Pergamon
0300-9629(94)E0013-I

Comp. Biochem. Physiol. Vol. 109A, No. 1, pp. 33–38, 1994

0300-9629/94 $7.00 + 0.00

MINI REVIEW

Aging in *Drosophila*

Theodore J. Nusbaum and Michael R. Rose

Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A.

Key words: Genetics of aging, physiology of aging, *Drosophila*.

Comp. Biochem. Physiol. 109A, 33–38, 1994.

Drosophila in Aging Research

Flies of the genus *Drosophila*, particularly *D. melanogaster*, have been mainstays of biological research on aging for more than 80 years. However, what was striking for most of those years was how little concrete progress was made on the problem of aging using the normally powerful *Drosophila* model system. To be sure, a great deal of longitudinal information was accumulated about *Drosophila* aging and various mutant or inbred stocks were characterized for longevity (Lints and Soliman, 1988). However, throughout the period from 1915 to 1980, there were none of the significant breakthroughs that characterized other research with *Drosophila*, from genetics to biochemistry to developmental biology to population genetics.

In this respect, *Drosophila* aging research was like that with other organisms. Up until 1980, little demonstrable progress was made in aging research. The one powerful experimental system, *in vitro* cell culture, had revealed the finite replicative potential of untransformed mammalian somatic cells, but the significance of this finding was, and remains, obscure (Finch, 1990). Otherwise, systems like the laboratory mouse had yielded little more than had *Drosophila*.

What was lacking was an experimental system which would allow definite tests of causal hypotheses. Unlike the genetics of eye colour, there were no genes of aging in *Drosophila*, or any other system. Unlike the biochemistry of such processes as digestion or biosynthesis, there were no defined 'aging pathways' to dissect. Unlike the study of diseases, there were no normal individuals to compare with aging individuals. In aging, all organisms age, and no separable genetics or biochemistry appear to be involved. In *Drosophila*, as in other systems, some studies attempted to study aging by using organisms that died much earlier; the early work of Pearl (e.g. 1922) with *vestigial* is an example of this approach. However, even to the extent to which such research has resolved the mechanisms of deterioration and death in such 'accelerated aging' syndromes, it has not advanced our understanding of normal aging. For all these reasons, aging research with *Drosophila* was as stymied as that with any other organism.

Correspondence to: Michael R. Rose, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A. Fax (714) 725-2181.
Received 5 October 1993; accepted 23 January 1994.

Creation of *Drosophila* with Postponed Aging

The first intentional creation of *Drosophila* with genetically postponed aging occurred in 1980. Flies with genetically postponed aging had been created before, but only inadvertently (Rose, 1991). In the 1970s, Rose set about the deliberate breeding of flies with postponed aging by the simple expedient of breeding them at later ages only. Under these conditions, flies that had a genetic tendency to die young or be sterile at later ages would be selected against. Flies that remained reproductively active at later ages, and thus healthier at later ages, were selectively favored. This early experiment produced a modest increase in lifespan of around 10%. Subsequent experiments of similar design by Rose and others (Rose, 1991) have produced still greater increases in lifespan, up to 100%. Recent experiments have broadened the range of selection experiments giving postponed aging. Selection for stress resistance also appears to produce increased life span in *Drosophila* (Rose *et al.*, 1992).

The end-result of this work has been the creation of experimental material with which it is possible to explore mechanistic hypotheses concerning the control of aging in *Drosophila*. Mutant flies that die earlier could do so because of pathological mechanisms unrelated to normal fly aging. On the other hand, flies that live longer must have abrogated or slowed normal aging mechanisms. Thus, they constitute ideal material for the study of those mechanisms, whatever they might be. A great deal of recent research activity in the area of *Drosophila* aging now employs postponed-aging stocks and their normal relatives. This research will be the primary focus of the following discussion.

