolation and gel loading buffers as well as interfering with enzyme activity assays. The efficiency of phenol extractions in DNA isolations can also be adversely affected by the acid.

Genetics

Recently, the standard of proof in molecular biology has required demonstration of gene function by knocking out or overexpressing a gene in an experimental system. This comparison is not yet possible at the organismal level in ants either through selective breeding or by gene transfer, but has been successfully achieved for primary tissue culture in *S. invicta* (Cônsoli, 2002; Chen, 2004). To date, no one has developed an immortal cell line for any social insect. Eventually, one may find a way to breed the ant model systems above, either by simulating the natural mating conditions or by artificial insemination as is done for honey bees, but neither has yet been accomplished.

The lack of a genome sequence for ants does not pose as much of a practical problem as one might think. Although *S. invicta* is the only ant species with a very large cDNA sequence database, differential gene expression studies with specifically targeted genes or suppressive subtractive hybridization are still possible using genomes already sequenced. Specific genes can be cloned by aligning sequences from the honeybee, fruit fly, mosquito, silk worm and fire ant sequence databases using the program CODEHOP (Rose *et al*., 1998) with the *Lasius niger* codon usage table selected. This technique has been successfully employed to clone numerous *L. niger* genes, including an extracellular SOD which was not thought to exist in insects (Parker *et al*., 2004b).

Comparative Studies

Ants as a group are extremely diverse and this diversity lends itself to many types of comparative studies across species and across populations. There are also exploitable differences across caste and sex within a single species. Some of these represent reversals of typical life span correlations with size and longevity as observed in humans and birds. For example, there are two sizes of workers in the weaver ant, *Oecophylla smaragdina*. The major (large) workers perform the dangerous tasks

outside the colony and have shorter life spans than the minor (small) workers who remain within the highly protected nest. But this difference in life span remains intact even in a protected laboratory environment (Chapuisat and Keller, 2002). It would be interesting to investigate if there are metabolic differences between these two sizes of worker ants.

The phenomena of monogynous/polygynous colonies have evolved independently many times across a wide range of ants and other social insect taxa. There is evidence that, within the same species, queens of monogynous populations live longer than queens of polygynous populations as predicted by evolutionary theory (Keller and Genoud, 1997). *Solenopsis invicta* is an excellent system to investigate life-span variation within a single species but across populations with different social structure as it has both polygynous and monogynous populations. In addition, the genetic basis for this difference in queen number is already known (Krieger and Ross, 2002).

One possibility for cross-species comparisons is the many cases of social parasites. Socially parasitic ants lack a worker caste and invade a host colony where their reproductive brood are raised by the host colony workers. In the genus *Pogonomyrex,* there are socially parasitic ants who live only 1–3 years and have recently evolved from a shared common ancestor of their longer lived hosts (*P. barbatus* and *P. rugous* (Johnson *et al*., 1996; Parker and Rissing, 2002). They represent an opportunity to investigate the evolution to a shorter life span once the relevant processes are discovered.

Reproductive rate correlates with life span such that highly fertile individuals have shorter life spans than less fertile members of the same species (Williams, 1957; Charlesworth, 1980; Partridge and Gems, 2002). Yet this fecundity/longevity tradeoff is reversed for queens and workers. The queen of a large social insect nest must lay eggs at a rapid rate to maintain the number of sterile workers in the colony and yet she has an extremely long life span (Rueppell *et al*., 2004). This reversal of the fecundity/longevity tradeoff has been shown true even without the morphological and physiological differences which are present between queen and worker ants. In the Ponerine ant *Platytyrea punctata*, all workers are capable of producing diploid workers yet the reproductive workers live significantly longer than their non-reproductive counterparts (Hartmann and Heinze, 2003).

