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Genetics of Increased Lifespan in Drosophila

Michael R. Rose

Summary

Natural selection in the laboratory has been used to produce populations of Drosophila *with genetically increased lifespan. These populations have been used to determine the physiological basis of postponed ageing and its pleiotropic concomitants. It appears that many loci and a number of physiological alterations are involved in increased lifespan.*

The Problem of the Genetics of Ageing

Genetics has often provided us with some of our most powerful tools for the analysis of biological phenomena, from energy metabolism to differentiation. Hence it is natural that ageing too should be probed using the tools of genetics, particularly ageing in the workhorse of metazoan genetics, *Drosophila melanogaster*. Genetics was first applied to lifespan in *Drosophila* in the early part of the century, but there was little progress until the 1980s.[1] The reason for this is that lifespan is not readily delimited from adult survival in general, while the latter will be affected by virtually all loci other than those involved in development and the maintenance of fertility. In effect, the problem is that lifespan loci are a large fraction of all loci, making the problem of ageing genetics one of the quantitative genetics[2] of fitness.

Leaving aside the number of loci involved, an additional problem is that inbreeding stocks to obtain pure lines for particular alleles affecting lifespan runs into problems of inbreeding depression in *Drosophila*, as it would in most organisms, other than self-fertilizing species.[1,3–4] With such inbreeding depression, lifespan is depressed due to effects at many homozygous loci, which will tend to obscure the allelic variation at the loci of initial interest.

An additional problem is that alleles which reduce longevity may do so by the introduction of novel deleterious effects, as opposed to modulating the processes normally determining ageing and lifespan. Therefore, methods are required that will produce longer-lived genetic stocks without inbreeding. Such methods can be readily developed using the evolutionary theory of ageing.[5–6] This theory proposes that ageing is a result of the fall in the intensity of natural selection with age, such that later ages are subject to the evolution of a variety of deleterious effects unopposed by selection. A direct corollary of this theory is that increasing the intensity of natural selection at later

ages should lead to the evolution of increased lifespan. Indeed, this theoretical prediction has inspired the creation of the *Drosophila* stocks discussed below. I begin by discussing these selection experiments and then consider the further analysis of the longer-lived stocks that selection has produced.

Selection for Postponed Senescence

The 1960s and 70s saw a number of experiments in which *Drosophila* stocks were cultured in such a way that selection intensities at later ages should have increased.[7–11] Specifically, cultures were derived in which eggs laid by older females exclusively were used to start the next generation; this procedure imposes selection for survival to, and reproduction at, later ages. Initially, the effects of this procedure were interpreted primarily in neo-Lamarckian terms, even though some of these experiments[7–8] gave results that seemed fully consonant with population genetic expectations, in that lifespan increased as a result of delayed breeding for a number of generations.

I performed comparable experiments in the late 70s using *D. melanogaster*, and obtained the expected increase in lifespan in a stock which had been characterized using quantitative genetics.[12–14] In order to establish further the reproducibility of the result, delayed breeding experiments were repeated with multiple lines by both myself[15] and Luckinbill *et al.*[16] The results of these studies left little doubt that natural selection could be used to produce longer-lived *Drosophila* simply by using older females only to reproduce a culture over a number of generations.

The discrepancy between these findings and those of the earlier workers was clarified by the work of Luckinbill and Clare.[17] They found that there was a genotype–environment interaction affecting longevity, such that at low rearing densities there is little selectable genetic variance for lifespan, while at high rearing densities such genetic variance is abundant. The difference between these experimental designs is shown in Fig. 1.

