Dual Modes of Aging in Mediterranean Fruit Fly Females

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The life history of medflies is characterized by two physiological modes with different demographic schedules of fertility and survival: a waiting mode in which both mortality and reproduction are low and a reproductive mode in which mortality is very low at the onset of egg laying but accelerates as eggs are laid. Medflies stay in waiting mode when they are fed only sugar. When fed protein, a scarce resource in the wild, medflies switch to reproductive mode. Medflies that switch from waiting to reproductive mode survive longer than medflies kept in either mode exclusively. An understanding of the physiological shift that occurs between the waiting and reproductive modes may yield information about the fundamental processes that determine longevity.

As a strategy for prolonging survival while maintaining reproductive potential, most organisms suspend reproduction in periods of food scarcity by entering a different physiological mode (*1*). The genes that regulate the transition to waiting mode and survival in this mode in nematode worms (dauer states) and yeast (stationary phase) are closely linked to longevity (*2*).

We hypothesized that fruit flies may also be able to enter a waiting mode and that they can live to and reproduce at extreme ages when subject to a lack of dietary protein. To test this, we maintained an initial pool of 2500 male and female Mediterranean fruit flies (*Ceratitis capitata*) from a large-scale rearing facility in single-pair cages on sugar and water (*3*). At 30, 60, and 90 days subgroups of 100 pairs were provided with a full diet ad libitum. Their reproduction and survival were monitored until the last female died (*4*). Lifetime reproduction and survival were also monitored from eclosion for two 100-pair control cohorts—one maintained on sugar-only (Control A) and the other on a full diet (Control B).

In all three treatments, the remaining life expectancy of flies increased on the days when they were given a full diet after having been maintained on a sugar-only diet (Table 1). Access to a full diet increased the remaining life expectancy of a 30-day-old sugar-fed fly 1.3-fold, that of a 60-day-old fly 2.3-fold, and that of a 90-day-old sugar-fed fly over 12-fold. In addition, the life expectancy of the full-diet control flies at eclosion (age zero) was similar to the remaining life expectancy of the treatment flies at the ages when they were first given a full diet. In most cases, the remaining life expectancies of these cohorts were also similar 30 and 60 days after they were first given a full diet even though their absolute ages differed substantially. Finally, remaining life expectancy declined rapidly after medflies were switched to a full diet. Remaining life expectancies in cohorts on full diets ranged from 8 to 16 days after 1 month on full diet and from 1 to 10 days after 2 months on a full diet. In contrast, the remaining life expectancy of the sugar-only cohort was 22 days after 1 month and 11 days after 2 months (*5*). This general response of a short-term gain in survival but a long-term reduction in life expectancy for flies switched from sugar to a full diet was observed in all three treatment cohorts.

Female medflies maintained on a sugar-only diet and then switched to a full diet were capable of producing eggs (Fig. 1), but lifetime egg production decreased as the age at the time of the switch increased (Table 1). Many sugar-only females were infertile (*6*). Most suppressed reproduction at ages younger than 30 days, and all suppressed reproduction at older ages (Fig. 2). Egg production for females switched to a full diet on day 30 was similar to that of the flies maintained on a full diet from eclosion except for the absence of a 4- to 6-day postreproductive period in the former group. One of the most notable findings was that 4- to 5-month-old medflies were capable of producing moderate numbers of eggs if they had been maintained on a sugar-only diet for the first 3 months.

Table 1. Longevity and reproductive data for medfly cohorts given access to a full diet at different ages. Data for the Control A flies are given in the "sugar-only" column and for the Control B flies under the $t = 0$ column, where t refers to the age at which flies were switched from a sugar-only to a full diet. Life expectancies for the ages when a cohort was first given access to a full diet are highlighted in bold. Life expectancies 30 days later are italicized. Life expectancies 60 days later are underlined.

	Lifetime sugar-only	Age given full diet (t)			
		0	30	60	90
Maximum age (days)	92	61	93	123	173
Remaining life expectancy*					
e_0	40.3	**32.8**	—	—	—
e_{30}	22.4	*8.0*	**29.6**	—	—
e_{60}	10.9	1.0	*8.8*	**25.1**	—
e_{90}	2.0	0	3.0	*8.3*	**25.8**
e_{120}	0	0	0	3.0	*15.8*
e_{150}	0	0	0	0	10.4
Maximum lifetime eggs	130	1686	1603	1127	1042
Reproductive rates (eggs/female)					
Gross†	27.1	794.1	715.3	540.2	394.1
Net ($x = t$)‡	23.8	657.8	456.3	160.1	105.7
Net ($x = 0$)§	23.8	657.8	305.7	25.6	1.1
% zero-days	94.7%	46.5	46.5	63.8	77.1
Lifetime egg production (% of cohort)‖					
0 eggs	43%	3	9	33	53
1 to 500 eggs	57	40	46	57	42
>500 eggs	0	57	45	10	5

