

MATERNAL AGE AND DEVELOPMENTAL VARIABILITY

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INTRODUCTION

In man maternal age has been shown to affect the incidence of various abnormal conditions. The incidence of still births, mongolism, central placenta praevia and congenital malformations such as anencephaly, hydrocephaly and spina bifida, all have been shown to increase with maternal age (see, for example, Penrose, 1934, 1939*a, b*; Murphy, 1954; Edwards, 1958). In some of these abnormalities such as mongolism and spina bifida, there is evidence for an initial small decrease in frequency in young women so giving parabolic variation with maternal age. In all cases parity (i.e. order of birth) and maternal age are necessarily correlated, but where investigated maternal age is the determining factor.

In mice the incidence of polydactyly, which in many stocks is determined by an incompletely penetrant recessive gene, *decreases* with age (Holt, 1945, 1948), and the same occurs for polydactyly in guinea-pigs (Wright, 1926), whereas white spotting in guinea-pigs increases with maternal age (Wright, 1926). It is perhaps surprising that polydactyly in mice and guinea-pigs does not follow the pattern observed for the human developmental abnormalities. However, other skeletal abnormalities in mice also decrease with age (Searle, 1954).

Bodmer (1961*a, b*) has shown that in mice the viability of both homozygous *fidget* and *pallid* mice decreases with age. In both cases the evidence suggests parabolic variation with age as the viabilities of offspring were less from very young mothers (2 months old) than from mothers 3-6 months old, and from mothers over 6 months old the viabilities of *fidget* and *pallid* mice decreased again. Similarly in man infant mortality varies parabolically with maternal age giving a minimum for mothers aged 20-35 years (see Treffers, 1954). Further evidence in man, mice and other organisms including plants is given in volume 57, Art. 5, of the *Annals of the New York Academy of Sciences* entitled 'Parental age and characteristics of the offspring'.

It is well known that in *Drosophila* recombination varies with age. Usually a U-shaped curve is found with a minimum between the 6th and 12th day of the female's age, but the recombination frequency in the first few days is higher than at any other time (see Bridges, 1915, 1929). In some cases there is evidence for a W-shaped curve with the central maximum from females aged about 20 days. In mice there is some evidence for decreasing recombination with maternal age (Fisher, 1949; Bodmer, 1961*a, b*), but data from old females are inadequate to test the possibility of a U-shaped curve. Wallace (1957), however, considers that her data and those of Fisher (1949) and of Owen (1953) are all at least suggestive of a U-shaped curve of variation.

Mather (1938) has stressed that crossing-over is inversely related to failure of pairing and non-disjunction. Perhaps part of the decrease in crossing-over with age is due to an increase in failure of pairing and non-disjunction (see also Bodmer, 1961*a*). In agreement is the increasing incidence of mongolism in man with maternal age, since mongolism is now known to be due to aneuploidy for one of the smaller somatic chromosomes (Lejeune, Gautier & Turpin, 1959) probably arising as a result of meiotic non-disjunction. Evidence for increasing frequency with maternal age of another cytological abnormality, the triple X-syndrome, has been presented recently (Johnston, Ferguson-Smith, Handmaker, Jones & Jones, 1961).

Polani, Briggs, Ford, Clarke & Berg (1960) stressed that the maternal environment of the primary oocytes, which are probably differentiated before birth, will change with age due perhaps to a progressive biochemical alteration. Thus developmental change induced by increasing age may affect the efficiency of chromosome pairing at meiosis. Developmental differences in plants, such as occur within an anther of a rye plant, have also been shown to affect chiasma frequency (Rees & Naylor, 1960).

It is relatively easy to obtain information on the varying incidence of congenital abnormalities in man, or the varying frequency of recombination and other characteristics in mice at ages in the centre of the reproductive age, but the more interesting information comes from very young or very old mothers. Collecting data at these periods in man is difficult but by no means impossible. Similarly, in mice it is difficult to collect adequate data from aged females. It was therefore decided to study developmental variability as affected by maternal age in *Drosophila melanogaster*, where data at extreme ages can be collected somewhat more easily, for the following characters: (1) egg size variability as measured by egg length, with hatchability as a concomitant observation; and (2) sterno-pleural chaeta number variability as measured by asymmetry between the two sides of the fly.

