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A density threshold for the expression of longevity in *Drosophila melanogaster*

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Long- and short-lived strains, selected for longevity for up to 27 generations, were raised under two treatments of developmental density. Selected stocks, controls and F_1 crosses between them are respectively long-lived, short-lived and intermediate, when raised at a high and uncontrolled developmental density. But when development takes place at a low density, longevity is sharply reduced, showing the existence of a developmental-density threshold for the expression of long life in selected strains and crosses. Selection for longevity is shown to have had effects in males comparable with those in females in the long-lived stock and in reciprocal F_1 crosses with the short-lived control. No age-specific effects on longevity were found in progeny from young, middle-aged and old adults.

INTRODUCTION

Two broad approaches have characterised the genetic study of longevity. One involves the use of mutant or mutagenised strains in which life span is reduced or otherwise altered in comparison with the wild-type (Clark and Maynard Smith, 1955; Gould and Clark, 1977; Bozcuk, 1978; 1981; Leffelaar and Grigliotti, 1984a, b; Klass, 1983). This approach has created useful stocks and proven effective in the study of crosses. But it seems limited in that mutations might shorten longevity in ways that have nothing to do with more rapidly advancing senescence, and a definite experimental modification of the ageing process is necessary for any such comparative approach. This is achieved directly in another method, by the selection of long-lived stocks for comparison with shorter-lived control strains (Rose, 1984; Luckinbill *et al.*, 1984; Luckinbill and Clare, 1985; Johnson and Wood, 1982). Strains are free of the effects of introduced mutations or mutagenesis in those comparisons, but they too have generated controversy.

Selection for improved longevity is applied in most such studies by favouring reproduction late in life at a point after mortality has begun to reduce population numbers. Wattiaux (1968), Rose and Charlesworth (1981) and Luckinbill and Clare (1985) have all used such a scheme, successfully producing strains of *Drosophila* that live longer than their short-lived ancestral stock. Lints and Hoste (1974) also used this plan, followed by Lints *et al.* (1979) with a modification of it and later Flanagan (1980), but all their efforts failed to obtain any effect of selection whatsoever. As selection proceeded in their studies, longevity either fluctuated wildly or varied slowly, but ultimately failed to separate selected from control lines.

Several important differences in experimental design distinguish the latter experiments from the former. These include differences in the treatment of strains that could affect the genetic variability available to selection as well as the circumstances of how selection was actually applied. All of the unsuccessful attempts (Lints and Hoste, 1974; Lints *et al.*, 1979; and Flanagan, 1980) differed from those in which life span did increase by one principal feature of selection. There the numbers of larvae were held to a constant and low density of 10 per vial during development.

Subsequent to these studies, Luckinbill and Clare (1985) used a dual design in a long-term selection study that incorporated separate density-controlled and uncontrolled treatments of the developing larvae. Both populations originated from the same ancestral population, but selection had markedly different outcomes. Where larval density was uncontrolled, selection increased life span by about 50 per cent. But where density was held low, longevity behaved as in Lints and Hoste

(1974), fluctuating wildly with no overall response.

We advanced a hypothesis to explain those results in terms of the effects that the different developmental environments had on the expression of genes for long life. A test of that hypothesis showed that raising larvae at a low population density during development substanstantially reduced adult longevity in selected long-lived lines and in F_1 crosses with short-lived control stocks. Thus, we showed that the larval environment could alter the expression of genes for long adult life. This suggested that the nonresponse of populations to selection in studies by Lints and his co-workers was artifactual, resulting from an altered genotype/phenotype correlation that prevented populations from responding to selection.

Based on their experiments, Lints and his co-workers advanced the hypothesis that longevity is either nongeneitc or else under the maternal control of minor genes. Clare and Luckinbill (1985), however, also performed the first crosses between selected long-lived stocks and short-lived controls. Longevity was definitely additive in F_1 populations, demonstrating both the existence and behaviour of genetic controls on life span. Thus, experiments in this laboratory have both obtained a conventional response to selection and also verified the unique outcome of Lints' studies, showing how their results could have been obtained.