Genetics of Aging in *Drosophila*

The number of genes involved in aging

One of the most elementary questions that can be asked about the genetics of a system is the number of loci involved. This is not a trivial problem where aging is concerned, because there are reasons for expecting the number of loci involved to be large (Rose and Finch, 1993). The estimation of this number is an important methodological question; a small number of loci can be investigated using Mendelian genetics, but a large number requires the use of quantitative genetics (Falconer, 1981).

Luckinbill *et al.* (1987) estimated the number of 'effective factors' underlying longevity in short-lived and long-lived stocks of *D. melanogaster*. Effective factors are in essence, linkage groups and are underestimates of the true number of genes underlying the expression of a character such as longevity (Wright, 1968; Lande, 1981). Estimates of the number of effective factors in this study ranged from 0.3 to 1.5, which the authors interpreted as representing one effective factor. In other work (Luckinbill *et al.*, 1988; Arking *et al.*, 1993), chromosome replacement studies with crosses between short-lived, long-lived, and balancer chromosome stocks were used to investigate the contribution of different chromosomes to aging. Longevity acted as a typical quantitative character with contributions from the three major chromosomes. Hutchinson and Rose (1990) also estimated the number of effective factors underlying longevity in postponed aging lines. Hutchinson and Rose concluded that there are multiple loci for longevity.

Using 2-dimensional protein gel electrophoresis, Fleming *et al.* (1993) surveyed 321 electrophoretic proteins in short-lived and long-lived stocks. Polymorphisms were identified at over 100 proteins. The authors tentatively conclude that just six of the 321 proteins were involved in the control of aging. Furthermore, they estimated that the total number of loci controlling aging in *Drosophila* is 200–400 based on the assumption that the 321 proteins studied are a random sample of the 10,000–20,000 transcribed proteins in *Drosophila*.

Specific loci

When trying to determine whether genes that cause aging have primarily large or small effects, it is particularly important to distinguish between genes causing normal aging and unusual mutations which influence lifespan. For example, in *D. subobscura*, the mutation *grandchildless* (also known as *ovariless*) causes the daughters of homozygotes to produce females

with longevity enhanced more than 50% over normal flies. However, these females lack ovaries and produce no offspring (Maynard Smith, 1958). Although *grandchildless* clearly has a substantial effect on longevity, characterizing normal aging on the basis of its effects might lead to faulty conclusions.

This is not to say, however, that genes affecting normal aging cannot have large effects. In a study of the molecular through ecological genetics of *D. mercatorum* (Templeton *et al.*, 1985, 1993), a common allele with large effects on longevity was identified. *Abnormal abdomen* (*aa*) significantly decreases lifespan but enhances early fecundity. Individuals homozygous for *aa* have a distinct fitness advantage in dry environments, which tend to kill flies early in their adult lives.

The cumulative, damaging effects of free radicals on cells and their constituent macromolecules have long been proposed as important facts in aging (Harman, 1956). Cu,Zn superoxide dismutase (SOD), which catalyzes the conversion of superoxide radicals into hydrogen peroxide, is a likely candidate as an anti-aging molecule. In natural populations of *D. melanogaster*, fast and slow ('F' and 'S', respectively) alleles of SOD are known to segregate. The S allele exhibits greater activity than the F allele. In the long-lived *D. melanogaster* laboratory populations of Rose (1984), the S allele is found at greater frequencies than in control lines (Tyler *et al.*, 1993). To determine whether this locus is causally related to aging and, if so, its effect on aging, Tyler *et al.* (1993) extracted SOD alleles from two natural populations and constructed different SOD genotypes on hybrid genetic backgrounds. Though samples sizes limited the statistical power of their analyses, the authors argue that the allelic difference between fast and slow SOD genotypes amounts to a 2–4% difference in life span. If this finding is generally true for SOD alleles in other species, the free radical theory of aging, while validated, is not particularly important by itself as an explanation of aging. Perhaps the most dramatic demonstration of the involvement of SOD in controlling longevity are the transformation experiments on *D. melanogaster* using bovine Zn,Cu superoxide dismutase (Reveillaud *et al.*, 1991). Among replicate transformants, lifespan was increased by as much as 10%.

