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Comparisons of lifespan and stress resistance between sexes in *Drosophila melanogaster*

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ABSTRACT

Animals exhibit different extents of sexual dimorphism in a variety of phenotypes. Sex differences in longevity, one of the most complex life history traits, have also been reported. Although lifespan regulation has been studied extensively in the fruit fly, *Drosophila melanogaster*, the sex differences in lifespan have not been consistent in previous studies. To explore this issue, we revisited this question by examining the lifespan and stress resistance of both sexes among 15 inbred strains. We first found positive correlations between males and females from the same strain in terms of lifespan and resistance to starvation and desiccation stress. Although the lifespan difference between male and female flies varied greatly depending on the strain, males across all strains collectively had a longer lifespan. In contrast, females showed better resistance to starvation and desiccation stress. We also observed greater variation in lifespan and resistance to starvation and desiccation stress in females. Unexpectedly, there was no notable correlation observed between lifespan and the three types of stress resistance in either males or females. Overall, our study provides new data regarding sexual dimorphism in fly lifespan and stress resistance; this information may promote the investigation of mechanisms underlying longevity in future research.

1. Introduction

Females and males often show different phenotypes in terms of morphology, physiology and behaviors [1–4]. Longevity, a complex life-history trait, is also sexually dimorphic in several animal species [5]. In humans, women live significantly longer than men in nearly all populations [6]. A recent study based on 101 wild mammal species also suggested that while longer male lifespans can be found in some species, females, in general, have longer lifespans than males [7]. Different models have been proposed to explain why females live longer [8,9]. For example, sex-specific selection suggests that differences in optimal trait values between males and females lead to sexual conflict over these traits, which results in different optimal lifespans for each sex [10]. The rates of extrinsic mortality, primary agents of mortality, and whether mortality is primarily random or condition-dependent all influence this process. Studying sex differences in lifespan regulation within a species may potentially enhance the understanding of the mechanisms underlying variation in longevity.

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The lifespan of wild animals is affected by multiple factors, including intrinsic factors (aging rates) and extrinsic factors (such as predation, starvation and other environmental factors) [11]. The study of protected animal populations, such as model organisms reared in laboratories, can therefore provide more information regarding variation or the mechanisms underlying longevity in a controlled environment. The fruit fly, *Drosophila melanogaster*, is one of the most popular model organisms. Its short lifespan makes it an ideal model for studying longevity. Many factors have been shown to regulate fly lifespan, including diet, temperature, housing density, and social environments [12–15]. In particular, mating is known to significantly reduce lifespan in females [16]. However, even focusing on nonvirgin flies, the direction of lifespan differences between male and female flies has not always been consistent in previous studies. While it is generally believed that female flies live longer than males [17–20], many reports have shown inconclusive results [21–23]. Surveys based on multiple strains even suggested longer male lifespans [12,24]. The sex differences in fly lifespan, therefore, is still controversial.

Stress resistance has also been studied extensively in fruit flies. It is generally believed that female flies are more resistant to various stresses than males [25]. In particular, better female resistance to starvation was documented in several studies [26,27]. However, resistance to oxidative stress is less consistent. While some studies have shown better female resistance to oxidative stress [18], surveys in multiple strains detected no significant difference [28,29]. Different oxidizing agents can even lead to opposite results [30]. In addition to simply focusing on stress resistance, the association between lifespan and stress resistance has also been investigated. In general, the ability to tolerate stress is believed to be related to lifespan and potentially shares underlying mechanisms with the aging process [18,31–33], although no relationship has been reported in some studies as well [34,35].

In summary, in fruit flies, the sexual differences in lifespan and stress resistance as well as their relationship were not always consistent in previous studies. Therefore, in this study, we revisited these questions by examining the lifespan and stress resistance of multiple inbred strains in both sexes. First, we asked whether males and females show differences in lifespan and stress resistance across these populations. Second, we investigated the difference in the variation in lifespan and stress resistance between the two sexes. Third, we examined the correlation between lifespan and stress resistance. By revisiting these questions, we hope that our results will provide additional information regarding the similarities and differences between the two sexes in terms of fly longevity and stress resistance.