To further test those findings, we repeat here the experiment designed to show the effects of developmental density on the expression of genes determining life span. Secondly, our previous studies have dealt with female longevity only. Therefore, we also describe the results of selection and effects of crosses on male longevity here. And finally, we test a further consideration of the hypothesis advanced by Lints and Hoste (1974), Lints *et al.* (1979) and Lints (1983), that longevity is controlled nongenetically.

METHODS

These experiments were conducted using the lines selected by Luckinbill and Clare (1985) with uncontrolled developmental density. The lines of that study were selected for reduced or increased longevity by reproducing them either early in life within a few days after eclosion, or late in life when most of the invididuals in a given generation had died. Populations under selection comprised 50 pairs of males and females. During selection, cultures were transferred to fresh medium at 48 hour intervals. Selection consisted of simply retaining the progeny produced in a given 48 hour interval between transfers to become the next generation. Selection has now been applied in this way for 27 generations to populations, with the result that the adult life span in long-lived stocks now exceeds that of the short-lived by about 60 per cent.

The media used and physical conditions of this study have been described elsewhere (Luckinbill *et al.*, 1984; Luckinbill and Clare, 1985). Longevity was measured here, as in former studies, in populations of 30 pairs of females with males isolated at one pair per standard shell vial and transferred daily to fresh medium. Also, as before, a set of sister lines taken from experimental lines and reared identically, provided replacements for occasionally escaping or infertile individuals.

Previous studies have established that reciprocal F_1 crosses between long- and short-lived stocks give identical results. Therefore, F_1 crosses here, except those in which male life span is measured, consisted of 25 pair of adults from each of the two component reciprocal populations (long-lived females × short-lived males and long-lived males × short-lived females), mated separately and combined for egg-laying into a single F_1 population of 50 pair. Where male longevity was measured, populations are from the 21st generation of selection and longevity is shown for 30 pairs of each separate reciprocal cross, raised with uncontrolled population density during development.

Where larval density was controlled, previously established procedures were also used. Eggs were first collected for 6–12 hours from populations to be measured on gelled acetic-acid-agar plates that had been spread with a live yeast culture and dried. The eggs were then removed and placed in vials at either 10, 70 or 120 per vial, where development took place. Males and females were sexed before eclosion and paired randomly before measurement of life span. Samples of the same population, raised in bottles with uncontrolled (and much higher) density were controls for this treatment. For the uncontrolled density treatment, 50 pairs of adults were allowed to lay eggs for two days, and progeny developed in a dense population, as took place under selection. Egg numbers there would equate to a density of about 200 or more eggs per vial. Males and females were also sexed before eclosion and paired randomly before measurement.

And finally, to determine whether the age of the parent has any effect on the longevity of the offspring they produce, life span was measured in replicate populations of progeny, collected from

females of both long- and short-lived stocks of generation 21, at the beginning, middle and late in life. Populations of offspring were collected by retaining the bottles from media transfers on days 3-5, 20-22 and 35-37 of adult life in the short-lived adult population. For the long-lived line, offspring were collected on days 3-5, 20-22 and day 50-52 of adult life. Offspring developed in uncontrolled-density conditions and were measured in populations of 30 separate pair, as elsewhere.

RESULTS

Fig. 1 compares long-lived lines and F_1 crosses after about 27 generations of selection with those from two generations before (Clare and Luckinbill, 1985). The long-lived stock underwent an increase in longevity in the two intervening generations. Therefore, both sets of measurements are expressed relatively, as per cent deviation, so that midparent values of crosses are equal and the response of strains to density manipulation can be compared relatively. Fig. 1 reveals that control populations and selected lines remained respectively short- and long-lived, and F_1 populations raised with larval-density uncontrolled show additive longevity, as before. The life span of the selected long-lived line raised at a controlled density of 70 or 120/vial is equvalent to that for an uncontrolled density, but if development takes place at a density of 10/vial, life span is sharply reduced. The longevity of the F_1 population raised at low density (10/vial) is less than the midparent value.