*The number of days remaining to the average individual at age x, denoted e_x. †Gross reproductive rate is the number of eggs laid by a hypothetical female that lives to the last day of possible life; this measure characterizes reproduction in a cohort in the absence of mortality. ‡$x = t$ denotes the number of eggs laid by the average female alive at age t. This net rate is the average number of eggs laid by a female that survived to the age at which her diet was switched from sugar to full. §$x = 0$ denotes the number of eggs laid by the average female maintained on sugar-only diet to age t and maintained on full diet thereafter. This net rate gives the average number of eggs laid by a female that was raised on a sugar-only diet from eclosion and then switched to a full diet at time t. ‖Only three females produced over 100 eggs when maintained on a sugar-only diet.

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Age-specific death rates (*7*) for the cohorts switched to a full diet at $t = 30$ and $t = 60$ days and the Control B cohort ($t = 0$) revealed marked similarities in their trajectories after they were given access to a full diet even though their chronological ages differed by up to 2 months (Fig. 3). The cohorts switched to a full diet on days 30 and 60 shifted to the same mortality trajectory as that of the cohort fed a full diet from emergence (Fig. 3, inset). Mortality in the cohort switched to a full diet on day 90 was similar to that experienced by the cohort maintained on a sugar-only diet from emergence. The switch to a full diet on day 90 appears to have set the mortality clock back 90 days. From this point on, we observe the slowly rising trajectory of the sugar-only cohort rather than the more rapidly rising trajectories of the other cohorts switched to full diets. The flies that survived to day 90 on a sugar-only diet may have had special genetic or induced physiological properties that enable exceptional longevity. These findings suggest that the life history of female medflies includes two distinct modes: a waiting mode with low mortality, in which few or even no eggs are produced; and a reproductive mode with prolific egg laying and low initial mortality followed by an acceleration in mortality and a reduction in egg laying.

The observed patterns of mortality and reproduction in the medfly may be associated with reproductive costs; an increment in reproduction at some age may result in a decrement in expected reproduction and an increase in mortality at later ages. Mortality may increase because reproduction diverts resources from somatic repair and maintenance, especially if the diet contains no protein (*8*). The observed patterns may also be attributable to reproductive determinism if the total supply of reproductive units (eggs) is limiting (*9*). The mammalian analog for reproductive determinism is the ovarian exhaustion of follicles as the pacemaker of reproductive senescence (*10*).

The finding that suppression of reproductive activity prolongs survival in the medfly is consistent with the results of other studies. Mortality decreases in male *Drosophila* when they are denied access to mates (*11*) and in female medflies when they are deprived of either mates or oviposition hosts (*12*) or when they are sterilized (*13*). Female rodents maintained on a restricted diet at young ages but subsequently fed ad libitum are, like medflies, capable of producing offspring at advanced ages (*14*). The effects of reduced food intake on reproductive aging may be mediated by retardation of the rate at which ovarial follicles are depleted (*15*).

Our findings have several important implications. (i) The assumption that there are two modes of aging provides an ecological and evolutionary context for aging research; the modes link the reproductive fate of individuals to the availability of food, mates, and hosts (*16*). (ii) The two modes enable flies to increase their longevity—individuals that experience both modes survive longer than those that remain in one mode exclusively. (iii) When resource availability is uncertain, older individuals may contribute more offspring than very young ones (*17*). For medflies protein is usually scarce in the wild (*18*), and thus it is likely that many female flies are quite old before they find protein and can reproduce. Induced demographic schedules

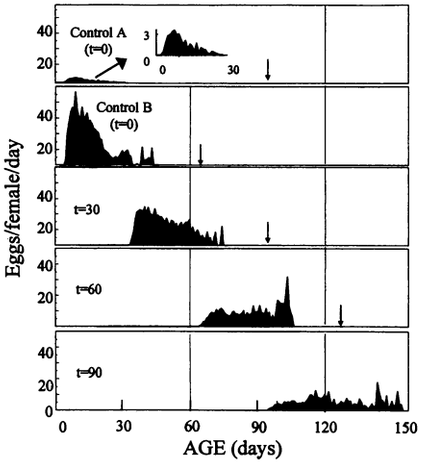

Fig. 1. Age-specific schedule of reproduction for female medflies maintained on a sugar-only diet (top panel and inset, Control A; note scale), on a full diet (sugar + protein hydrolysate) throughout their lives second from top; Control B ($t = 0$), or on a sugar diet from eclosion and then switched to a full diet on days 30, 60, and 90 (lower three panels). The vertical arrows specify the oldest age attained by the cohort. For $t = 90$, this was 173 days.