2. Materials and methods

2.1. Fly stocks and environmental details

The laboratory stocks of 15 inbred strains of flies were obtained from the Fly Core in Taiwan, including Canton-S (CS), Oregon-R (OR), RAL-208, and RAL-313 originally from North America; GA125, GA129, GA130, RG2, RG3, RG5, Z11, Z30, and Z53 originally from Africa; and FR310 and FR361 originally from Europe. Flies were kept at 25 °C and 60% relative humidity with a 12 h light/12 h dark cycle and provided with standard white food mainly containing cornmeal, yeast, and sucrose. The detailed composition of the fly food is provided in Supplemental Table S1.

2.2. Lifespan and stress resistance assays

Lifespan and stress resistance assays followed those in a previous study [15]. Flies were collected within 3 days after eclosion and then transferred to new bottles and allowed to freely mate for 24 h. Male and female flies were then sorted under carbon dioxide anesthesia and placed into vials (height: 9.5 cm; diameter: 2.5 cm) filled with 5 mL of standard white food, with 30 flies in each vial.

For the lifespan assay, more than 200 flies of each sex were collected from approximately 10 bottles for each strain. Thirty flies of the same sex were placed in one vial with 8–10 replicate vials for each strain. Flies were transferred to new vials every 2–3 days, and mortality was recorded. Since flies were sometimes lost during transfer, the exact numbers of flies for each strain are listed in Table 1.

Table 1
Number of flies (males/females) included in each experiment.

Strain	Lifespan (♂/♀)	Starvation resistance (♂/♀)	Desiccation resistance (♂/♀)	Oxidative stress resistance (♂/♀)
Z53	285/284	294/287	290/292	292/282
FR310	277/289	277/240	279/226	278/219
RAL-313	186/242	270/258	290/260	253/254
RG2	279/292	288/266	279/233	240/261
RG3	284/287	260/223	241/230	280/234
GA125	279/290	295/291	293/288	304/281
GA130	292/286	282/277	293/225	289/292
OR	291/291	–	96/24	151/99
CS	274/288	270/251	287/243	286/259
RAL-208	293/291	179/107	186/104	170/79
GA129	287/290	229/165	–	100/61
Z11	270/289	289/285	281/277	284/271
Z30	268/286	207/134	191/124	211/122
FR361	263/279	–	–	–
RG5	269/274	261/206	245/216	237/176

Referring to previous studies [36,37], the early stage of lifespan was calculated as the average survival days for the first 10% of total flies to die for each strain. The median stage of lifespan was calculated as the average survival days for the middle 10% of total flies to die for each strain. The late stage of lifespan was calculated as the average survival days for the last 10% of total flies to die for each strain.

For the stress resistance assays, more than 200 flies of each sex were collected from approximately 10 bottles for each strain. Thirty flies of the same sex were placed in one vial with 8–10 replicate vials for each strain in each experiment. Flies were transferred to fresh vials every 2–3 days for two weeks. The flies that survived after two weeks were subsequently utilized for the stress assays. We conducted three stress assays simultaneously for all strains to ensure consistent conditions. However, due to limited fly numbers, we had to exclude certain strains from specific assays to maintain an adequate sample size for the remaining assays. The exact numbers of flies for each strain in the three assays are listed in Table 1.

(1) Starvation resistance: flies that survived after two weeks were placed in vials containing only 5 mL of 5% agar under carbon dioxide anesthesia, with 30 flies in each vial. Mortality was scored every 8 h until all the flies had died.
(2) Desiccation resistance: flies that survived after two weeks were placed in empty vials without any food and water under carbon dioxide anesthesia, with 30 flies in each vial. Mortality was scored every 8 h until all the flies had died.
(3) Oxidative stress resistance: flies that survived after two weeks were placed in vials containing 1/16 pieces of Kimwipe tissue (Kimtech Cat no. 34155) under carbon dioxide anesthesia, with 30 flies in each vial. A total of 340 μL of paraquat solution (5% sugar and 10 mM paraquat) was added on the first day [38,39], and an extra 150 μL was supplied each subsequent day to prevent it from drying out. Mortality was scored twice a day until all the flies had died.