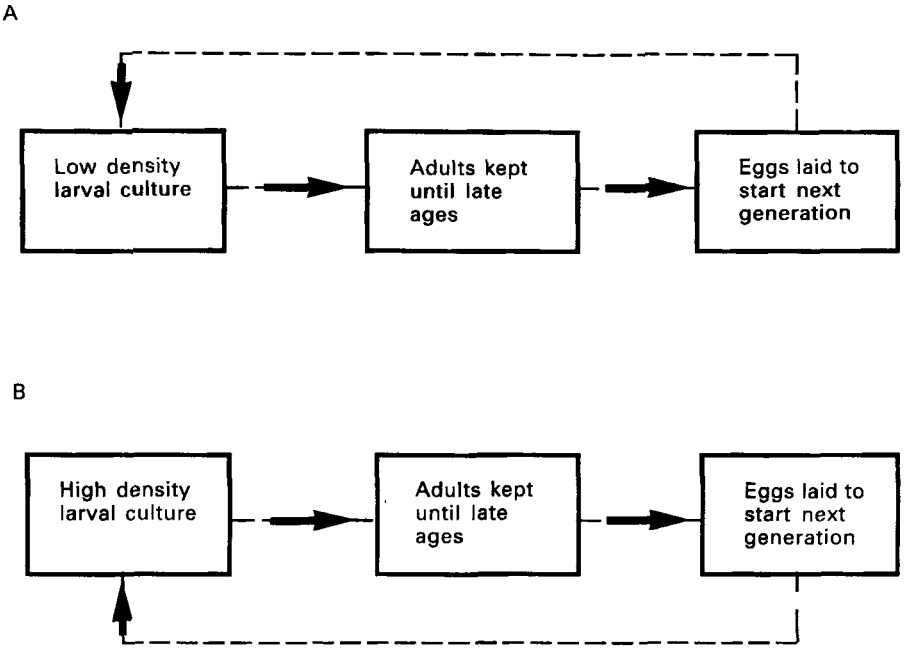

Fig. 1. *Alternative schemes for selecting for increased lifespan using delayed breeding. Both schemes involve the retention of adults until late ages before eggs are collected with which to start each generation. (A) Larvae reared at low densities. In this case, there is little response to selection when it is sustained for a moderate number of generations. (B) Larvae reared at high densities. In this case, there is a response to selection after ten or more generations, lifespan increasing.*

Physiological Genetics of Increased Lifespan

The long-lived *Drosophila* stocks that have been produced by selection provide material for investigating the physiological genetics of increased lifespan. The basic experimental design in this research has been the comparison of one or more lines with postponed ageing with one or more control lines that have not undergone selection. Ideally, multiple lines of each type are used, with each population mean constituting a single datum, since evolutionary theory offers predictions about population means, not the characters of individuals. The overall range of results is given in Table 1.

Performance characters are an obvious starting point for the biology of postponed ageing. Longer-lived lines have enhanced later fecundity, but reduced early fecundity.[14–17] Similarly, later locomotor activity is enhanced in longer-lived lines, but early locomotion is reduced.[18] (This locomotor assay uses time in motion in a space small enough to prevent flight.) Flight duration is enhanced over a wide range of ages in longer-lived flies, with no depression in early performance.[19–21]

Resistance to potentially lethal stresses is another context in which longer-lived lines appear to be improved. Time to death under conditions of starvation, desiccation, or low levels of ethanol is increased.[22] However the time to death from exposure to heat with high humidity is unaffected.[22]

In terms of constitutional changes, overall body weight and the weight of most body components appear to be unaltered.[19,23] The weight of the early ovary is substantially decreased,[23] presumably a reflection of the changes associated with depressed early fecundity. On the other hand, the lipid content of longer-lived flies is increased,[18] this increase following the genotype-, age-, and gender-dependent pattern of starvation resistance. This suggests that the enhancement of starvation resistance is a direct reflection of the altered lipid level.[18]

Overall, the picture that emerges from these physiological analyses is one of a fly that is superior with respect to most 'somatic' characters. Longer-lived flies can fly, walk, lay eggs, and resist stresses better at later ages, as well as being able

Table 1. Concomitants of genetically increased lifespan

Enhanced characters
Later fecundity
Starvation resistance
Ethanol vapor resistance
Desiccation resistance
Later locomotor activity
Flight duration
Lipid content
Diminished characters
Early metabolic rate
Early locomotor activity
Early fecundity
Early ovary weight
Unchanged characters
Total body weight
Weight of body parts other than ovaries
Later metabolic rate
Survival at elevated temperatures

to fly more and resist stresses better at earlier ages. On the other hand, these flies have some reductions in early performance, particularly in early fecundity, early metabolic rate, and early locomotor activity. However, all three of these losses of performance could reflect changes in physiology associated with diminished reproduction at earlier ages but with little deleterious impact on general somatic maintenance.