Egg-size variability and hatchability

Two strains of flies, an Oregon-R mass bred strain and a Samarkand inbred strain were used. Eggs were laid on watch-glasses on which was placed roughened agar mixed with a little acetic acid-alcohol mixture to encourage laying. Yeast was added also. Flies were exposed to these watch-glasses for about 6 hr. every 24 hr. For the remainder of the time they were incubated on a normal treacle-oatmeal medium seeded with excess yeast. Eggs were measured for length immediately following laying on the agar-acetic acid-alcohol medium and incubated for at least 24 hr. to determine hatchability. Sixty eggs were measured daily except from old females when insufficient eggs were laid.

In Fig. 1 the means, variances and coefficients of variation of egg length, and percentage hatchability are plotted against maternal age in days for the Oregon-R stock. There was a slight decrease in egg length with age and an initial decrease in variance followed by an increase. The coefficients of variation followed a pattern similar to the variances. Hatchability, after staying above 90% for the first 13 days of life, fell rapidly in eggs from older females.

To test these trends further and to assess their significance, regressions against age were calculated for each variable using the technique of orthogonal polynomials (Fisher, 1954) to test for quadratic as well as linear trends in view of the possibility

of significant curvature. In the case of hatchability the angular transformation (Bliss, 1938) was applied to avoid correlations between the variance and the mean. The analysis was carried out up to the 22nd day of maternal age. Data from 2 or more successive days were necessary for sufficient accuracy to plot points from older flies. These are the points to the right of the vertical dotted line in Fig. 1. They were omitted from the regression analysis. The fitted regression curves are plotted in Fig. 1.

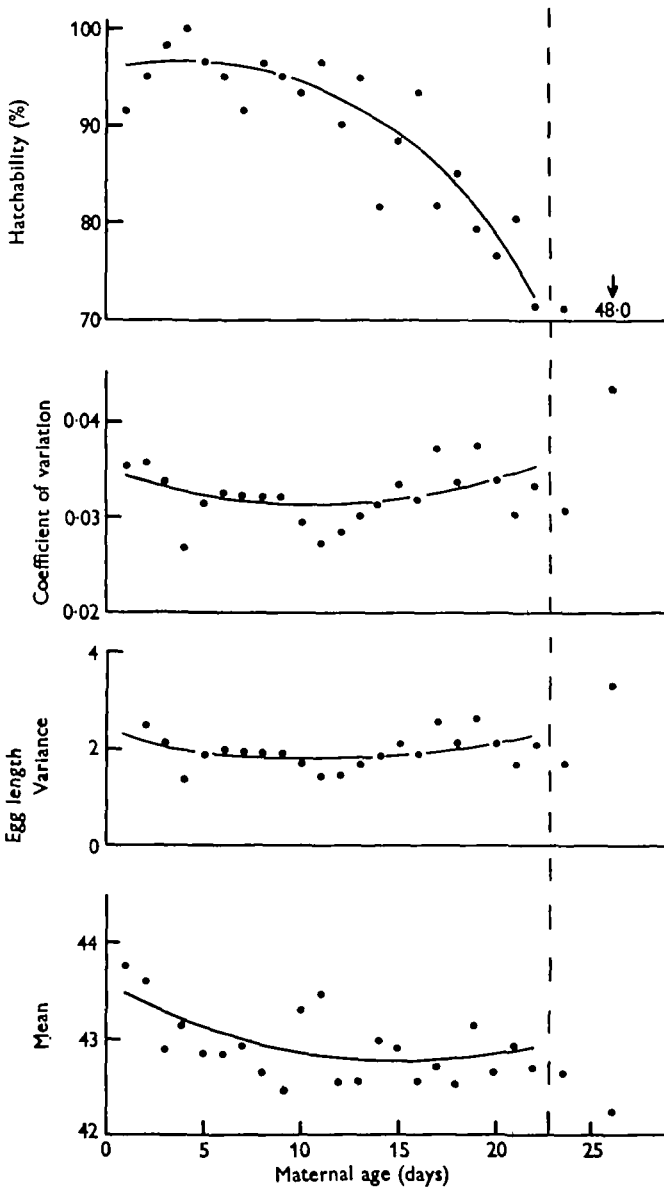


Fig. 1. Means, variances and coefficients of variation of egg length and percentage hatchability for the Oregon-R stock plotted against maternal age. Multiplying the means by 0.0118 will express them in mm.