2.3. Statistical analysis

Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad), SPSS 22.0 (IBM) and R (R Core Team, 2023). Sexual differences in lifespan, starvation, desiccation, and oxidative stress resistance within each strain were examined by the Cox proportional hazards model. Sexual differences in lifespan (total lifespan, early, middle and maximum lifespan), starvation, desiccation, and oxidative stress resistance across multiple strains were examined by the generalized linear mixed model (GLMM), with strain considered a random effect. Because data distributions were not normally distributed, data for lifespan and stress resistance were first log-transformed to calculate the coefficient of variation. The variations between males and females within each strain were tested by Levene's test. The variations between males and females across strains were examined by comparing CoV using paired t-test (normally distributed) or Wilcoxon matched-pairs signed-rank test (nonnormally distributed). Pearson correlation analysis was used to study the correlation.

3. Results

3.1. Males tended to have a longer lifespan than females

By examining the lifespan of 15 different inbred lines in both sexes, we first found a positive correlation of the average lifespans between males and females among these strains (Figure S1A). Direct comparisons between the two sexes in the same strain revealed multiple significant differences (Figure S2). Six strains (RAL-313, RG3, OR, CS, Z11, Z30) showed significantly longer male lifespans. Six strains (RG2, GA125, GA130, RAL-208, GA129, FR361) showed significantly longer female lifespans, although the differences in three strains were relatively small (GA130, GA129, FR361). When we collapsed all strains and assigned strain as a random effect, a generalized linear mixed model (GLMM) suggested a significantly longer lifespan of males than females ($F = 390.658$, $df1 = 1$, $df2 = 8325$, $p < 0.001$) (Table 2), along with a significant interaction effect between sex and strain ($F = 41.699$, $df1 = 25$, $df2 = 8325$, $p < 0.001$). These results suggested that although male flies collectively have longer lifespans, the extent of the difference between the sexes can vary significantly depending on the strain.

Since the shorter lifespan of females might be due to the humid environment caused by larval growth in the early stage of the lifespan, we also applied GLMM to examine the survival days in the early, middle and late stages of the lifespan, which were calculated as the average lifespan of flies with the shortest, middle, or longest survival (10% of flies each) in each strain (Table 2). The comparisons between the two sexes suggested that females showed shorter survival days than males not only in the early stage (the first

Table 2
Results of GLMM for the sexual difference in lifespan.

	Fixed effect	Coefficient	SE	t value	p value	Random effect	Variance	SE
Total lifespan	Intercept	3.849	53.481	0.072	0.943	Strain	0.204	0.003
	Sex	−0.657	0.038	−17.192	<0.001			
Early stage of lifespan	Intercept	2.339	0.070	33.379	<0.001	Strain	0.133	0.007
	Sex	−0.846	0.097	−8.693	<0.001			
Middle stage of lifespan	Intercept	3.386	0.010	372.365	<0.001	Strain	0.003	0.000
	Sex	−0.757	0.014	−52.998	<0.001			
Late stage of lifespan	Intercept	4.385	1.458	3.008	0.003	Strain	0.019	0.001
	Sex	−0.298	0.036	−8.169	<0.001			

10% to die; F = 81.024, df1 = 1, df2 = 818, p < 0.001) but also in the middle stage (the middle 10% of flies; F = 4522.459, df1 = 1, df2 = 818, p < 0.001) and the late stage (the last 10% to die; F = 168.107, df1 = 1, df2 = 818, p < 0.001), suggesting that the shorter female lifespan was not simply due to the suboptimal environment in the early stage.

3.2. Females showed greater variation in lifespan than males

The variations in lifespan between the two sexes were compared by the coefficient of variation (CoV) and Levene's test (Table 3). Except for FR310 and RAL313, most strains showed greater CoV in females than males, although the differences according to Levene's test were only significant in Z53, OR, GA129, Z11 and Z30. Direct comparison of CoV across strains between the two sexes also indicated greater variation in females than in males. Therefore, for both within and across stains, the lifespan variations were greater in females than in males.

