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Female Sensitivity to Diet and Irradiation Treatments Underlies Sex-Mortality Differentials in the Mediterranean Fruit Fly

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Abstract

Large-scale experiments on medflies that were subjected to sterilizing doses of ionizing radiation (plus intact controls) and maintained on either sugar-only or full, protein-enriched diets revealed that, whereas the mortality trajectories of both intact and irradiated male cohorts maintained on both diets are similar, the mortality patterns of females are highly variable. Mean mortality rates at 35 days in male cohorts ranged from 0.2 to 0.3 but in female cohorts ranged from 0.09 to 0.35, depending on treatment. The study reports three main influences: (a) qualitative differences exist in the sex–mortality response of medflies subjected to dietary manipulations and irradiation, (b) the female mortality response is linked to increased vulnerability due to the nutritional demands of reproduction, and (c) female sensitivity to environmental changes underlies the dynamics of the sex-mortality differential.

Differences in the reproductive biology of males and females (1) underlie sex-specific nutritional requirements in virtually all species (2–5). Whereas female fruit flies are required to manufacture a large quantity of eggs with high protein content (6,7), males are required

to produce only a small volume (relatively speaking) of sperm consisting of minimal amounts of protein. Because of sex differences in energetic and protein requirements related to reproductive demands, we hypothesized that changes in diet will have a substantial effect on the mortality trajectory of females but will have little or no effect on the mortality trajectory for males. Consequently, female sensitivity to dietary conditions will underlie changes in the sex-mortality differentials. We report the results of a test of this hypothesis involving male and female Mediterranean fruit flies (*Ceratitis capitata*) subjected to sterilizing doses of cobalt^{60} (CO^{60}) irradiation (plus intact controls) and maintained on both sugar-only and full (i.e., protein-enriched) diets.

Methods

Studies were conducted at the Moscamed medfly mass rearing facility located in Metapa, Chiapas, Mexico (8). Adult flies were maintained under the following environmental conditions: 12:12 light:dark cycle, 24°C (± 2°) and 65% relative humidity (± 9%). Approximately 3,800 medflies (total of both sexes) were placed in 15 × 60 × 90 cm aluminum frame cages. Each of four cages collectively constituted a replicate for a 2 × 2 design (two diets, irradiated and not irradiated). For irradiated flies, two days before emergence pupae were subjected to a sterilizing dose of 14 krad CO^{60} in hypoxia (9). The two diets were sugar-only and full diet consisting of a 1:3 ratio of enzymatic yeast hydrolysate and sugar. Although irradiated females and males both experience increased oxidative damage to their DNA, protein, and lipids in a dose-dependent manner (10,11), the primary reason for irradiation treatment was to destroy their gamete-producing gonadal germaria and therefore eliminate the nutritional demands for egg production in females. Irradiation would likely have little impact on reducing the reproductive costs in males because their baseline reproductive requirements are minimal (12).

Each day dead flies were removed, counted, and their sex was determined. A total of 35 different cages (replicates) were used for each of the 4 treatments with a grand total of 140 cages containing over 536,000 flies in the study. The statistical methods included a multivariate analysis of variance (MANOVA) for life expectancies. A methodological innovation in this study was the recognition that the independent experimental units are the cages in which the flies are raised. Thus, the sample size corresponds to the number of cages, $N = 140$. Each cage contains two cohorts, one male and the other consisting of the female flies raised in the cage. To allow for dependencies between the recorded lifetimes between male and female cohorts living in the same cage, a MANOVA was used. Because male and female flies in the same cage may be affected by the same cage effect and thus cause a dependency between male and female lifetimes, a 2-way MANOVA was applied. Such dependencies may be caused by shared environmental conditions such as fly density. These dependencies must be included in a fully appropriate statistical model, and we demonstrate that this dependency structure can be addressed by using a MANOVA approach. The proposed approach may be useful for other, similar analyses of experimental data on aging. A second class of statistical methods involved the nonparametric estimation of hazard functions from life tables. These methods are explained in the Appendix of (13), and additional methodological details are provided in (14,15).

