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Folic acid enriched diet genotype and sex dependent effects on *Drosophila melanogaster* imago life expectancy: chronic experiment

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Introduction

The negative effects of excessive folate consumption by insects are widely known, including disruption of the gut microbiota, accumulation of reactive oxygen species, and aberrant DNA methylation. At the same time, a high requirement for folate has been demonstrated during the larval stage, including for adequate DNA and histone methylation. This study is a part of project aimed to evaluate the effects of folic acid enriched diet on *D. melanogaster* fitness components. Specifically here we would like to focus attention on such index as life expectancy, that is a part of species fitnes.

Materials & Methods

We took to the experiment two *D. melanogaster* stocks: *Canton-Special* (*C-S*) and *radius incompletus* (*ri*) from "Drosophila stocks collection of Genetics and Cytology Department of V.N. Karazin Kharkiv National University" that is National Heritage of Ukraine. For each stock we had formed two groups - Control (CxC) and Experimental (ExE). Individuals of CxC group developed in a standart medium, without any suplements, but ExE group rared in medium suppied with 1 mg/ml folic acid. In order to detect the presence of a persistent effect through the impact on the epigenome or changes in gametes we added two more «hybrid» groups in the experiment (ExC & CxE). Offsprings from hybrid groups developed in control medium and weren`t introduced into further croses. Each stock was analysed separately in parallel. Scheme of crosses of randomly chosen virgin females and males is presented below (Fig. 1. NB! yellow lightnings indicate that individuals developed in experimental medium). Experiment lasted for six generations for main (CxC & ExE) and for five generations for hybrid (ExC & CxE) groups.

We evaluated life expectancy for offspings of each group in each generation. We collected virgin females and males and put the separately in vials with temporary medium. During the experiment we periodically while changing vial into one with fresh medium (every 3 days) inspected the number of survived individuals and noted the number. The smallest group was n=34 and largest - n = 154, total number of individuals n = 8734. This dataset was analysed in R with two models: Cox's Proportional Hazards (CPH) and Aalen's Aditive Regression (AAR) from the «survival» package. The surviving curves were plotted as well. (Fig. 2)