3.3. Females showed greater resistance and greater variations than males in starvation and desiccation resistance

In addition to lifespan, we also investigated the stress resistance of these inbred strains, including resistance to starvation, desiccation, and oxidative stress. The correlations between males and females were all positive for these stressors, although the correlation was not significant for oxidative stress (p = 0.088) (Figure S1B-D). For starvation and desiccation stress, females showed better resistance in all tested strains (Figure S3 and S4). GLMM also indicated better resistance to starvation (F = 3197.307, df1 = 1, df2 = 6315, p < 0.001) and desiccation (F = 4221.626, df1 = 1, df2 = 5967, p < 0.001) in females than males (Table 4). In contrast, the differences between males and females in oxidative stress resistance were not consistent across strains. There were eight strains (FR310, RAL-313, GA125, CS, Z11, Z30, GA129, OR) with better resistance in males and three strains (Z53, RG2, RG5) with better resistance in females (Figure S5). Collapsing data from all strains in GLMM suggested better oxidative stress resistance in males than in females (F = 104.84, df1 = 1, df2 = 6237, p < 0.001). (Table 4).

The variations in these three stress resistances were also examined. CoV and Levene's test together suggested that, for most strains, female flies exhibited greater variation in starvation and desiccation resistance than males. There were 8 strains with significantly greater variation in starvation resistance in females (Table 5) and 10 strains with significantly greater variation in desiccation resistance in females (Table 6). Comparisons of CoV from multiple strains also indicated larger variations of females across strains in these two stress resistances. For oxidative stress resistance, there were five strains (FR310, RAL-313, GA125, CS, Z11) with significantly greater variations in males, but three strains (Z53, RG2, GA130) with significantly greater variations in females (Table 7). The difference in CoV between the two sexes across strains, therefore, was not significant.

3.4. Lifespan and stress resistance were not correlated among strains

Since lifespan is often suggested to be related to stress resistance, we assessed whether stress resistance can predict lifespan across these tested inbred strains. Surprisingly, examining the relationships between lifespan and the three types of stress resistance showed no significant correlation in either sex (Fig. 1A–F).

We were also interested in whether the abilities to resist different stresses were similar among these strains. By examining the correlations among these three types of stress resistance, we found no significant correlation in most cases (Fig. 1G–L). Positive correlations were detected only in males between oxidation resistance and desiccation resistance (Fig. 1I) and in females between starvation resistance and desiccation resistance (Fig. 1J).

Table 3
Comparison of variation in lifespan between males and females.

	Lifespan CoV		p value
	Male	Female	Levene's test
Z53	9.201	**9.855**	**0.0462**
FR310	7.296	6.811	0.0614
RAL-313	15.911	15.546	0.6986
RG2	7.244	9.213	0.1441
RG3	14.462	18.540	0.5143
GA125	8.300	8.979	0.0705
GA130	15.889	17.150	0.1393
OR	24.925	**25.982**	**0.0012**
CS	24.126	27.151	0.8374
RAL-208	11.996	15.125	0.5517
GA129	12.880	**16.313**	**<0.0001**
Z11	17.694	**25.023**	**0.0007**
Z30	33.442	**37.125**	**0.0001**
FR361	10.932	11.176	0.8161
RG5	12.294	11.143	0.1371
			Wilcoxon signed-rank test
Cross strain average	15.106	**17.009**	**0.0043**

Table 4
Results of GLMM for the sexual difference in stress resistance.

	Fixed effect	Coefficient	SE	t value	p value	Random effect	Variance	SE
Starvation resistance	Intercept	4.225	0.017	242.147	<0.001	Strain	0.088	0.002
	Sex	0.458	0.025	18.530	<0.001			
Desiccation resistance	Intercept	14.626	4.753	3.077	0.002	Strain	44.957	0.823
	Sex	17.782	0.568	31.322	<0.001			
Oxidative stress resistance	Intercept	49.412	14.831	3.332	0.001	Strain	437.786	7.84
	Sex	−9.464	1.777	−5.326	<0.001			

Table 5
Comparison of variation in starvation resistance between males and females.