Thus, these populations validate the findings of our previous study. F_1 crosses are additively positioned between parental lines and populations show a strong reduction in life span when raised at a low density (although not as strong for the long-lived stock as before).

Table 1 compares fecundity and development time in long-lived parental strains under the two extremes of developmental density from the replicate measurements of fig. 1. Holding developmental density low substantially shortens life span but developmental time and fecundity are changed

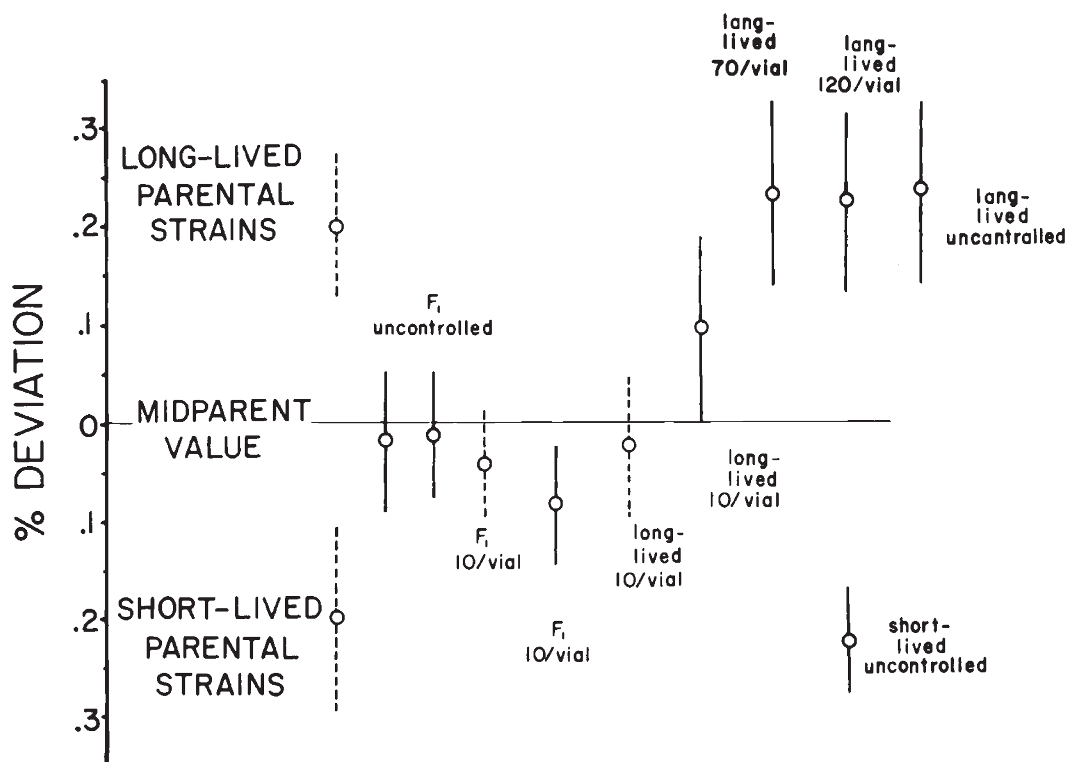

Figure 1 The life span of long-lived and short-lived lines and their F_1 crosses are shown for various treatments of population density during development. Circles indicate population means and vertical lines show the 95 per cent confidence intervals of life span in current estimates. Dashed lines show values of corresponding measurements made two generations before.

Table 1 Comparison of the average fecundity (number of eggs laid per female) and development time of long-lived parental stocks raised under two treatments of developmental density

	Population density at development	Average eggs/ female	S	Development (days)
Replicate I	Uncontrolled density	1037	257·3	10
	Controlled (10/vial)	1161	375·7	10
Replicate II	Uncontrolled density	965	144·6	11
	Controlled (10/vial)	1067	165·0	10

little. Daily fecundity is increased in lines developing at low density.