The regression analysis (Table 1) reveals a significant decrease in egg length with age ($P < 0.05$). The variance and coefficients of variation of egg length at first decreased, then increased and this is confirmed by significant quadratic components. A linear regression analysis of the coefficients of variation for the first 11 days on maternal age was significant ($P < 0.05$), thus confirming the initial decrease. Hatchability decreased significantly with age with a significant quadratic component.

For the Samarkand inbred stock (Fig. 2) the regression analysis was carried out up to the 16th day of maternal age (Table 1). Egg length decreased significantly with age, but rather more than for the Oregon-R stock, and there is some evidence of a significant quadratic component. Egg-length variance and coefficients of variation decreased slightly at first, then increased as shown by the significant quadratic components. In contrast with the Oregon-R stock, however, there are significant linear components, since the increase in egg length variability after the 10th day of maternal age far exceeded the initial decrease. The initial decrease was not, however, significant on a linear regression analysis using the coefficients of variation. Hatchability decreased significantly with age and, as in the Oregon-R stock, with a subsignificant quadratic component.

Table 1. *Regression of egg-length means, variances and coefficients of variation, and of hatchabilities on maternal age*

	D.F.	Egg length			Egg-length variance			Egg-length coefficient of variation			Hatchability		
		M.S.	F	P	M.S.	F	P	M.S.	F	P	M.S.	F	P
1. Oregon-R stock													
Linear regression	1	0.59	6.18	< 0.05	0.01	0.09	> 0.20	2.07	0.29	> 0.20	865.81	46.23	< 0.001
Quadratic regression	1	0.32	3.36	< 0.10	0.57	5.47	< 0.05	32.73	4.53	< 0.05	99.30	5.30	< 0.05
Residual	19	0.09	—	—	0.11	—	—	7.23	—	—	18.73	—	—
2. Samarkand inbred stock													
Linear regression	1	2.69	30.18	< 0.001	1.79	9.98	< 0.01	141.06	12.60	< 0.01	261.10	16.95	< 0.01
Quadratic regression	1	0.81	9.09	< 0.01	1.18	6.56	< 0.05	58.89	5.26	< 0.05	60.43	3.92	< 0.10
Residual	13	0.09	—	—	0.18	—	—	11.20	—	—	15.40	—	—

As maternal age increased, therefore, hatchability decreased and egg-length variability increased. Thus developmental stability deteriorates with age as might be expected. Of interest is the possibility of an initial fall in variability in the very young females to a minimum between about the 5th and 12th day of maternal age, showing that in the first few days developmental stability may be somewhat lower than between the 5th and 12th days. We might now ask ourselves whether the adult flies derived from females of differing maternal ages vary in developmental stability in a similar way to the eggs from which they are derived, or whether all the extreme types are eliminated during development. In the next section an attempt is made to answer these questions using sterno-pleural chaeta number asymmetry as a measure of variability in the adult.

Sterno-pleural chaeta number asymmetry

Oregon-R flies at 25° C. were permitted to lay eggs on watch-glasses with food seeded with yeast. When the eggs had hatched, two replicates in vials were set up daily each containing 40 newly hatched larvae. On emergence 30 flies (15 of each sex

per replicate) were scored for sterno-pleural chaeta number. Asymmetry was computed as the sum of the absolute differences between the left- and right-hand sides of the flies, A , divided by the total number of chaetae, T , on the 60 flies scored daily, A/T . This measure, which has been used in other studies (see, for example, Thoday, 1955, 1958; Beardmore, 1960), is more correct than using just the sum A as it takes into account variability of fly size (Parsons, 1961), but at the level of competition