Results

Sex-Specific Life Expectancy

The between-treatment variation for female medfly life expectancies greatly exceeds that for males (table 1). For example, the life expectancies for females range from a low of 13.7 days for intact flies maintained on sugar-only diets to 18.4 days for sterilized flies maintained on a full diet—a difference of 4.7 days. In contrast, the life expectancy of males ranged from a low of 14.3 days for sterile males maintained on a full diet to a high of 15.1 days for flies maintained on sugar-only diets—a difference of less than 1 day. Note that longevity is the greatest for females but the lowest for males for the "sterile-full diet" treatment. Table 2 contains the MANOVA table (decomposition of sum of squares), which shows that the Fertility × Diet interaction is not significant ($F \leq .01$; $p = .996$). Based on a significance level of $p < .001$, fertility status and diet are significant factors for mean lifetimes. The results on treatment effects of diet and fertility separated by sex are shown in table 3. To determine whether there was a significant interaction between sex and fecundity, we compared the model effect parameters for fecundity between the two sexes. The effect of fecundity was significantly different between males and females, $p < .001$. Because interactions were insignificant overall, they were not considered. We find that fertility status and diet significantly affect mean lifetimes for females but do not affect mean lifetimes for males apart from very small changes that are not significant and likely due to chance variation. Specifically, female mean life expectancy is changed by 2.9 days depending on fertility status, where fertile flies have significantly lower mean life expectancies. Female mean life expectancy is changed by 1.84 days depending on diet, where sugar-only flies have the lowest mean life expectancy. Thus, the results of table 3 imply an interaction of sex with fertility status. The sex life expectancy ratio is reversed from favoring males to favoring females, not only when the diets are switched from sugar-only to protein as previously reported (13) but also in cohorts of irradiated flies regardless of diets. Specifically, there is a 10% male advantage in life expectancy for intact flies maintained on sugar-only diets. However, there is a 10% male disadvantage when both sexes are maintained on a full diet and a 10% and a 30% male disadvantage in life expectancy, respectively, when flies are irradiated and maintained on both a sugar-only and a full diet. Table 1 shows that not only are female life expectancies more variable in response than males, their responses to irradiation are in the opposite directions—sterilized males do worse but sterilized females do better.

Table 1. Expectation of Life in Days (e_0), Standard Deviation (*SD*), and Number of Cages (*n*) for Male and Female Medflies Subjected to Four Different Treatments*

Sex	Fertility Status†	Diet	e_0	*SD*	*n*
Female	Intact	Sugar	13.70	1.73	35
		Full	16.65	2.02	35
	Sterile	Sugar	15.58	2.42	35
		Full	18.44	3.74	35
Male	Intact	Sugar	15.12	2.13	35
		Full	14.76	2.26	35
	Sterile	Sugar	14.68	2.46	35
		Full	14.28	2.91	35

*A total of more than 536,000 flies, or 260,000 individuals of each sex, were used in the study.
†"Sterile" and "intact" refer to irradiated and nonirradiated, respectively.

Table 2. MANOVA Table for Mean Lifetimes, Subject to Different Diets and Irradiation Treatments

	Wilks's Lambda			Numerator Denominator		
Main Effects	*df*	Λ	*F*	*df*	*df*	*p* Value
Fertility	1	.4758	74.3614	2	135	< .0001
Diet	1	.6541	35.6931	2	135	< .0001
Interactions	1	.9999	.0045	2	135	.9955
Residuals	136					
Total	139					

Notes: Experimental unit is cage, and the dependent variable is the bivectors consisting of mean lifetime of the female and male cohort in a cage (n = 140 cages). Fertility refers to the fertility status of flies (intact vs. sterile), depending upon whether they were irradiated or not. Whereas main effects are highly significant, the interactions are not significant. The underlying MANOVA model is: $(X_{ijk1},X_{ijk2}) = (\mu_1,\mu_2) + (\alpha_{i1},\alpha_{i2}) + (\beta_{j1},\beta_{j2}) + (\alpha_{i1}\beta_{j1}, \alpha_{i2}\beta_{j2}) + (\alpha_{i1}\beta_{i1},\alpha_{i2}\beta_{i2}) + (\varepsilon_{ijk1},\varepsilon_{ijk2})$. These are bivectors where each component corresponds to one sex and i = 1, 2 denotes fertility, j = 1, 2 denotes diet, k = 1, . . ., 35 denotes cage, $(\varepsilon_{ijk1},\varepsilon_{ijk2})$ are bivariate normal errors with zero mean; the interaction terms for diet and fertility are given by the products.