In table 2 the longevity of males is compared from the 25th generation of selection in parental and reciprocally crossed F_1 populations. Males clearly undergo changes during selection that are compatible with those in females. Although males have a greater life span than females in every case, F_1 crosses show clear additivity, just as females do. Average male/female longevity reflects the same trends.

Table 3 shows the mean life span of two replicate populations of offspring from young, middle-aged and old parents in short- and long-lived strains. The replicate-by-age source of variation from this two-way mixed model factorial analysis of variance indicates no significant difference is detectable in the life span of offspring from parents at different ages in either the short-lived or long-lived stock ($P=0{\cdot}79$, $0{\cdot}99$).

Table 2 The mean longevity of 30 males and females each in long- and short-lived stocks and their reciprocally crossed F_1 populations. Male and female longevity is also shown averaged for each line

	Short-lived (control)	Short-lived females × Long-lived males	Long-lived females × Short-lived males	Long-lived (selected)
Males	70·2	78·5	82·3	86·8
S	10·6	12·4	16·3	14·7
Females	52·5	60·9	65·6	76·9
S	16·9	18·7	13·8	15·4
Average male/female longevity	61·4	69·7	74·0	81·8

CONCLUSIONS

These experiments show that:

1. The expression of longevity is highly sensitive to the density at which larvae develop. Strains that are long-lived under dense developmental conditions become short-lived when raised at low density, though not so strongly as in our previous study. F_1 crosses show additivity in life span as before and their longevity is also reduced by low developmental density.
2. A threshold for the expression of genes for long-life appears to exist at somewhere between 10 and 70 larvae per vial. The length of devleopment is unchanged by low density and about the same average fecundity is found within the reduced adult life span. Populations raised at a controlled but high density of larvae have the same life span as uncontrolled populations.
3. The changes in male longevity under selection closely parallels that in females, with males also showing additivity in crosses. Average pair longevity corresponds with this.
4. The life span of progeny show no significant trend or deviation with the age of the parent from which they issue.

The highly controlled experiments of Lints and Hoste (1974), Lints *et al.* (1979) and Flanagan (1980) have been interpreted widely as posing a dilemma for understanding the genetic and evolutionary basis of the aging process (Lints and Hoste, 1974; Lints, 1978; 1983; Lints *et al.*, 1979). Though others have obtained positive responses to such selection (Wattiaux, 1968; Rose and Charlesworth, 1981; Rose, 1984), their studies offer no strict basis of comparison with those by Lints and his co-workers. Luckinbill and Clare (1985) and Clare and Luckinbill (1985) have shown that response to selection is dependent, in part, on developmental environment. Stocks that had increased as much as 50 per cent in longevity under selection, had substantially reduced life spans when raised at the developmental density used in Lints and Hoste (1974), Lints *et al.* (1979) and Flanegan (1980). Elegant precedence for such an environment-dependent expression of genes is found in Robertson (1961; 1963; 1964; 1966).

These experiments verify the results of our earlier studies, using the same strains at later generations. Stocks continue to be long-lived and crosses with short-lived controls continue to show additivity. All are sensitive to developmental density. Long-lived stocks show the same abrupt decrease when raised at low density, but their longevity is regained at high (but controlled) densities of 70

Table 3 Longevity in replicate populations of progeny from young, middle-aged and old parents in short- and long-lived lines are compared by ANOVA

Short-lived (early-selected) lines

Replicate	Young parents		Middle-aged parents		Old parents	
	Mean	S	Mean	S	Mean	S
1	51·9	11·3	52·7	12·8	56·7	8·4
2	51·1	14·7	54·1	11·2	55·6	8·0

Source of variation	Sum of squares	d.f.	*F*	Attained significance
Error	22262·5	174		
Replicate	2·0	1	0·016	0·90*
Age of parent	675·8	2	2·641	0·07*
Replicate by age of parent	59·1	2	0·230	0·79*

Long-lived (late-selected) lines

Replicate	Young parents		Middle-aged parents		Old parents	
	Mean	S	Mean	S	Mean	S
1	68·2	10·9	67·9	13·8	68·2	15·0
2	68·1	11·9	68·2	11·4	69·1	12·3