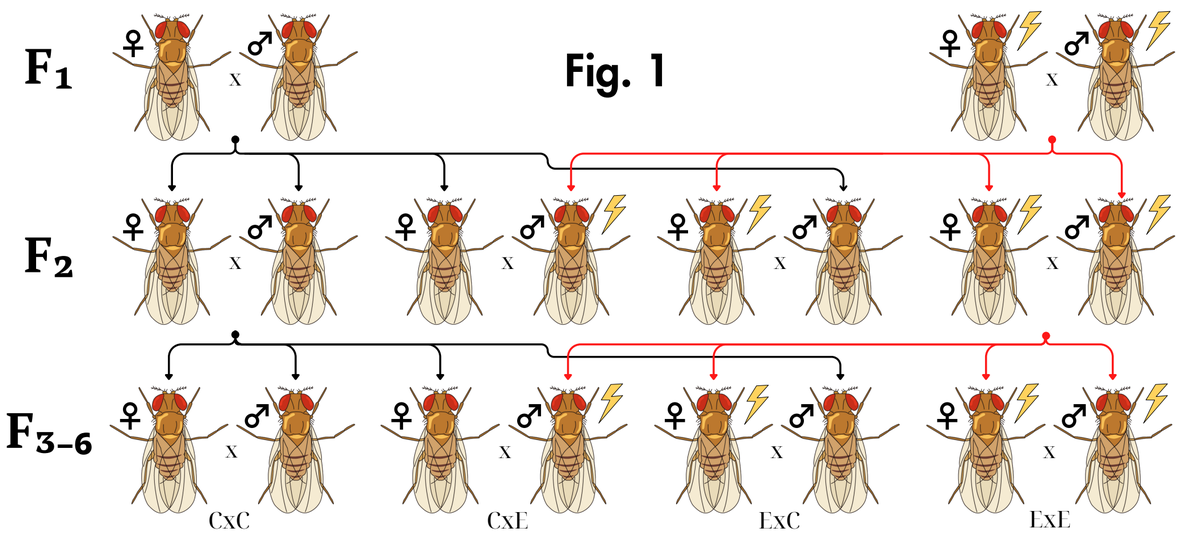


Fig. 1

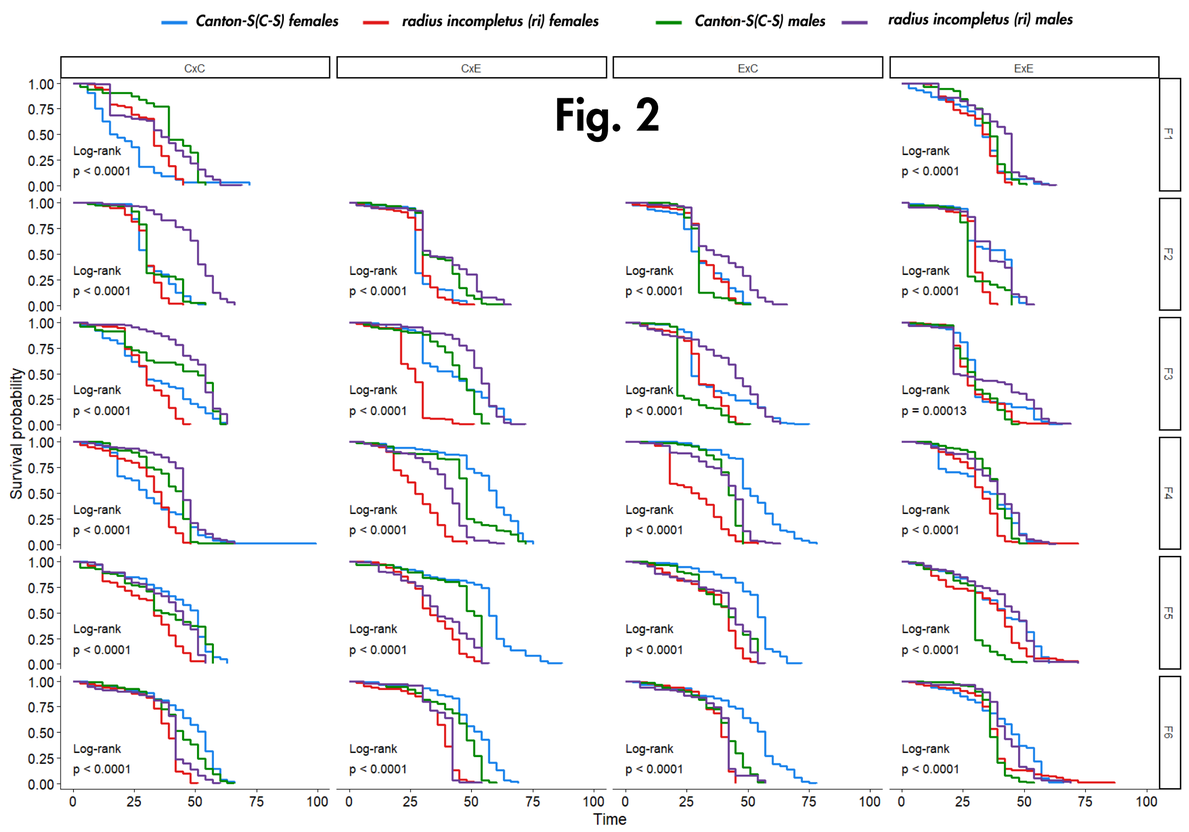


Fig. 2

Results & Discussion

CHP model appeared to be not applicable to collected data because Schoefild residuals were statistically significant (global p = 2e-16), it means that proportional hazard (PH) assumption is violated. Unlike CHP model, AAR doesn't need PH assumption to be satisfied. Results of AAR model are presented below. For baseline hazard the following set of covariates was choosen: first generation, *C-S* stock, CxC group, females.