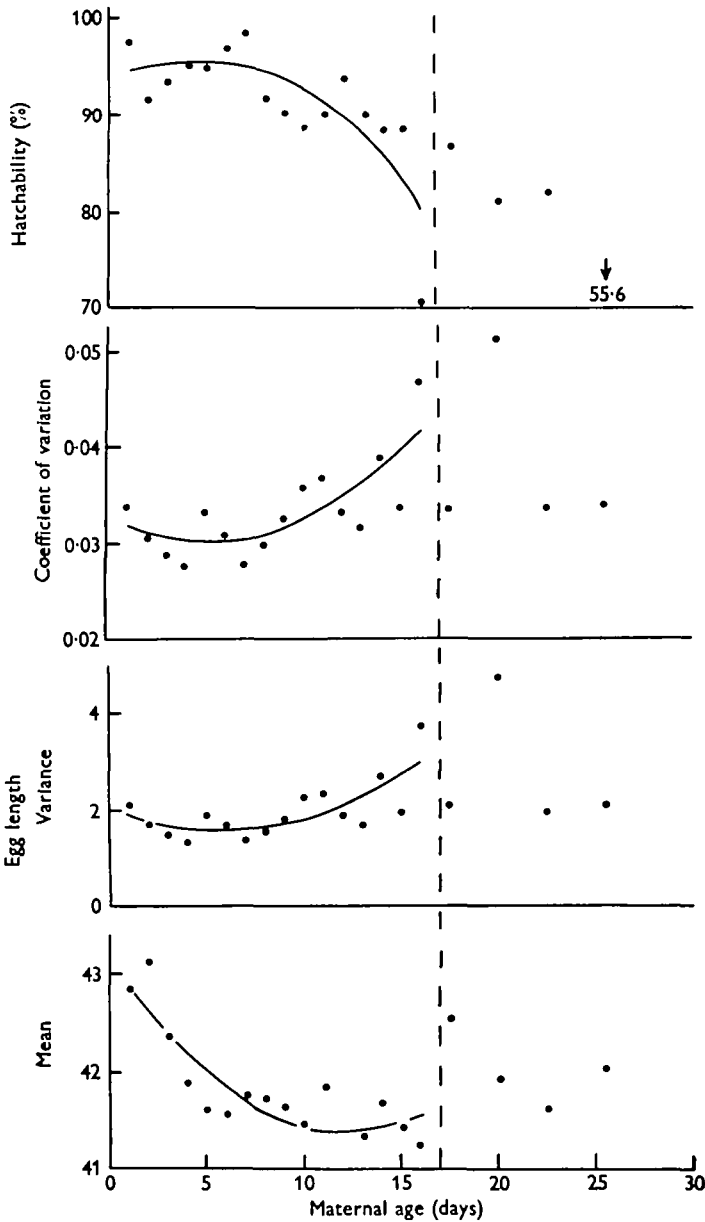


Fig. 2. Means, variances and coefficients of variation of egg length and percentage hatchability for the Samarkand inbred stocks plotted against maternal age. Multiplying the means by 0.0118 will express them in mm.

used both measures were equivalent. In Fig. 3 A/T is plotted against maternal age. A fairly clear parabolic type of variation with age is apparent. Asymmetry decreased for the first 5 days then increased after about the 15th day to a level far exceeding the initial level. In Table 2 the regression analysis is given. It was taken up to the quartic level since the curve appeared at first sight to have two minima, but the cubic and quartic components are not significant. The linear and quadratic components are, however, significant confirming a general increase in variability with age combined with significant parabolic variation. In this experiment a linear regression analysis carried out up to the 15th day of maternal age, which is the minimum of the regression curve in Fig. 3, was significant ($P < 0.05$) so confirming the initial decrease of asymmetry with age.

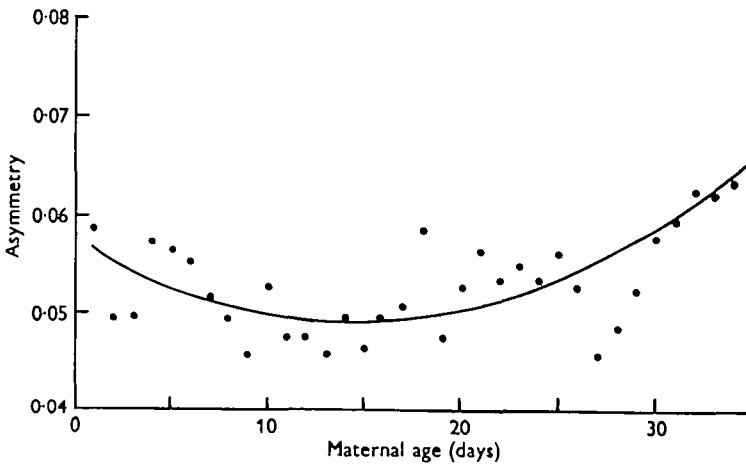


Fig. 3. Asymmetry of sterno-pleural chaeta number plotted against maternal age for the Oregon-R stock grown at 25° C.