With respect to mediating mechanisms for these physiological changes, hormone levels and other internal signalling systems are obvious candidates for further investigation. In addition, the enzymes of intermediary metabolism could be altered in these flies.

Pleiotropy in Lifespan Genetics

There are three fundamental alternatives for allelic variation affecting lifespan: (i) alleles enhancing lifespan also enhance early fitness-components; (ii) alleles enhancing lifespan have deleterious effects on early fitness-components; and (iii) alleles enchancing lifespan have negligible effects on early fitness-components. The first type of allele will normally be close to fixation in natural populations, because natural selection would respond to its beneficial effects on early fitness-components, so it should not be possible to select for, or otherwise isolate, alleles of this kind. This is not true, however, of the second and third types of allele.

When alleles have opposed effects on fitness-components, they are said to exhibit 'antagonistic pleiotropy'.[24] This arises, for example, when an allele enhances early fertility, but does so at the expense of continued survival. Such alleles are often maintained polymorphically in natural populations because antagonistic pleiotropy readily gives rise to heterozygote superiority.[24] This in turn is expected to generate negative genetic correlations between early and late components of fitness, such as early fecundity and lifespan. Evidence for such correlations has been found in *Drosophila*.[12–13] In addition, with antagonistic pleiotropy between early reproduction and lifespan, selection for increased lifespan should result in decreased early reproduction, as discussed above, a result that has been obtained often.[7,12,14–17] In general, there is abundant evidence for the existence of alleles that have antagonistic pleiotropic effects early and late.

Turning to the third type of allele, some negative evidence against the existence of alleles with effects confined to late ages alone has been published,[12–13] but it appears that that evidence is undermined by the existence of antagonistic pleiotropy among the characters used.[25] A variety of other studies have found evidence for alleles with effects largely specific to later ages.[25–27] The best evidence of this kind is what appears to be 'mutation-accumulation' at later ages: alleles with effects specific to later ages will be largely free of the action of natural selection, leaving deleterious alleles free to accumulate by mutation pressure.[5] This implies that characters like later female fecundity and later male mating success should exhibit increased genetic variation, relative to early reproductive characters. Both results have been found in *Drosophila*.[26–27]

Taken together, the available evidence suggests that selection increases lifespan in *Drosophila* by means of alleles with beneficial effects at late ages only. While these alleles have no early beneficial effects on fitness-components, and therefore are normally at low frequency in natural populations, they may have either deleterious or negligible effects on early fitness-components. Evidence for both patterns of early effect is available in *Drosophila*.

How Many Loci are Involved?

While it is apparent that loci with varying patterns of pleiotropy are involved in the response to selection for increased lifespan, such results do not indicate the total number of loci involved. Two basic methods have been used to address this problem: effective factor analysis and chromosome substitution.

Quantitative genetic methods can be used to provide a minimum estimate of the total number of 'effective factors', or loci of roughly equivalent effect, affecting a character, providing the inheritance of the character is additive. As shown in Fig. 2, increased lifespan is inherited approximately additively. Two studies have used effective factor estimation procedures to determine the number of loci underlying increased lifespan in similar *Drosophila* stocks.[28–29] The essential parameter estimates found in these studies were quite similar, but the data were analysed in different ways. In particular, it was found that negative effective factors should not be discarded, as was done in one study,[28] because they indicated the involvement of many loci.[29] The larger and statistically more robust of the two studies found that there was no statistically significant evidence for fewer than an arbitrarily large number of loci involved.[29]

The results of chromosome substitution experiments corroborate this conclusion. In *Drosophila*, it is possible to use stocks with large-scale chromosome rearrangements that prevent

