circadian systems—and in autotrophically grown wild type *Euglena* (*2*)—to damp out.

The fact that the P_4ZUL mutant can be synchronized by LD cycles when grown at 19°C (Fig. 1, curve A), but not when grown at 25°C (Fig. 1, curve B), leads to some interesting theoretical possibilities. One of the consequences of the chronon model (*13*) for circadian clocks is that only and all eukaryotic cells possess regulatory capacities for circadian timekeeping, but that these capabilities can be expressed only when the cells are in the circadian-infradian growth mode (GT $\geqq$ 24 hours). This phenomenon has been termed the GET effect (*7*) after the *Gonyaulax* (*5*), *Euglena* (*2*), and *Tetrahymena* (*7*) systems, since in each of these organisms a single transition from bright to dim illumination (switch-down) occurring during the infradian growth phase of an exponentially increasing culture is sufficient to elicit a free-running, circadian rhythm of cell division.

Our data for the mutant further support (although they do not demand) this hypothesis: as predicted, entrainment did not occur at 25°C when the GT was only 10 hours (ultradian mode), but was observed at 19°C when the overall GT was increased to about 24 hours (Fig. 1). Furthermore, a single switch-up in irradiance (Fig. 3) was sufficient to generate a persisting rhythm of cell division in cultures that had been exponentially increasing in the circadian-infradian mode (*7*) at 19°C.

It may well be that the paucity of reports of light-induced synchrony in heterotrophically grown unicells may be due to the fact that at commonly used laboratory temperatures (21° to 25°C) cell division may be taking place so rapidly that the rhythm cannot be expressed. Indeed, we have found that the rhythm can be entrained by a *L*D: *10*,14 cycle in the wild type of *E. gracilis* Klebs (Z strain) when the cells are grown heterotrophically at 19°C (but not at 25°C) on either the low *p*H medium or on a 0.02*M* sodium acetate medium (*14*); the synchrony persists for at least several cycles in continuous darkness. Similarly, Mitchell reports (*15*) LD-induced synchronization of an ultraviolet-bleached mutant of this same strain also maintained on acetate, although it is not yet clear whether the observed synchronous cell division in this case will persist under constant conditions.

In addition, reports from our laboratory (*10*, *16*) concerning the breakdown of division synchrony in autotrophic cultures of wild type *Euglena* maintained on a minimal salt medium in LD at 25°C (GT of 24 hours) by either exposure to continuous illumination (at 3500 lux) or by the addition of 0.025*M* sodium acetate, 0.006*M* ethanol, or 0.34*M* L-glutamic acid to the medium are also consistent with the circadian-infradian "rule" (*7*) that the source of constant illumination or the introduction of utilizable, exogenous carbon resulted in a decrease in the GT to 13 to 15 hours (that is, constituting a switch to the ultradian growth mode), which, in turn, obliterated the cell division rhythm.

In conclusion, our results with the P_4ZUL mutant corroborate our thesis that a cellular circadian clock in some manner periodically gates cell division in *Euglena* grown under appropriate conditions which allow for the expression of this rhythm. Of course, this hypothesis is at best only a superficial description of what is surely a complex phenomenon; we do not yet know the nature or location of the photoreceptor system that is implicated, the identity of the molecular components of the postulated underlying biological clock, or the manner in which information regarding period and phase is transduced from this circadian oscillator to the observed rhythm of cell division. It is not clear to what extent interaction among individual cells in a population of oscillators may contribute to the extended persistence of the rhythm under constant conditions (*1*, *17*). Nevertheless, we believe that the photosynthetic mutant, as well as other strains of *Euglena* that lack chlorophyll and plastids, provides useful experimental systems.

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Senescence and Genetic Load: Evidence from Tribolium

Abstract. *After 40 generations in which young adults were killed shortly after the onset of reproduction, strains of* Tribolium castaneum *with significantly decreased median longevity evolved. These findings support the hypothesis that the longevity of a species is controlled by genetic factors, and represents a compromise between selection for longer reproductive period and the limit set by environmental hazards.*

Edney and Gill (*1*) have discussed possible reasons behind differences in longevity (onset of senescence) among organisms. Elaborating on earlier models (*2*) they reason that the intrinsic, genetically controlled longevity of each species must have developed as a compromise between the forces tending to prolong it (for increased reproduction) and hazards tending to curtail it (random accidents and environmentally induced senescence, including mechanical deterioration of the organisms). This hypothesis could be tested in two ways—rearing organisms in an environment in which hazards are reduced should eventually result in longer lived populations, although this may take some time in view of the genetically programmed life-span

Table 1. Median longevities of *Tribolium* for selected and stock strains (by sex) and probability P for given differences by one-tailed t-tests. Longevities are expressed as census intervals which are coded as multiples of 4 weeks minus 2.