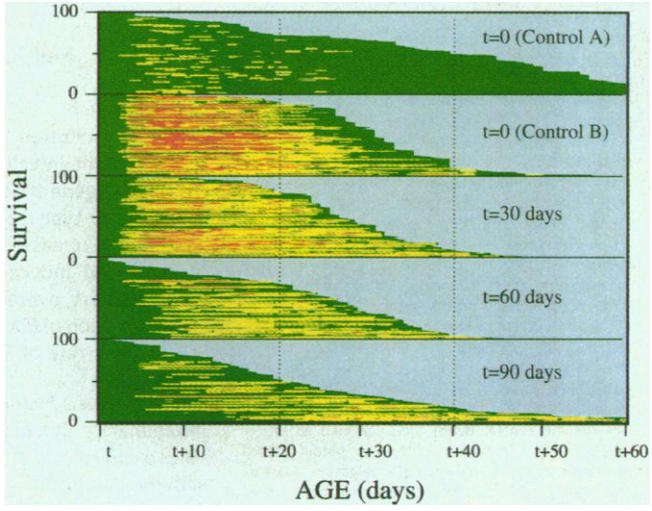

Fig. 2. Event history diagrams (*6*) for survival and reproduction in the five cohorts of 100 females. Each horizontal line represents the life course of an individual fly, the length of which is proportional to its lifespan. Segments within each lifeline are color-coded to depict egg-laying levels at each age: green = 0 eggs, yellow = 1 to 40 eggs, red ≥ 40 eggs. Control A (top) and Control B (second from top) refer to flies maintained throughout their lives on a sugar-only and a full diet, respectively. The symbol t denotes the time at which sugar-fed cohorts were fed a full protein diet.

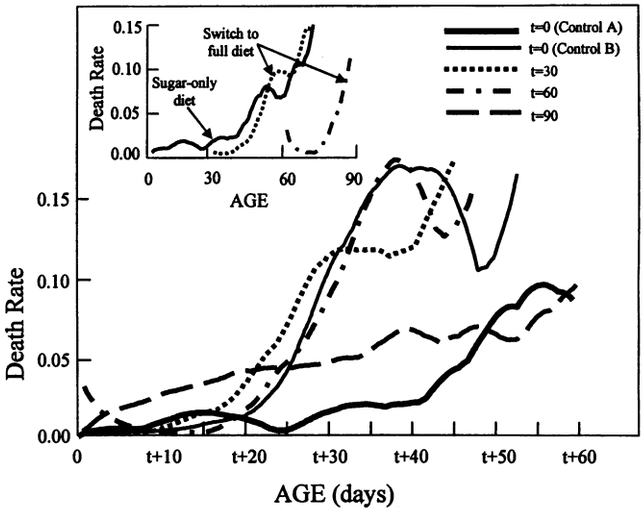

Fig. 3. Smoothed hazard functions (trajectories of mortality) for the five study cohorts starting at the time (t) when they were first switched from a sugar to a full diet (except for the Control A flies, which were fed a sugar-only diet throughout their lives). The inset highlights the significant reductions in hazard rates when flies on a sugar-only diet were switched to protein diets at days 30 and 60 (*7*).

of the kind observed in this medfly experiment do not fit the simplistic formulation of the Lotka equation (*19*), and thus new equations will have to be developed to incorporate the observed plasticity of fertility and survival (*20*). (iv) The effects on longevity of dietary restriction may be mediated by gonadal activity or through the rate of ovarial depletion (*15*). The causal mechanism underlying the dietary restriction response that has been observed in a wide range of species (*21*) may be linked with physiological adaptations to nutritional stress in yeast (stationary phase) and nematodes (dauer stage) and to host and mate deprivation in insects (*12*, *22*).