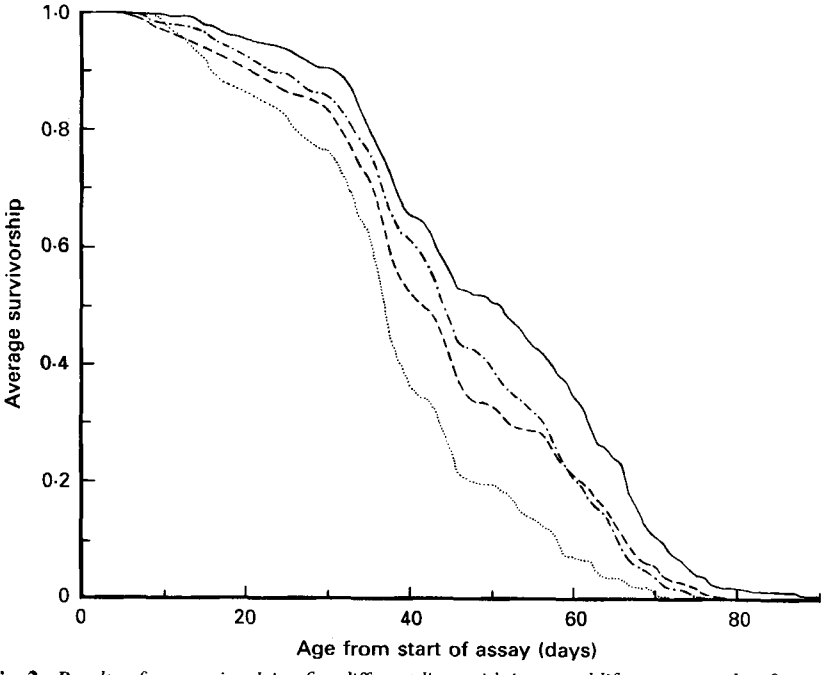

Fig. 2. *Results of crosses involving five different lines with increased lifespan crossed to five control lines. The solid line gives the average survivorship pattern over the five longer-lived lines. The dotted line gives the average survivorship pattern over the five control lines. The dashed and dash-dot lines give the averages of both types of reciprocal cross between the five control and the five long-lived lines. Statistically, the crossed lines do not deviate significantly from the mid-point between parental-line longevities, indicating average additivity in the inheritance of increased lifespan.*

crossing-over. With these stocks, entire chromosomes can be manipulated, so that genetic lines with arbitrary combinations of chromosomes can be created. In particular, chromosomes from one line can be substituted for the chromosomes of another line, individually or in combination. With this procedure, it is possible to estimate the magnitude of the contribution that each chromosome type makes to the differentiation of lines for any particular character. Using these techniques, it has been found that all three major chromosomes contribute to genetically increased lifespan in *Drosophila*,[30] suggesting the involvement of many loci.

Given the correspondence of the results from two very different types of experiment, effective factor analysis and chromosome substitution, it seems reasonable to conclude that many loci are involved in postponed ageing in the selected *Drosophila* stocks. Here, 'many' could mean as few as 5 loci or as many as 500. However, since the lifespan character and its correlated characters are subject to a great deal of environmental variability in their expression, this is enough to render impractical the use of Mendelian genetics in the analysis of these stocks.

Conclusions

Drosophila evolutionary geneticists have managed to create flies with genetically increased lifespans. This increased lifespan has been achieved as a result of genetic changes at many loci, involving alleles with varying patterns of pleiotropy. These pleiotropic effects involve a variety of physiological mechanisms that are being studied at the organismal level but which might also be amenable to study at cell and molecular levels. One major task for further research would be to ascertain how far genetically increased lifespan in *Drosophila* can be understood using non-genetic avenues of physiological research.

The other major task that remains is the resolution of the specific loci involved using molecular genetic techniques. Since many loci contribute to increased lifespan, simple Mendelian techniques alone will not provide useful starting points. Instead, molecular methods will be required to find the individual loci from the very outset. Large-scale screening methods, such as 2-D gel electrophoresis, might be one method of surveying the numerous loci involved. Whether such gels use protein or DNA, consistent spot or band differences of replicated selection stocks compared with replicated controls could indicate specific sites of allelic substitutions. It is particularly important that any such studies use replicated selected and control lines, because a single pair of selected and control lines could exhibit differentiation at loci through accidental genetic drift and fixation rather than selective substitution.

Beyond *Drosophila*, the significance of this line of research is that it indicates how biologists interested in ageing might go about determining the genetic and physiological mechanisms that can act so as to postpone ageing in any outbred metazoan. Evidently, it would be medically interesting to discover mechanisms for postponing ageing in mammals. To achieve this goal, analogous selection and genetic studies could be performed in a small, short-lived rodent, such as *Mus musculus*. The *Drosophila* findings may indicate the general range of possibilities for ageing genetics, and one may hope that some of the fruitful lines of research have been found.

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