As we see males have less chances of death at the age of 20-50 days with great increase from 50-s days. However cumuilative effect shows that males generally have less chances to die in comparsion with females from the same group.

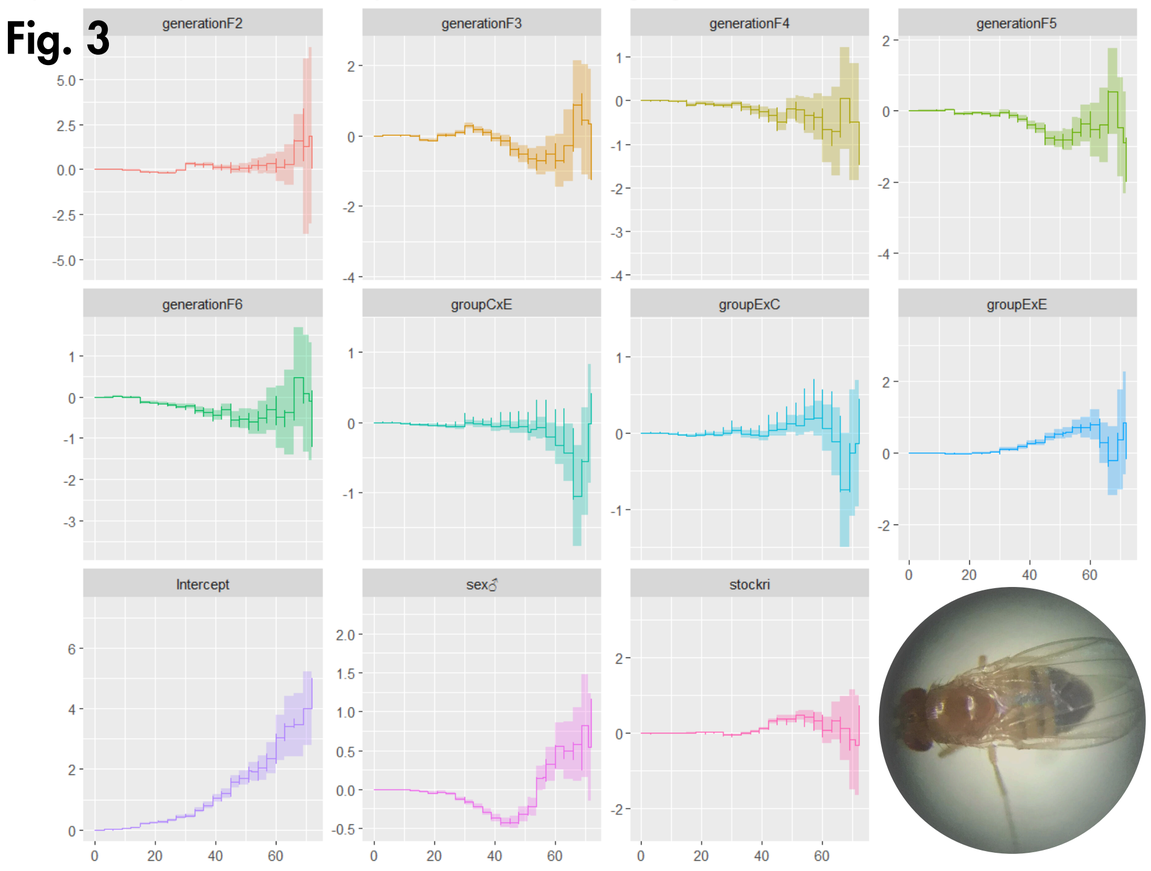
Folate addition (group ExE) in concentration of 1 mg/ml shows increasing risk of death from 30s days of drosophila's imago life. Plots for group covariates (Fig.3) CxE and ExC are pretty simmilar and their confidence intervals fluctuate around zero. Effect of ExC covariate is not significant, unlike CxE. However we can't say that there are any serious consequnces for offsprings if one of the parents was developed in experimental medium and had in their pedigree similar individuals.