One of the most dramatic findings for a single locus was the 18–33% increase in the lifespan of virgin males obtained by Shepherd *et al.* (1989) when they transformed inbred stocks of *D. melanogaster* with an additional copy of EF-1α, the gene for elongation factor. Unfortunately, this initial study suffered from a lack of replication and controls. Stearns and Kaiser (1993) repeated this study with extensive replication as well as variation in mating status, genetic background, and gender. Their findings were more equivocal. Under some conditions, an additional copy of the EF-1α gene continued to enhance life span, but under others, it had no effect, or even reduced life span. Interestingly, when it increased life span, EF-1α reduced early fecundity, a characteristic finding when selecting for postponed aging (Rose, 1991). This study is particularly important because, as with SOD, the physiological basis of the genetic effect may be known: altered rates of protein synthesis.

Physiology of Aging in *Drosophila*

Physiological mechanisms

Other than the SOD work (see above), studies of the energetic metabolism of flies with genetically postponed aging provide the best-known example of physiological involvement in the aging of *Drosophila*. Changes in a number of physiological characters have been observed in flies selected for postponed aging compared to their controls.

For example, resistance to starvation, desiccation, and low-level ethanol are all enhanced in postponed aging flies (Rose, 1984; Service *et al.*, 1985). Flies selected to enhance their resistance to starvation not only responded to the selection for starvation resistance, but their longevity also increased (Rose *et al.*, 1992). This result shows that some alleles that increase starvation resistance also increase lifespan and that some fraction of the increased lifespan is due to the physiology of starvation resistance.

Lipid content of long-lived flies is greater at most adult ages (Service, 1987). Furthermore,

the early fecundity of the long-lived stocks is reduced (Rose, 1984; Luckinbill and Clare, 1985). The allocation of lipids to ovaries or fat bodies is consistent with the differences in early fecundity and starvation resistance. Normal flies allocate lipids to their ovaries, which enhances egg output early in their lives. There is evidence that longer-lived flies allocate lipids to their fat bodies, increasing their ability to resist starvation, but decreasing their early fecundity (Chippindale *et al.*, 1993). In experiments that did not involve lines selected for starvation resistance, the association between increased lifespan, enhanced starvation resistance, and high lipid levels were also observed (Zwaan *et al.*, 1991). However, this does not demonstrate that lipids are the only energy source utilized by aging adult *Drosophila*.

Desiccation resistance has also been shown to be causally related to increased lifespan in stocks selected for postponed aging (Service *et al.*, 1985; Rose *et al.*, 1990). Selection for desiccation resistance was accompanied by increased lifespan (Rose *et al.*, 1992). The physiological mechanism appears to be glycogen metabolism, in part, because increased levels of stored glycogen are associated with both increased desiccation resistance and increased longevity (Graves *et al.*, 1992). In other experiments (e.g. Hoffman and Parsons, 1989 a,b), flies selected for desiccation resistance also had lower metabolic rates and motor activity than controls, suggesting that glycogen metabolism may not be the exclusive physiological mechanism mediating the relationship between desiccation resistance and longevity.

Physiological independence or dependence?

Selection of *D. melanogaster* for desiccation resistance does not significantly increase starvation resistance (Rose *et al.*, 1990, 1992), indicating a lack of shared pleiotropic alleles and a seeming independence between these two physiological systems of aging. However, selection for starvation resistance (Rose *et al.*, 1992) does give rise to a significant increase in desiccation resistance, indicating the presence of shared pleiotropic alleles and a lack of independence between these physiological mechanisms of aging. These seemingly contradictory results can be reconciled if we assume that starvation resistance depends primarily on lipids and to a much smaller extent on glycogen. Selection to increase starvation resistance entails increasing the storage of both lipids and glycogen. Therefore, selection to enhance starvation resistance will also enhance desiccation resistance. However, flies selected for desiccation resistance, which entails increased capacity to store glycogen, will have only a very small increase in starvation resistance, which depends almost entirely on lipids (Rose *et al.*, 1992).