Given that castes share the same genome, the differences in life span must be based on differential gene expression at some point(s) in their life history. This provides the foundation for testing gene expression differences already associated with aging in model systems. If the mechanisms hypothesized for life span extension in model systems are truly general, then there should be evidence of these same mechanisms being used in queen ants. One such mechanism, the resistance to oxidative stress conferred by superoxide dismutase (SOD), was recently tested in a comparison of cytoplasmic SOD activity and expression levels across all castes (Parker *et al*., 2004a). The study found that a high level of cytoplasmic SOD does not correlate with life span as for previous work in *Drosophila* (Orr and Sohal, 1994; Sohal *et al*., 1995; Hari *et al*., 1998; Sun and Tower, 1999; Arking *et al*., 2002; Spencer *et al*., 2003) and is in agreement with previous comparative studies (Perez-Campo et. al, 1998; Barja, 2002).

These small, highly fecund and extraordinarily long-lived queens must overcome all of the physiological problems of maintaining mitochondria, proteins which are degraded, damaged and/or incorrectly folded, as well as accumulative damage to DNA and membranes that are associated with long life. Queens in particular must do all of these things an order of magnitude longer than workers, and while reproducing at a high enough rate to sustain a colony. All of these individual molecular processes represent potentially fruitful lines of inquiry that could reveal novel aging resistance mechanisms.

Conclusions

It should not be overlooked that only those processes verified by both unnatural life-span manipulations in the laboratory and naturally occurring evolved differences can be considered as true proven causes of life span variation. Without verification, one will never know whether the mechanisms uncovered in the traditional model systems are artifacts of laboratory stress or specific to short-lived organisms. Testing in the laboratory will guard against making up ''just-so'' stories based solely on observed natural correlations. The challenge is to combine studies across both types of systems to discover what processes truly underlie the human aging process. Ants offer one of the best insect models to build this bridge.

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REFERENCES

Arking, R., Buck, S., Novoseltev, V.N., Hwangbo, D., and Lane, M. (2002). Genomic plasticity, energy allocations, and the extended longevity phenotypes of *Drosophila*. *Ageing Res. Rev*. 1, 209–228.

Barja, G. (2002). Rate of generation of oxidative stress-related damage and animal longevity. *Free Radical Biol. Med.* 33, 1167–1172.

Chapuisat, M., and Keller, L. (2002). Division of labour influences the rate of ageing in weaver ant workers. *Prod. R. Soc. Lond. B.* 269, 909–913.

Charlesworth, B. (1980). *Evolution in Age-Structured Populations*. Cambridge University Press, Cambridge.

Chen, M.-E., Lewis, D.K., Keeley, L.L., and Pietrantonio, P.V. (2004). cDNA cloning and transcriptional regulation of the vitellogenin receptor from the imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Insect Mol. Biol.* 13, 195–204.

Cole, A.C., Jr. (1968). *Pogonomyrmex Harvester Ants. A Study of the Genus in North America*. University of Tennessee Press, Knoxville.

Cônsoli, F.L., and Vinson, S. B. (2002). Hemolymph of reproductives of *Solenopsis invicta* (Hymenoptera: Formicidae) amino acids, proteins and sugars. *Comp. Biochem. Physiol.* 132, 711–719.

Fjerdingstad, E.J., Gertsch, P.J., and Keller, L. (2002). Why do some social insect queens mate with several males? Testing the sex-ratio manipulation hypothesis in *Lasius niger*. *Evolution*. 56, 553–562.

Fjerdingstad, E.J., Gertsch, P.J., and Keller, L. (2003). The relationship between multiple mating by queens, within-colony genetic variability and fitness in the ant *Lasius niger*. *J. Evol. Biol.* 16, 844–853.

Fjerdingstad, E.J., and Keller, L. (2004). Relationships between phenotype, mating behavior, and fitness of queens in the ant *Lasius niger*. *Evolution*. 58, 1056–1063.

Hari, R., Burde, V., and Arking, R. (1998). Immunological confirmation of elevated levels of CuZn superoxide dismutase protein in an artificially selected long-lived strain of *Drosophila melanogaster*. *Exp. Gerontol.* 33, 227–237.

Hölldobler, B., and Wilson, E.O. (1990). *The Ants*. Springer-Verlag, Berlin.

Jemielity, S., and Keller, L. (2003). Queen control over reproductive decisions: No sexual deception in the ant *Lasius niger*. *Mol. Ecol.* 12, 1589–1597.