Table 3. The Fitted MANOVA Models

		Females			Males		
Effect[a]		Mean Value Life Expectancy	*SD*	*p* Value	Mean Value Life Expectancy	*SD*	*p* Value
Overall mean		16.09			14.71		
Fertility effect	Fertile	−1.45	0.31		.19	.29	
				<.001			.54
	Sterile	1.45	0.31		−.19	.29	
Diet effect	Sugar	−.92	0.31		.23	.29	
				<.01			.31
	Full	.92	0.31		−.23	.29	

Notes: The fitted MANOVA models are given by $(X_{ijk1},X_{ijk2}) = (\mu_1,\mu_2) + (\alpha_{i1},\alpha_{i2}) + (\beta_{j1},\beta_{j2}) + (\varepsilon_{ijk1},\varepsilon_{ijk2})$, i = 1, 2 (fertility), j = 1, 2 (diet), k = 1, . . ., 35 (cage) for given fertility-diet combination (without interactions). The quantities in the model are bivectors, with one component for each sex. The fitted means are the overall mean effect + the fertility effect (sterile or fertile) + the diet effect (sugar or full diet). The estimated effects, their standard deviations (*SD*), and significance levels are listed below, separately for male and female flies. Differences from the means listed in table 1 are due to residual effects not included in the MANOVA model.

a. Checking for interactions between sex and diet, respectively sex and fecundity within the MANOVA model, the null hypotheses of no interactions would be $\alpha_1 = \alpha_2$ and $\beta_1 = \beta_2$, respectively. Because the 99.9% confidence interval for $\alpha_1 = \alpha_2$ is found to be (.9025, 2.3775) and that for $\beta_1 = \beta_2$ is found to be (.4125, 1.8875), we conclude that both interactions are highly significant ($p < .001$). The p values for the significance of the differences were $p = 3.0442 \times 10^{-13}$ for the alphas and $p = 1.5164 \times 10^{-7}$ for the betas. More details about the MANOVA can be found in reference (36).

Sex Differences in Mortality Trajectories

To assess the dynamics underlying the life expectancy differentials, we plotted smoothed mortality curves for all 35 cohorts by sex for each of the four treatments (figs. 1 and 2). Three aspects of these figures merit comments. First, although slopes of the male mortality schedules in each of the four treatments vary widely, the trajectories themselves are remarkably similar—monotonically increasing through 20–30 days followed by leveling off. Mortality in some male cohorts decreased at older ages (fig. 1A–D). Second, the opposite is seen in female medfly cohorts inasmuch as their mortality patterns (a) are unique in each of the four treatments, and (b) bear little resemblance to any of the male mortality patterns (fig. 2A–D). Third, the patterns of female mortality in cohorts of both nonirradiated and irradiated flies are similar if they are maintained on sugar-only diets (fig. 2A,B). However, these patterns are substantially different in the nonirradiated and irradiated groups maintained on a full diet (fig. 2C,D). For example, mortality in the nonirradiated cohorts maintained on a full diet is initially low but then increases. However, mortality in cohorts of irradiated females maintained on a full diet is low at both young and old ages.

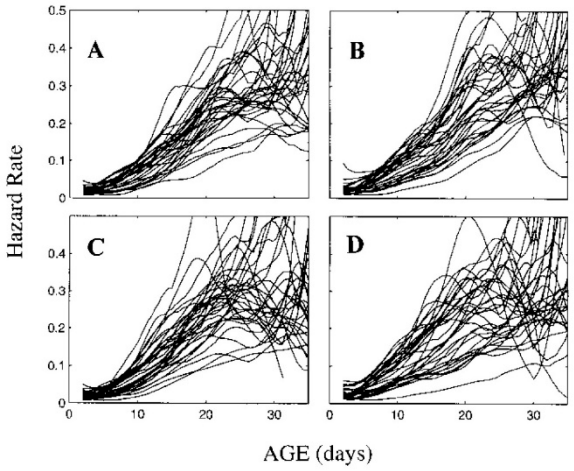

Figure 1. Smoothed hazard rates for 35 male Mediterranean fruit fly cohorts in each of four treatments: **A**, sugar-fed, intact; **B**, sugar-fed, irradiated; **C**, full diet, intact; **D**, full diet, irradiated. Each curve is based on deaths in approximately 1,900 flies.