A better case can be made for the relationship between ethanol resistance and desiccation resistance. Ethanol resistance is correlated with longevity in lines selected for increased longevity (Service *et al.*, 1985), though no definitive causal relationship has been established. In selection experiments to increase desiccation resistance, ethanol resistance also increased (Rose *et al.*, 1990, 1992). Although the physiological system by which these flies resist ethanol has not been identified, the results of these selection experiments indicate that pleiotropic alleles are shared by these two stress resistance characters. Hence, these stress resistance characters and the physiological systems underlying their expression are not independent.

The relationship between ethanol resistance and starvation resistance seems to indicate independence in their physiological underpinnings. Back selection for early fecundity on flies originally selected for late-life fecundity decreased starvation resistance. However, ethanol resistance remained unchanged (Service *et al.*, 1988). This result might be interpreted as independence between the physiology of ethanol resistance and starvation resistance, due to the lack of shared pleiotropic alleles. However, it may also be the case that the two characters do share pleiotropic alleles. Under this alternative scenario, the alleles held in common are not also shared with early fecundity, i.e. starvation resistance is genetically correlated with early fecundity, but ethanol resistance is not.

Conclusions

While there may be individual genes with large effects on aging, more often than not

aging phenotypes are typical quantitative characters involving many genes, each with comparatively small effects. No single physiological system is responsible for aging. In the best-known cases, energetic metabolism and SOD account for less than 40% of the average difference in lifespan between long-lived and normal flies (Rose *et al.*, 1992; Tyler *et al.*, 1993). Instead, aging is controlled by multiple physiological processes having complex relationships with one another.

References

Arking R., Dudas S. P. and Baker G. T. (1993) Genetic and environmental factors regulating the expression of an extended longevity phenotype in a long lived strain of *Drosophila*. *Genetica*, **91,** 127–142.

Chippindale A. K., Leroi A. M., Kim S. B. and Rose M. R. (1993) Phenotypic plasticity and selection in *Drosophila* life-history evolution I. Nutrition and the cost of reproduction. *J. evol. Biol.* **6,** 171–193.

Falconer D. S. (1981) *Introduction to Quantitative Genetics*. 2nd ed. Longman, London.

Finch C. E. (1990) *Longevity, Senescence, and the Genome*. University of Chicago Press, Chicago.

Fleming J. E., Spicer G. S., Garrison R. C. and Rose M. R. (1993) Two-dimensional protein electrophoretic analysis of postponed aging in *Drosophila*. *Genetica* **91,** 183–198.

Graves J. L., Toolson E. C., Jeong C., Vu L. N. and Rose M. R. (1992) Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. *Physiol. Zool.* **65,** 268–286.

Harman D. (1956) Aging—A theory based on free radical and radiation chemistry. *J. Gerontol.* **11,** 298–300.

Hoffman A. A. and Parsons P. A. (1989a) An integrated approach to environmental stress tolerance and life-history variation: desiccation tolerance in *Drosophila*. *Biol. J. Linn. Soc.* **37,** 117–136.

Hoffman A. A. and Parsons P. A. (1989b) Selection for increased desiccation resistance in *Drosophila melanogaster:* Additive genetic control and correlated responses for other stresses. *Genetics* **122,** 837–845.

Hutchinson E. W. and Rose M. R. (1990) Quantitative genetic analysis of postponed aging in *Drosophila melanogaster*. In *Genetic Effects on Aging II* (Edited by Harrison D. E.), pp. 57–63. Telford, Caldwell, NJ.

Lande R. (1981) The minimum number of genes contributing to quantitative variation between and within populations. *Genetics* **99,** 541–553.

