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Reconciliation of *daf-2* suppression by *elt-3* in *C. elegans* from Tonsaker et al., 2012 and Kim et al., 2012

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In Tonsaker et al., 2012, the authors claim that they cannot reproduce one of the genetic results from Budovskaya et al., 2008. Specifically, *elt-3(vp1)* was found to suppress the longevity phenotype of *daf-2(e1370)* in Budovskaya et al., 2008 but not in Tonsaker et al., 2012. In response to Tonsaker et al., 2012, the original result was validated several ways, including using different alleles of *elt-3* as well as *elt-3* RNAi (Kim et al., 2012). One possible explanation for the different results between the two reports is that the *daf-2(e1370)* strain from one of the labs had acquired a background mutation affecting its lifespan (either shorter or longer).

In order to test whether the *daf-2(e1370)* strain used by the Kim and McGhee labs are the same, we compared their lifespans. We found that the *daf-2(e1370)* strain from the Kim lab lives slightly longer than *daf-2(e1370)* strain from the McGhee lab. Specifically, the median lifespan of the Kim lab strain is ~16% longer than that of the strain from the McGhee lab ($p < 0.0001$) (Figure 1). This suggests that one, the other or both have picked up background mutations that affect their lifespans. To minimize confusion, we renamed the *daf-2(e1370)* strain used by the McGhee lab as YB1 and renamed the strain used by the Kim lab as YB2.

In order to discern the phenotype of *daf-2(e1370)* without the effect of the background mutations, we backcrossed YB1 and YB2 to the wild-type strain (*N2*) two times and reisolated animals that were homozygous for *daf-2(e1370)* (Table 1). From YB1, we generated the backcrossed strain YB3. From YB2, we generated the backcrossed strain YB4. We performed each life span experiment four times with very similar results. Table 2 presents all statistical analysis for the last two replicates. We found that the lifespan of YB3 was 17% longer than the lifespan of YB1 ($p < 0.0001$) (Figure 2). The lifespan of YB4 did not change than that of YB2 ($p = 0.707$ and $p = 796$) (Figure 3). These results indicate that YB1 has a background mutation that significantly reduces its lifespan.

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Finally, we compared the lifespan of *daf-2(e1370); elt-3(vp1)* to the 2x-backcrossed *daf-2(e1370)* strain (YB3). We used the *daf-2(e1370); elt-3(vp1)* strain from the McGhee lab (JM161) as well as the *daf-2(e1370); elt-3(vp1)* strain from the Kim lab (SD1294). We found that JM161 and SD1294 had comparable lifespans indicating no significant difference in the *daf-2(e1370); elt-3(vp1)* strains between the two labs (Figure 4). We found that both JM161 and SD1294 had significantly shorter lifespans than YB3 (*daf-2(e1370)*) (Figure 5). This result indicates that the *elt-3(vp1)* mutation suppresses the longevity phenotype caused by the *daf-2(e1370)* mutations. This result is consistent with the original results in (Budovskaya et al., 2008) but not with (Tonsaker et al., 2012). The results from (Tonsaker et al., 2012) are confounded by a background mutation contained in the *daf-2(e1370)* strain (YB1). This background mutation reduces the longevity of YB1 (*daf-2(e1370)*), such that it does not live longer than JM161 (*daf-2(e1370); elt-3(vp1)*) (Tonsaker et al., 2011). When the background mutation is removed by backcrossing to wild-type, the *elt-3(vp1)* mutation suppresses the longevity phenotype of *daf-2(e1370)*.

References

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Figure 1.

daf-2(e1370) strain from the Kim lab (YB2) lives longer than the *daf-2(e1370)* strain from the McGhee lab (YB1). The median life span of YB1 and YB2 is 42 (± 0.6 days) days and 49 (± 0.9) days, respectively (log rank test $p = 0.0001$).

Figure 2.

The median lifespan of the 2x outcrossed *daf-2(e1370)* strain YB3 is 17% longer than that of YB1 ($p < 0.0001$).

Figure 3.
The median lifespan of the 2x outcrossed YB2 strain, YB4, is the same than that of YB2.

Figure 4.
The median and maximal life spans of the *daf-2(e1370); elt-3(vp1)* strain from the Kim (SD1294) and McGhee (JM161) labs are not significantly different.

Figure 5.
The *daf-2(e1370); elt-3(vp1)* strain from the McGhee lab (JM161) has a shorter lifespan than the 2x backcrossed *daf-2(e1370)* strain (YB3).

Table 1
Strains used in this study

Strain:	Genotype:	Derivation:		Reference:
YB1	<i>daf-2(e1370)III</i>	McGhee Lab	Renamed from CB1370	(Tonsaker et al., 2012)
YB2	<i>daf-2(e1370)III</i>	Kim Lab	Renamed from CB1370	(Budovskaya et al., 2008)
YB3	<i>daf-2(e1370)III</i>	2x backcrossed from YB1		This
YB4	<i>daf-2(e1370)III</i>	2x backcrossed from YB2		This
JM161	<i>daf-2(e1370)III, elt-3(vp1)X</i>			(Tonsaker et al., 2012)
SD1294	<i>daf-2(e1370)III, elt-3(vp1)X</i>			(Budovskaya et al., 2008)

Table 2
Statistical analysis of life span experiments

Strains:	p-value	
	Experiment#1	Experiment#2
<i>N2</i> vs. <i>JM161</i>	<0.0001	<0.0001
<i>N2</i> vs. <i>SD1294</i>	<0.0001	<0.0001
<i>JM161</i> vs. <i>SD1294</i>	0.844	0.596
<i>YB1</i> vs. <i>YB2</i>	0.0006	0.0011
<i>YB1</i> vs. <i>YB3</i>	<0.0001	<0.0001
<i>YB2</i> vs. <i>YB4</i>	0.707	0.796
<i>YB3</i> vs. <i>JM161</i>	<0.0001	0.0002
<i>YB3</i> vs. <i>SD1294</i>	<0.0001	0.007