Johnson, R.A., Parker, J.D., and Rissing, S.W. (1996). Rediscovery of the workerless inquiline ant *Pogonomyrmex colei* and additional notes on natural history (Hymenoptera, Formicidae). *Insectes Sociaux*. 43, 69–76.

Johnson, R.A. (2000). Seed harvester ants (Hymenoptera: Formicidae) of North America: An overview of ecology and biogeography. *Sociobiology*. 36, 89–122. [Erratum: 2000. v. 36,597].

Johnson, R.A. (2001). Biogeography and community structure of North American seed-harvester ants. *Annu. Rev. Entomol.*, 46, 1–29.

Keller, L. and Genoud, M. (1997). Extraordinary lifespans in ants—A test of evolutionary theories of ageing. *Nature*. 389, 958–960.

Krieger, M.J.B., and Ross, K.G. (2002). Identification of a major gene regulating complex social behavior. *Science*. 295, 328–332.

Kutter, H., and Stumper, R. (1969). Hermann Appel, ein leidegeadelter Entomologe (1892–1966). *Proceedings of the Sixth International Congress of the IUSSI (Bern)*, pp. 275–279.

Orr, W.C., and Sohal, R.S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science*. 263, 1128–1130.

Parker, J.D., Parker, K.M., Sohal, B.H., Sohal, R.S., and Keller, L. (2004). Decreased expression of Cu-Zn Superoxide Dismutase 1 in ants with extreme lifespan. *Proc. Nat. Acad. Sci. USA*. 101, 3486–3489.

Parker, J.D., Parker, K.M., and Keller, L. (2004). Molecular phylogenetic evidence for an extracellular Cu Zn superoxide dismutase gene in insects. *Insect Mol. Biol.* 13, 587–594.

Parker, J.D., and Rissing, S.W. (2002). Molecular evidence for the origin of workerless social parasites in the ant genus *Pogonomyrmex*. *Evolution*. 56, 2017–2028.

Partridge, L., and Gems, D. (2002). Mechanisms of ageing: Public or private? *Nat. Rev. Genet.* 3, 165–175.

Perez-Campo, R., Lopez-Torres, M., Cadenas, E., Rojas, C., and Barja, G. (1998). The rate of free radical production as a determinant of the rate of aging: Evidence from the comparative approach. *J. Comp. Physiol. B.* 168, 149–158.

Porter, S.D., and Jorgensen, C.D. (1988). Longevity of harvester ant colonies in southern Idaho. *Journal of Range Management* 41, 104–107.

Rose, T.M., Schultz, E.R., Henikoff, J.G., Pietrokovski, S., McCallum, C.M., and Henikoff, S. (1998). Consensus-degenerate hybrid oligonucleotide primers for amplification of distantly related sequences. *Nucleic Acids Res.* 26, 1628–1635.

Rueppell, O., Amdam, G.V., Page, R.E., and Carey, J.R. (2004). From genes to societies. *Sci. Aging Knowl. Environ.* 5, pe5.

Sohal, R.S., Agarwal, A., Agarwal, S., and Orr, W.C. (1995). Simultaneous overexpression of copper- and zinc-containing superoxide dismutase and catalase retards age related oxidative damage and increases metabolic potential in *Drosophila melanogaster*. *J. Biol.Chem.* 270, 15671–15674.

Solley, G.O., Vanderwoude, C., and Knight, G.K. (2002). Fire ants in Australia: A new medical and ecological hazard. *Med. J. Aust.* 176, 521–523.

Spencer, C.C., Howell, C.E., Wright, A.R., and Promislow, D.E.L. (2003). Testing an 'aging gene' in long-lived *Drosophila* strains: Increased longevity depends on sex and genetic background. *Aging Cell* 2, 123–130.

Sun, J., and Tower, J. (1999). FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Mol.Cell. Biol.* 19, 216–228.

Taber, S.W. (1998), *The world of the harvester ants*. Texas A and M University Press, College Station.

Williams, G.C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, 11, 398–411.