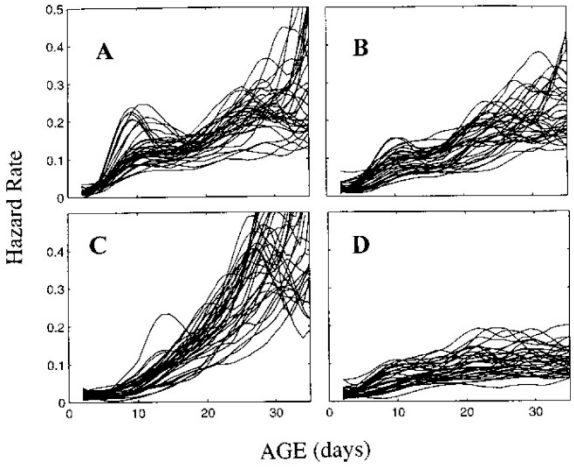

Figure 2. Smoothed hazard rates for 35 female Mediterranean fruit fly cohorts in each of four treatments: **A**, sugar-fed, intact; **B**, sugar-fed, irradiated; **C**, full diet, intact; **D**, full diet, irradiated.

Mean Sex-Mortality Trajectories

The mean curves of the 35 cohorts in each of the treatments provide a collective summary of the broad sex-mortality patterns (fig. 3). Although the mortality patterns for both intact and irradiated female flies maintained on sugar-only diets (fig. 3, bottom) are similar, their overall levels differ—mortality in cohorts of irradiated females is lower than in cohorts of intact females. After day 20, cohorts of both sterilized males and females maintained on full diets experience the lowest mortality of any treatment. Not only are the qualitative

patterns (slopes) of male mortality similar among the four treatments, their levels are also nearly identical through day 20. This similarity in male mortality is remarkable considering the high doses of radiation and the large differences in the nutritional quality of the diets. Similar mortality patterns exist for both intact and sterilized female cohorts that are maintained on sugar-only diets. There is a surge in mortality at young ages followed by a shoulder around 10 days and then a gradual increase in mortality thereafter (fig. 3). An interesting feature of these parallel patterns is that not only does the mortality in sugar-fed, intact females exhibit a "shoulder" as observed previously (13), but sugar-fed sterile females did as well. This raises interesting questions about the physiological mechanism underlying this surge in mortality at young ages.

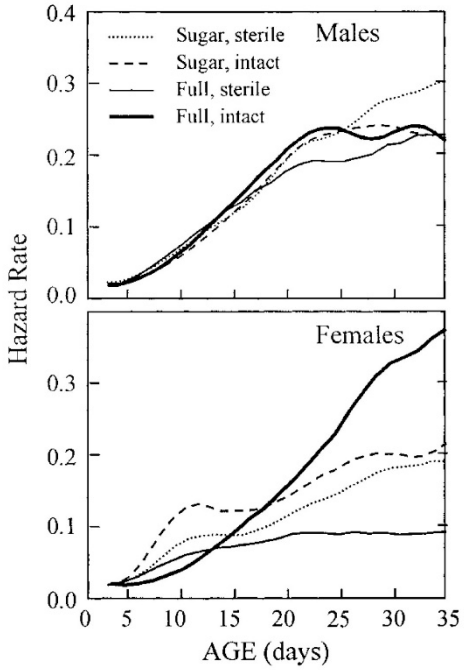

Figure 3. Mean hazard rates for the 35 male (top) and female (bottom) Mediterranean fruit fly cohorts shown in figures 1 and 2. Each curve is based on deaths in a total of more than 66,000 individuals of each sex. Note that the solid lines denote flies on full diets and broken lines flies on sugar-only diets.

