alone, median survival 78 days (Log-rank: $P = 0.0677$) (Figure 1). Overexpression of of *α-synuclein* with $PI3K^{+}$ shows a slight increase in lifespan, median survival 52 days, compared to overexpression of $PI3K^{+}$ alone, median survival 46 days (Log-rank and Wilcoxon: $P < 0.0001$). This, however, is not rescued to the level of the *α-synuclein* or *Ddc-Gal4* controls (Log-rank and Wilcoxon: $P < 0.0001$). The engagement of protective proteins such as Pink1 during α-synuclein cytotoxicity may not have enough of an effect to increase the *PI3K* phenotype; alternatively, the detrimental effects of *PI3K* may be too severe on its own, or is initiated before *α-synuclein* induced cytotoxicity takes effect.

In conclusion, it appears that, in dopominergic neurons, overexpression of $PI3K^{+}$ results in a severe decrease in lifespan, likely due to the sensitivity of this particular cell type coupled with the inability of these cells to progress through cell cycle, thereby triggering apoptosis. A substantial difference was not observed with the addition of *α-synuclein* overexpression, and therefore, no conclusion can be made regarding possible links between the PI3K signaling pathway and α-synuclein cytotoxicity.

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Copulation duration in *Drosophila melanogaster* reared on different diets: a multiple choice test.

Pavković-Lučić, Sofija, Dragana Miličić, and Luka Lučić. Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia. E-mail addresses: sofija@bio.bg.ac.rs, draganam@bio.bg.ac.rs, luka@bio.bg.ac.rs

Duration of copulation in *Drosophila* is a species-specific trait, which considerably varies among different species (Spieth, 1952; Grant, 1983; Kraaijeveld *et al*., 2008). Variation in this trait was also recorded among geographical or inbred strains (for review see Hirai *et al*., 1999). Mating duration in *Drosophila* is related to many traits, like courtship vigor (Gromko *et al*., 1991), fertility (Gromko *et al*., 1991; Singh and Singh, 1999), female fitness (Friberg, 2006), and paternity (Gilchrist

and Partridge, 2000). Different *Drosophila* species may also vary in copulation duration during first and second mating (Singh and Singh, 2004; Pavković-Lučić and Kekić, 2006). Factors influencing variation in duration of copulation are very complex and described for some *Drosophila* species (for review see Singh and Singh, 2004). Furthermore, this component of mating behavior may be influenced by temperature (Parsons and Kaul, 1966), exposure to rival males prior to and during mating (Bretman *et al*., 2009), and female cuticular hydrocarbon profile (Friberg, 2006).

In order to investigate which of the sexes holds control over the duration of copulation in *D. melanogaster*, it has been found to be largely under male control (MacBean and Parsons, 1967). When considering body size, it was recorded that mating duration in *D. melanogaster* depends on female body size, but either not or much less on male body size when size variation was created by varying the degree of crowding (starvation) among larvae from an inbred strain (Lefranc and Bundgaard, 2000).

Here we report the results of measuring duration of copulation in *D. melanogaster* after long-term rearing (for more than one year) on two diets: standard laboratory food (cornmeal-sugar-agar-yeast medium, "C strain"), and tomato substrate ("T strain"). Contrary to standard laboratory food, tomato medium was made without sugar and yeast (Kekić and Pavković-Lučić, 2003). Flies of those strains were scored for size and copulation duration in *female choice* test, when only C females were used (Pavković-Lučić and Kekić, 2010). Here we scored those flies for size and duration of copulation in a *multiple choice* test, which counts the instances of four types of matings, when males and females of two strains were placed together.

Before the experiment started, flies were maintained in 250 ml glass bottles, without competition, at 25°C, relative humidity of 60%, and 12h:12h L:D cycle. Flies used in the mating experiment were sexed without anesthesia every few hours after eclosion. They were kept separately according to sex and strain for 3-5 days in food vials until they were used. Flies of alternative types (males and females maintained on two different foods) were marked by fluorescent UV dust (red and green) 24 h before testing.

Experiments were conducted in the morning (7:00-11:00 am), at the room temperature. Flies were crossed as follows: 10 females (C) + 10 females (T) + 10 males (C) + 10 males (T). Ten trials were run. Mating bottles were observed constantly for 60 min (*per* replicate); each copulating pair was gently aspirated and carefully transferred to a separate glass vial, and the time from the beginning up to the end of copulation (*i.e*., duration of copulation) was recorded. After mating, the male and female types were recorded under binocular using UV lamp. Then, flies were put into separate eppendorfs filled with 70% ethanol for further analyses. One of the parameters measured was body size, which was scored as wing length (Partridge *et al*., 1987).

Table 1. Mean wing length ($\overline{X}$ ± S. E.) in mated flies reared on cornmeal-sugar-agar-yeast (C) and tomato (T) diet. 1mm = 62 scale units.

Fly strain	N	$\overline{X}$ ± S. E.	t	df	P
C males	84	81.88 ± 0.39	2.130	154	0.0348
T males	72	80.58 ± 0.47			
C females	76	93.67 ± 0.42	5.352	154	< 0.001
T females	80	90.36 ± 0.44			

Out of possible 200 copulations, 78% of matings occurred (156 copulations). Mating males and females maintained on different food significantly differed according to body size, *i.e*., flies reared on cornmeal-sugar-agar-yeast diet were larger than those reared on tomato (Table 1). No significant difference in mean duration of copulation between different mating types was observed, *i.e*., it was very uniform among different

types of mating (in several cases, flies interrupted copulation during transferring into glass vial; these couples were excluded from statistical analysis) (Table 2).

When flies from C and T strains were used in the *female choice* test, no difference in duration of copulation was scored between C × C and C × T mating types, when only C females were used; however, males reared on standard laboratory food were larger than those maintained on tomato (Pavković-Lučić and Kekić, 2010). Similarly, in the *female choice* test, when difference in body size of full-sib males was significantly induced by another environmental factor, *i.e*., variation in growth temperature (18°C vs. 25°C), no difference in mating duration was observed (Kekić *et al*., 2007). In this experiment, flies of both sexes maintained for many generations on different diets significantly differed in size, while the difference in mean duration of copulation among different mating types was not recorded. It seems that, when flies were long-term maintained on different food regimes, the size of both sexes did not influence examined behavioral trait. However, this is only a preliminary report, and other and more precise analyses should be done.

Table 2. Comparison of mean durations of copulation (in seconds) among different mating types in a *multiple choice* test. Abbreviations: C - cornmeal-sugar-agar-yeast medium, T - tomato medium.

Mating type	N	Duration of copulation $\overline{X}$ ± S. E.	t	df	P
♂ C × ♀ C	43	1165.21 ± 21.03	1.249	82	0.215
♂ T × ♀ T	41	1199.32 ± 17.19			
♂ C × ♀ T	36	1219.19 ± 19.12	1.524	63	0.132
♂ T × ♀ C	29	1173.90 ± 23.09			
♂ C × ♀ C	43	1165.21 ± 21.03	1.868	77	0.065
♂ C × ♀ T	36	1219.19 ± 19.12			
♂ C × ♀ C	43	1165.21 ± 21.03	0.272	70	0.786
♂ T × ♀ C	29	1173.90 ± 23.09			
♂ T × ♀ T	41	1199.32 ± 17.19	0.775	75	0.441
♂ C × ♀ T	36	1219.19 ± 19.12			
♂ T × ♀ T	41	1199.32 ± 17.19	0.901	68	0.370
♂ T × ♀ C	29	1173.90 ± 23.09			

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