Lints F. A. and Soliman H. M. (1988) *Drosophila* as a model organism for ageing studies. Blackie and Son, Ltd., Glasgow.

Luckinbill L. S. and Clare M. J. (1985) Selection for lifespan in *Drosophila melanogaster*. *Heredity* **55,** 9–18.

Luckinbill L. S., Clare M. J., Krell W. L., Cirocco W. C. and Richards P. A. (1987) Estimating the number of genetic elements that defer senescence in *Drosophila*. *Evol. Ecol.* **2,** 85–94.

Luckinbill L. S., Graves J. L., Reed A. H. and Koetsawang S. (1988) Localizing the genes that defer senescence in *Drosophila melanogaster*. *Heredity* **60,** 367–374.

Maynard Smith J. (1958) The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura*. *J. exp. Biol.* **35,** 832–842.

Pearl R. (1922) *The Biology of Death: Monographs on Experimental Biology*. J. B. Lippincott Co., Philadelphia.

Reveillaud I., Niedzwiecki A. and Fleming J. E. (1991) Expression of bovine superoxide dismutase in *Drosophila melanogaster* augments resistance to oxidative stress. *Mol. cell. Biol.* **11,** 632–640.

Rose M. R. (1991) *Evolutionary Biology of Aging*. Oxford University Press, Oxford.

Rose M. R. (1984) Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38,** 1004–1010.

Rose M. R., Graves J. L. and Hutchinson E. W. (1990) The use of selection to probe patterns of pleiotropy in fitness characters. In *Insect Life Cycles: Genetics, Evolution, and Coordination*. (Edited by Gilbert E.), pp. 29–42. Springer, London.

Rose M. R., Vu L. N., Park S. U. and Graves J. L. (1992) Selection on stress resistance increases longevity in *Drosophila*. *Exp. Gerontol.* **27,** 241–250.

Rose M. R. and Finch C. E. (1993) The Janiform genetics of aging. *Genetica* **91,** 3–10.

Service P. M. (1987) Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* **60,** 321–326.

Service P. M., Hutchinson E. W., MacKinley M. D. and Rose M. R. (1985) Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* **58,** 380–389.

Service P. M., Hutchinson E. W. and Rose M. R. (1988) Multiple genetic mechanisms for the evolution of senescence in *Drosophila melanogaster*. *Evolution* **42,** 708–716.

Shepherd J. C. W., Walldorf U., Hug P. and Gehring W. J. (1989) Fruit flies with additional expression of the elongation factor EF-1α live longer. *Proc. natn. Acad. Sci. U.S.A.* **86,** 7520–7521.

Stearns S. C. and Kaiser M. (1993) The effects of enhanced expression of elongation factor EF-1α on lifespan in *Drosophila melanogaster* IV. A summary of three experiments. *Genetica* **91,** 167–182.

Templeton A. R., Crease T. J. and Shah F. (1985) The molecular through ecological genetics of *abnormal abdomen* in *Drosophila mercatorum*. I. Basic genetics. *Genetics* **111,** 805–818.

Templeton A. R., Hollocher H. and Johnson J. S. (1993) The molecular through ecological genetics of *abnormal abdomen* in *Drosophila mercatorum*. V.

Female phenotypic expression on natural genetic backgrounds in natural environments. *Genetics* **134,** 475–485.

Tyler R. H., Brar H., Singh M., Latorre A., Graves J. L., Mueller L. D., Rose M. R. and Ayala F. J. (1993) The effects of superoxide dismutase alleles on aging in *Drosophila*. *Genetica* **91,** 143–150.

Wright S. (1968) *Evolution and the Genetics of Populations. Vol. 1. Genetic and Biometric Foundations.* University of Chicago Press, Chicago.

Zwaan B. J., Bijlsma R. and Hoekstra R. F. (1991) On the developmental theory of ageing. I. Starvation resistance and longevity in *Drosophila melanogaster* in relation to pre-adult breeding conditions. *Heredity* **66,** 29–39.