Survival of individuals from *ri* stock is lower in control, but if we compare *ri* with *C-S* in conditions when individuals from both stock are exposed to folic acid, *ri* stock shows better survival, so addition of folate doesn`t simply increase effect of genetic instability on lifespan.

We did not observe clear persistent effect of conseqently exposure of generations of fruit flies to folic acid, because there is a decrease of risk of death from generation to generation in all groups (including CxC) caused by unknown (discussive) reason. And this decrease overlaps the increasing effect in ExE group from generation to generation and turns it off. Still, we can roughly evaluate this effect by compasion of AAR intergroup results (NB! Baseline hazard function for "group CxC" is F1, *C-S*, CxC, females and for "group ExE" is F1, *C-S*, ExE, females). Thus, the negative effect of folic acid consumption decreases from generation to generation ($F2 = 1.17*10^{-4}-(-2.04*10^{-4}) = 3{,}21*10^{-4}$; $F6 = (-2{,}97*10^{-4})-(-4{,}68*10^{-4}) = 1.71*10^{-4}$).

Thus differences between covariates' risk emerge at ≈20 - 30s days of life cycle, we suggest this might be consequences of some processes that take place in drosophilla at late reproductive period and maybe connected to aging.

Fig. 3



Results of Aalen's Additive Regression Model

Covariate	Slope	Coefficient	SE of coefficient	z	p
Intercept	0.057700	2.28e-04	1.18e-05	19.400	1.80e-83***
sex ♂	-0.015100	-4.59e-05	4.82e-06	-9.510	1.86e-21***
group CxE	-0.000845	-1.57e-05	6.25e-06	-2.500	1.23e-02*
group ExC	0.002610	6.19e-06	6.49e-06	0.953	3.41e-01
group ExE	0.010500	5.41e-05	6.73e-06	8.040	9.19e-16***
stock *ri*	0.006290	3.67e-05	4.83e-06	7.600	2.99e-14***
generation F2	0.008350	2.71e-05	1.19e-05	2.270	2.33e-02*
generation F3	-0.001240	-2.49e-05	1.21e-05	-2.060	3.93e-02*
generation F4	-0.014700	-5.36e-05	1.16e-05	-4.610	3.94e-06***
generation F5	-0.019500	-7.93e-05	1.16e-05	-6.820	9.01e-12***
generation F6	-0.023800	-7.98e-05	1.14e-05	-6.980	2.90e-12***

* — p < 0.05, *** — p < 0.001

Intergroup results of Aalen's Additive Regression Model

Group	Covariate	Slope	Coefficient	SE of coefficient	z	p
CxC	generation F2	-0.014400	-2.04e-04	7.87e-05	-2.60	9.45e-03**
CxC	generation F3	-0.024100	-4.02e-04	7.59e-05	-5.30	1.15e-07***
CxC	generation F4	-0.021200	-2.49e-04	7.61e-05	-3.27	1.08e-03**
CxC	generation F5	-0.026600	-3.58e-04	7.63e-05	-4.68	2.81e-06***
CxC	generation F6	-0.037000	-4.68e-04	7.28e-05	-6.43	1.31e-10***
CxC	stock *ri*	0.000141	1.13e-04	3.69e-05	3.06	2.18e-03**
ExE	generation F2	0.008760	1.17e-04	5.93e-05	1.98	4.83e-02*
ExE	generation F3	0.017900	1.09e-04	6.70e-05	1.63	1.03e-01
ExE	generation F4	-0.004910	-7.47e-05	5.42e-05	-1.38	1.68e-01
ExE	generation F5	-0.010500	-2.69e-04	5.56e-05	-4.84	1.33e-06***
ExE	generation F6	-0.018700	-2.97e-04	5.48e-05	-5.41	6.23e-08***
ExE	stock *ri*	-0.006030	-7.38e-05	3.38e-05	-2.18	2.89e-02*

* — p < 0.05, ** — p < 0.01 *** — p < 0.001

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