Strain and sex	Strains		P
	Selected	Stock	
Wild type female	5.0	7.5	0.0013
Wild type male	12.5	14.5	.0936
Black female	5.6	5.9	.3804
Black male	5.0	8.8	.0102

adjusted to the previously experienced severity of the hazard factor. Another test would be to enhance and advance the action of the hazard factor by removing adults from a population after permitting them to reproduce for only the very earliest portion of their life-span. Such an experiment carried on for "50 or so generations" should result in a population in which the load of deleterious mutations affecting the late reproductive and postreproductive periods would be permitted to accumulate, shortening the life-span.

In connection with a long-term selection experiment carried out for a different purpose (*3*), material was at hand for the second test suggested by Edney and Gill. Two strains of the flour beetle *Tribolium castaneum*, one the wild type UPF strain, a second marked with the autosomal semidominant *black*, had been reared in my laboratory for 40 generations. In each generation from 500 to 2000 recently eclosed adults were permitted to oviposit for 3 days and were killed thereafter. These selected beetles of both strains could be compared with the laboratory stocks from which they had originated. The latter were kept in mass culture, the flour being changed monthly, at which time the entire adult population was transferred to a new jar, being thinned out somewhat whenever necessary. Although no detailed age distributions are available, it is known that under such conditions many adults will live for several generations, being quite capable of coexisting with their F_5 generation offspring.

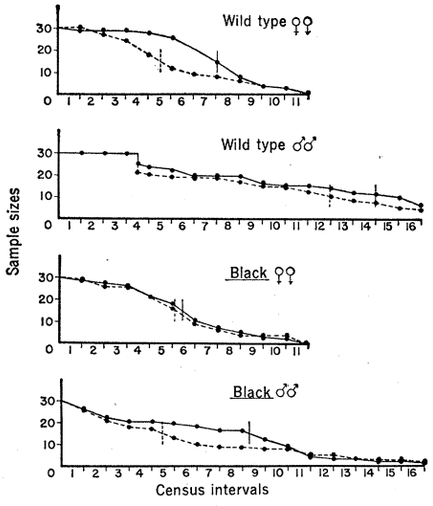

Fig. 1. Survivorship curves for the males and females of the four strains employed in the study. Ordinate: sample size (initially 30); abscissa: census intervals representing 4-week intervals commencing 22 May 1968. For purposes of computation the census intervals had been coded 1, 2, 3. . . . Thus a median longevity of 5.0 is equivalent to 18 weeks. Solid lines represent stock cultures; broken lines, selected strains (*7*). Medians are indicated by short vertical lines.

Sixty pupae (30 female, 30 male) were taken at random from each of the two selected cultures, and an equal number were sampled from the two stock cultures. The pupae were placed singly in small vials containing 1 g of standard flour medium. The 240 vials were maintained at 29°C, 70 percent relative humidity, and constant illumination. Starting in May 1968 the vials were examined every 4 weeks and all vials whose occupant had died since the last census were recorded and discarded. The experiment was terminated in August 1969, at which time 13 beetles, all males, were still alive.

Males of every strain lived considerably longer than females (Fig. 1 and Table 1). The selected strains had the same range of longevity as the stock cultures; however, the distributions of individual life-spans in the selected cultures differed appreciably from those in the stock cultures. During most of the experiment mortality was higher in the selected cultures. This is best illustrated by differences in median longevity (Table 1). All four deviations between medians were in the expected direction and two were highly significant (wild type females and *black* males). Combining probabilities (*4*) of the four separate tests we obtain $-2\Sigma\ln P = 29.109$. This is greater than the critical chi-square value of 21.955 for $P = .005$ (d.f. = 8).

These data support Edney and Gill's views that intensification of hazard factors (early death) permits the accumulation of deleterious mutants acting during the late reproductive and postreproductive period whose aggregate effect it is to reduce the life-span of a significant proportion of the population. The observed changes were in distribution of mortality rather than in range of longevity. This would suggest nonadditive genetic factors for longevity. Others have observed unequal contributions by various chromosomes to the variability of this character in *Drosophila* (*5*). All selected cultures had evolved a marked increase in length of developmental period (*3*), which is believed to be due to elimination of early pupating individuals by cannibalism. This may have counteracted the selection of early reproducing adults, and without it the effect noticed in this study might have been even more pronounced. The increase in developmental period was especially marked in the *black* selected strain, and possibly the lack of difference in median longevity of the *black* females might be related to this fact. Alternative explanations of the phenomenon observed here are, of course, possible, since we have no direct evidence of an increase in genetic load in the selected strains. It is possible, as suggested by Edney and Williams (*6*), that selected genes favoring early oviposition may also pleiotropically shorten the life-span. However, the accumulation of late acting deleterious mutations seems far more plausible.

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7. The perpendicular drop in the wild type male graphs represents mortality due to a malfunctioning of the humidification system during census interval 4. Water was sprayed into 16 vials, drowning the beetles. There had been no mortality in their lots prior to that time. The subsequent computations for these strains were adjusted to allow for this occurrence.
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