Table 2. *Regression of asymmetry on maternal age in the Oregon-R stock at 25° C.*

	D.F.	M.S.	F	P
Linear regression	1	27,754.09	15.85	< 0.001
Quadratic regression	1	45,092.72	25.74	< 0.001
Cubic regression	1	2,299.90	1.31	> 0.20
Quartic regression	1	73.22	0.04	> 0.20
Residual	30	1,751.52	—	—

It was then thought reasonable to repeat this experiment, and to combine it with another contrast whereby two replicates of newly hatched larvae were placed daily in an incubator at 30° C. to complete their development. It is well known that developmental stability decreases if flies are grown at temperatures differing from those in which they are normally grown (Thoday, 1955, 1958; Beardmore, 1960). Thus we are testing whether the effects of age and temperature are cumulative, or whether different maternal ages behave differentially. In Fig. 4 the data are plotted and the regression analyses given in Table 3.

At 25° C. asymmetry increased with age, but in contrast with the experiment just reported, the quadratic component was not significant. The mean square for the

quadratic component is usually smaller and may depend to some extent on the precise environment, so we may expect it not always to be significant, but only in a fair proportion of cases. At 30° C. there is far more asymmetry than at 25° C., especially in flies derived from young females, when developmental instability is very high. There is a great deal of variability from day to day which tends to obscure any trends but the points appear to fit a parabola. In confirmation is a subsignificant quadratic component (Table 3). The extreme variability at 30° C. might be expected since this temperature is a severe environmental stress, as it is close to the limit of survival of *Drosophila*. No doubt slight changes in the micro-environment from day to day, such as variable humidity, account for some of this extreme variability.

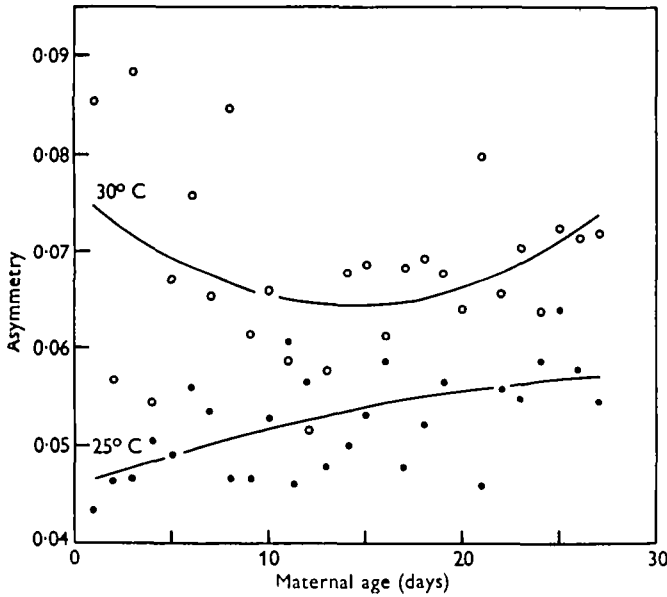


Fig. 4. Asymmetry of sterno-pleural chaeta number plotted against maternal age for the Oregon-R stock grown at 25° C. (●) and 30° C. (○).

Table 3. Regression of asymmetry on maternal age in the Oregon-R stock at two temperatures, 25° C. and 30° C.

	D.F.	25° C.			30° C.		
		M.S.	F	P	M.S.	F	P
Linear regression	1	26,440.52	11.97	< 0.01	236.19	0.03	> 0.20
Quadratic regression	1	697.61	0.31	> 0.20	27,272.37	3.49	< 0.10
Residual	24	2,243.26	—	—	7,809.91	—	—

In general these experiments show that variability of the adult fly as measured by sterno-pleural chaeta number asymmetry does depend on the age of the mother and that many extreme types are not eliminated during development. It might be postulated that if competition were severe enough to lead to a substantial elimination of flies before eclosion, then the adults may be less variable due to the action of stabilizing selection eliminating extreme, and presumably less fit, flies.