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4. Pairs were housed in 6.5 cm by 6.5 cm by 12 cm clear plastic containers. Dead males were replaced with virgin males of the same age. The adult diet consisted of yeast hydrolysate and pure sucrose (1:3 ratio by volume). The yeast hydrolysate (ICN Biomedicals) contained 60% protein as well as vitamins and minerals.
5. Considering simultaneously all possible comparisons of the life expectancy for protein groups t = 0, 30, 60, and 90 days (the 95% confidence intervals using Tukey's method for the differences in the mean post-protein), lifetime differences between any two means are at most 9.21 days. For example, for the differences between protein groups t = 0 and t = 30, 60, and 90 we obtain the 95% confidence intervals 3.22 ± 4.72, 4.49 ± 4.72, and −0.70 ± 4.72 days, respectively.
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7. Age-specific central death rates, defined as $m_x = 2d_x/(n_{x-1} + n_x)$, were smoothed to obtain hazard function estimates, applying case weights n_x. Here d_x is the number of observed deaths and n_x the number at risk at day x. The 95% pointwise confidence bands were calculated based on the variance of these estimates. We found that the lower bound of the 95% confidence band for the hazard function for the Control A (sugar-only) is above the upper bound of the 95% confidence band for the hazard function for the t = 30 cohort from day 33 through 53 (with a few days of minor overlapping). Similarly, the lower bound of the confidence band for Control A is consistently above the upper bound of the confidence band for the t = 60 cohort from day 63 through 80. For details of methods see H.-G. Müller, J.-L. Wang, W. B. Capra, *Biometrika* **84**, 881 (1997).
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IEX-1L, an Apoptosis Inhibitor Involved in NF-κB–Mediated Cell Survival

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Transcription factors of the nuclear factor–κB/rel (NF-κB) family may be important in cell survival by regulating unidentified, anti-apoptotic genes. One such gene that protects cells from apoptosis induced by Fas or tumor necrosis factor type α (TNF), *IEX*-1L, is described here. Its transcription induced by TNF was decreased in cells with defective NF-κB activation, rendering them sensitive to TNF-induced apoptosis, which was abolished by transfection with *IEX*-1L. In support, overexpression of antisense *IEX*-1L partially blocked TNF-induced expression of *IEX*-1L and sensitized normal cells to killing. This study demonstrates a key role of *IEX*-1L in cellular resistance to TNF-induced apoptosis.

Tumor necrosis factor type α (TNF), a major inflammatory cytokine, simultaneously activates a cell suicide program and an anti-death activity that results in resistance of many cancer cells to TNF-mediated killing, thus limiting its use in cancer therapy (*1*). TNF-stimulated anti-death activity, unlike TNF-induced cell death, depends on de novo protein synthesis and the genes involved appear to be transcriptionally activated by transcription factors of the nuclear factor–κB/rel (NF-κB) family (*2, 3*). Hence, cells lacking NF-κB subunit RelA (p65) or overexpressing a mutated inhibitor IκBα gene showed enhanced susceptibility to TNF-mediated killing (*4*). Using the mRNA differential display technique (*5*), we cloned a gene that appeared to be the same as a previously reported immediate-early response gene *IEX*-1 (*6*), except that it had an in-frame insertion of 111 nucleotides at position 211 of the coding region for *IEX*-1, and it could encode a longer polypeptide with a 37–amino acid insertion relative to *IEX*-1 (*7*). The longer *IEX*-1 [referred to here as *IEX*-1L; the original *IEX*-1 is referred to as *IEX*-1S (short)] was found to be generated from *IEX*-1 in the absence of RNA splicing as it contained the entire intron sequence of *IEX*-1 (*8*).

IEX-1L protein was demonstrated in 293 cells transiently transfected with a pcDNA-HA·Tag–*IEX*-1L plasmid by using a monoclonal antibody (mAb) to influenza virus hemagglutinin (HA) (Fig. 1A, arrow L-HA) (*9*). The difference between the molecular mass of HA-IEX-1L (32 kD) and of HA–IEX-1S (28 kD) could be accounted for by a 37–amino acid insertion present in IEX-1L. Endogenous IEX-1L protein was also detected by using a polyclonal antibody (Ab) to IEX-1 (*10*) (Fig. 1B, arrow L), which was larger than the reported IEX-1S protein (*6*) (Fig. 1B, arrow S). When McF-7 cells expressed IEX-1L or IEX-1S fused to green fluorescence protein (GFP) (*9*), a typical pattern of fluorescence around the nuclear periphery and endoplasmic reticulum membrane was observed (Fig. 1C), which was distinct from the diffuse distribution of fluorescence visible throughout the entire cell when GFP alone was expressed (Fig. 1D).

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