Source of variation	Sum of squares	d.f.	*F*	Attained significance
Error	27760·8	174		
Replicate	23·5	1	0·147	0·70*
Age	15·6	2	0·049	0·95*
Replicate by age of parent	4·7	2	0·015	0·99*

* Not significant.

or more per vial. This shows that the effect of density we observe is not contingent upon the vials used for rearing larvae but on the numbers of developing *Drosophila* themselves. The physiological mechanism by which this threshold is established is unknown, but these data suggest it is rooted in the complex of physical effects and feeding relationships that develop as burrowing larvae alter the medium and mature.

Of interst also is the fact that long-lived lines did not undergo as strong a reduction in life span when raised at 10/vial as those in Clare and Luckinbill (1985). The cause of this is not evident but it may indicate that the expressivity of genes for long-life selected is improving. Under sustained selection, it might be expected that longevity would eventually become less sensitive to the effects of larval density. Certainly, more replicates raised across a range of developmental densities would be necessary to distinguish such a trend.

Comparative male and female longevity typically varies widely in studies of life span. Male longevity exceeds that of females here by 13–34 per cent here, yet shows identical overall trends to those in females. Males are about 25 per cent longer lived in the selected line than in the short-lived control line. The average longevity of males alone or of male/female pairs in stocks and crosses is consistent with that of females alone, on which previous studies have been based.

The conclusion of Lints and Hoste (1974) and its reaffirmation by Lints *et al.* (1979) and Lints (1978; 1983) that the control of longevity is maternal and non-heritable derives from its evident dependency on parental age at reproduction and rapid reversibility in their experiments. But, Lints and co-workers are not alone in such a finding. Lansing (1947; 1954), O'Brian (1961), Callahan (1962), and Flemmings and Ludwig (1964) have all recorded somewhat similar outcomes. In every case, reproduction at a late age in life caused a rapid decline in longevity, resulting in some instances in extinction. Reproduction at an early age appeared necessary for continuation of stocks. The fact that such findings have often preceded the extinction of the lines under examination, suggests

that their conclusions may be the result of the intense inbreeding some lines were subjected to and/or artifacts of their culture and maintenence. Certainly the idea of the non-heritable basis of life span has arisen *post hoc*, as an explanation for the empirical findings of those studies, and not from any prospective reasoning.

Our studies are inconsistent with this hypothesis in two ways:

1. By obtaining a conventional response to selection for long-life (Luckinbill and Clare, 1985). Also, in crossing long- with short-lived stocks, the demonstration that longevity is additive is of particular relevance because additivity in the F_1 is inconsistent with maternal theories of inheritance (Clare and Luckinbill, 1985 and here).

2. We have also shown that experiments on which the hypothesis of nongenetic control of longevity is based may have been influenced by a powerful experimental artifact, demonstrable with our selected strains (Luckinbill and Clare 1985; Clare and Luckinbill, 1985 and here). To the foregoing we add the examination of life span's dependency on parental age at reproduction here. And again, our findings agree only with a conventional expectation.

Given the weight of our previous results (Luckinbill and Clare, 1985; Clare and Luckinbill, 1985), their verification and extension here, and those of others preceding us (Wattiaux, 1968; Rose and Charlesworth, 1981; Rose, 1984), we conclude that little is convincing in arguments for the non-genetic or maternal control of longevity. In fact, the most powerful current evidence suggests only that genes with an environmental component to their expression, control life span. The fact that stocks can be selected and a stable phenotype obtained, attests to the genetic control of this character above all. This finding in particular, has been independently verified with similarly selected lines (Rose and Charlesworth, 1984).

If the preceding works and ours are valid, it means only that longevity has a conventional genetic basis. Such a conclusion hardly seems to demand proof or even independent verification to be convincing. But the consideration of longevity in the same evolutionary light as other characters has evoked controversy from its earliest inception. Final resolution of this issue may require the mapping and identification of some of the contributing genetic elements.

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