Relative Cost of Reproduction in Females

Differences in the levels and patterns of the female mortality schedules shown in figure 3 provide insights into the relative cost of reproduction (16–18). For example, the difference between curves in figure 3 (bottom) shows the cost of:

a. actually maturing eggs—mortality differences between sugar-fed, intact (S-I) and full diet, sterile (F-S). Thus, S-I females must draw on larval reserves to mature eggs as

well as use for maintenance, whereas F-S females have external source of protein for maintenance and have no egg production demands.

b. attempting to mature eggs under sterility—mortality differences between sugar-fed, sterile (S-S) and full diet, sterile (F-S). Thus, neither S-S nor F-S females in this case have protein demands associated with egg production, since both are sterile. However, S-S females must draw on larval protein reserves only for maintenance but F-S females have an external source of protein for maintenance. The smaller difference between these mortality curves (relative to differences in former comparison) suggests that maintenance costs are substantially less than those for egg production, as would be expected; and

c. maturing eggs with full diet—mortality differences between full-diet, intact (F-I) females and full diet, sterile (F-S). The cross-over at day 13 between the F-I and F-S mortality curves suggests that fertile flies are initially protected, whereas sterile flies are not. This is reflected in the sharper rise in mortality under full diet and speaks for a protective effect caused by the presence of eggs (19). Although the MANOVA did not show a significant interaction pattern between diet and fertility status, we see from figure 3b that the mortality trajectories of female flies are much more affected when the flies are on full diet rather than when on restricted diet. This interaction demonstrates a close association between reproduction (which is affected by diet) and longevity. It is not captured in mean life time analysis but clearly in the dynamics of mortality; this shows the importance of analyzing the entire mortality trajectory.

Discussion

This study leads us to infer the following: (a) qualitative differences exist in the sex-mortality response of medflies subjected to dietary manipulations and irradiation; (b) the female mortality response is linked to increased vulnerability due to the nutritional demands of reproduction; and (c) greater female than male vulnerability to perturbation underlies changes in the sex-mortality differential. These results are consistent with those presented in previous studies on sex-mortality differentials of the medfly (13,20–28). Whereas considerable variation exists in the mortality trajectories of female cohorts over a wide range of experimental conditions including density (20), diet (13), mating status (23), ionizing radiation (25), and periodic starvation (28), the mortality patterns for male cohorts are similar between treatments [also see (29)].

The expression of a mortality shoulder in both irradiated and intact female cohorts maintained on sugar suggests that this vulnerable period is not due solely to weakening of protein-deprived females attempting to produce eggs from their larval-derived proteins (13). The prevalence of the shoulder (or peak) can be seen in figure 2a,b for protein-deprived females: it is much more pronounced in fertile female cohorts but also occurs for most sterile female cohorts. It also appears occasionally in the mortality curves for full diet female medflies. Because irradiated females cannot produce eggs, the shoulder in irradiated female cohorts maintained on sugar must be due to increased vulnerability resulting

from protein demands in other reproductive contexts, such as ovarian requirements unrelated to the manufacture of eggs [e.g., manufacture of accessory gland products (30)]. The absence of a pronounced mortality shoulder in male cohorts (e.g., fig. 3, where the average hazard curves provide no evidence for a shoulder for the male cohorts) suggests that the shoulder in female cohorts is also not related to the lack of a basic metabolic protein requirement that is independent of sex; otherwise the shoulder would have been expressed in protein-deprived male cohorts.

Our inferences have four implications. First, an implicit assumption underlying sex-mortality differentials is that the mortality of both sexes is environment-specific. However, our findings demonstrate that male medfly mortality is independent of at least one type of environmental change (i.e., dietary manipulation). Therefore, changes in the sign and magnitude of male-female life expectancy differentials are linked to the mortality response to dietary change in females. Second, the large differences in the sex-specific responses cast doubt on the transferability of the findings from life table studies on one sex to the other. The longevity response of one sex may be substantially different from the response of the other; therefore, the outcome of a longevity selection study on females may not apply to males (31). Third, greater female sensitivity to changes in environmental conditions will create sex-mortality crossovers; that is, when age-specific death rates favor one sex at younger ages but the other sex at older ages. Understanding mortality crossovers between two cohorts is important because crossovers often point toward fundamental differences in the underlying biology between two cohorts (32)—the protective effect of eggs at young ages in intact females maintained on a full diet versus females subject to all of the other treatments. Fourth, the conventional explanations for differences in male-female mortality including the behavioral [high risk/high stakes male strategies; (33)] and chromosomal [homogametic sex advantage; (34,35)] hypotheses may be misleading because the outcome is context-specific. Indeed, the results in the current report reinforce earlier findings that it is impossible to classify one sex as longer lived than the other without considering the environment in which they are maintained or the treatments to which each sex is subjected (22).

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