DISCUSSION

It has been shown that maternal age influences the variability of egg length and sterno-pleural chaeta number asymmetry which are both of some adaptive value. In fact it is likely that all variable characters contributing to overall fitness will vary with maternal age. In the introduction it was pointed out that maternal age can affect characters as diverse as recombination, developmental abnormalities and the viability of deleterious recessive genes. Litter size in mice is affected by maternal age and often increases initially then decreases, showing parabolic variation (Table 4—see also Russell, 1954). Maximum litter size probably occurs at a period when the viability of deleterious recessive genes is highest. Furthermore, litter-size variability is often greater from very young mothers than from rather older mothers (Table 4); but from very old mothers, litter-size variability is very much increased again. Thus again we have parabolic variation with age with a minimum for mothers aged 4–8 months. Similarly, in *Drosophila* parabolic variation in the number of eggs laid per viable female may occur with maternal age. In Table 5 eggs laid per female per hour are given for the egg-length variability study reported in this paper. There is maximum reproductive ability at the time of maximum developmental stability and hatchability. The time of maximum developmental stability will be the time when stabilizing selection is most effective in producing a low proportion of extreme (and hence less fit) offspring.

Table 4. *Litter size and its variability in mice at three maternal ages (from mothers having five or more litters)*

Stock		Litter		
		First	Middle*	Last
Sd (Danforth's short tail) (data from 28 matings)	Mean	4.9	5.6	4.9
	Variance	4.5	3.9	6.1
	Coefficient of variation	0.43	0.35	0.51
Linkage group XIII (data from 40 matings)	Mean	6.4	7.0	5.9
	Variance	5.3	5.6	7.7
	Coefficient of variation	0.36	0.34	0.47

* If the total number of litters = n , the middle litter was taken as $\frac{1}{2}n$ for even numbers and $\frac{1}{2}(n+1)$ for odd numbers.

Table 5. *Eggs laid per female per hour*

Maternal age (days)	Oregon-R	Samarkand inbred
	2-6	1.71
7-11	2.03	1.76
12-16	1.77	1.86
17-21	1.46	0.70
22-26	1.74	—

Somewhat surprising is the somewhat increased variability in young females, which although not significant in all cases allows us to conclude, in agreement with other studies, that there is an optimum age when the offspring will be fittest, as measured by maximum developmental stability and viability. This will also be a time when the development of the offspring will be least affected by external factors of the environment, as the study carried out at 30° C. suggests.

It might be supposed that the increased variability late in age corresponds to, or just precedes, the onset of the 'dying' process in the life span, which is initiated when ageing has proceeded to a stage at which the individual is no longer capable of maintaining a steady state (Clarke & Maynard Smith, 1961). The higher variability early in life perhaps occurs because the organism does not move into a stage of maximum stability until it is a few days old.

The results of this study in general agree with those obtained in mouse and man, but provide more detailed data at critical maternal ages. However, the situation differs in mammals from that in *Drosophila* in that the parental expenditure on the offspring in mammals, in particular in man, is much higher. In *Drosophila* parental expenditure ceases as soon as the egg is laid. In mammals, parental expenditure may vary between parents and may obscure trends to a certain extent. Parental expenditure could, for example, affect the frequency of congenital abnormalities determined by incompletely penetrant genes where the penetrance depends very much on the environment. In mammals parental expenditure must be divided into that incurred before birth and that incurred after birth. Parental expenditure after birth would refer to factors such as pattern of lactation and care of the young which may vary with maternal age. Sawin (1954) has studied some of these factors in rabbits with some suggestive results.

SUMMARY

1. Evidence is presented showing that in *Drosophila* egg-length variability and sterno-pleural chaeta number asymmetry, which are both measures of developmental stability, usually increase with age parabolically, so that both these characters decrease at first to a minimum between the 5th and 12th days of maternal age, then increase to a maximum, although the initial decrease in variability may not necessarily be significant.

2. Maximum hatchability probably occurs at the time of maximum developmental stability.

3. At the time of maximum developmental stability, growing the flies at the extreme temperature of 30° C. has least effect on sterno-pleural chaeta number asymmetry. At this time, therefore, the organism is best buffered against the stresses of the external environment.

4. It is concluded from these studies, and from studies in the mouse and from the literature, that there is an optimum maternal age when the offspring will, in general, be fittest as measured by maximum developmental stability, viability and reproductive ability.

5. It is argued that these results are of application to man, although factors such as variable parental expenditure in man could complicate the situation.

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