	Starvation resistance CoV		p value
	Male	Female	Levene's test
Z53	6.87	**9.36**	**<0.0001**
FR310	10.87	12.88	0.1352
RAL-313	11.82	11.83	0.6289
RG2	7.89	6.95	0.3544
RG3	10.04	**15.08**	**0.0008**
GA125	7.31	**7.91**	**0.0125**
GA130	6.49	**8.12**	**<0.0001**
CS	6.59	**8.21**	**0.0048**
RAL-208	7.63	**15.63**	**0.0005**
Z11	6.71	7.23	0.5574
Z30	6.48	**13.08**	**0.0009**
RG5	8.70	**13.74**	**0.0001**
GA129	8.65	10.29	0.5036
			Wilcoxon signed-rank test
Cross strain average	8.16	**10.79**	**0.0017**

Table 6
Comparison of variation in desiccation resistance between males and females.

	Desiccation resistance CoV		p value
	Male	Female	Levene's test
Z53	10.84	**11.62**	**<0.0001**
FR310	10.95	**11.88**	**0.0005**
RAL-313	8.62	**9.01**	**<0.0001**
RG2	8.01	**11.68**	**<0.0001**
RG3	11.04	**14.99**	**<0.0001**
GA125	6.97	**9.73**	**<0.0001**
GA130	9.04	9.07	0.9888
CS	8.64	**9.21**	**<0.0001**
RAL-208	10.91	11.70	0.8610
Z11	**12.52**	8.93	**0.0039**
Z30	10.54	12.24	0.1522
RG5	10.54	**12.24**	**<0.0001**
OR	6.24	**12.40**	**<0.0001**
			Paired *t*-test
Cross strain average	9.60	**11.13**	**0.0365**

4. Discussion

By examining multiple inbred strains of flies, we validated some previous findings but also observed some unexpected results related to lifespan and stress resistance. Consistent with previous reports, both lifespan and resistance to starvation and desiccation among these strains were highly correlated between the two sexes. While females showed better resistance to starvation and desiccation stress in most strains, the difference in lifespan and oxidative stress resistances varies depending on the strain. GLMM even suggested a longer lifespan and better oxidative stress resistance in males than in females. Our results also suggested greater variations within or across populations in lifespan, starvation resistance and desiccation resistance in females than in males. Finally, to our surprise, we did not find any significant correlation between lifespan and stress resistance among these strains.

We used 15 inbred strains to investigate the sex differences in lifespan in this study. The advantage of the inbred strain is control of genetic variation, providing a homogeneous genetic background for the two sexes. However, similar genetic backgrounds between the two sexes may result in similar lifespans, which could probably explain the high correlation between male and female lifespans

Table 7
Comparison of variation in oxidative stress resistance between males and females.

	Oxidative stress resistance CoV		p value
	Male	Female	Levene's test
Z53	7.43	**10.60**	**<0.0001**
FR310	**17.87**	10.81	**<0.0001**
RAL-313	**11.00**	8.64	**<0.0001**
RG2	7.71	**9.46**	**0.0243**
RG3	9.77	10.09	0.9647
GA125	**12.20**	9.18	**0.0054**
GA130	11.21	**14.22**	**0.0097**
CS	**13.69**	10.71	**<0.0001**
RAL-208	13.34	15.53	0.1454
Z11	**14.08**	9.94	**<0.0001**
Z30	13.31	14.77	0.6005
RG5	9.61	10.02	0.1232
GA129	12.43	12.16	0.4089
OR	11.81	12.54	0.8870
			Paired *t*-test
Cross strain average	11.82	11.33	0.5560

observed in our study. In addition, inbreeding can lead to reduced lifespan due to the accumulation of harmful mutations and the expression of deleterious recessive alleles, known as inbreeding depression [40]. In particular, the unguarded X hypothesis suggests that inbreeding may decrease female lifespan more than male lifespan due to the inbreeding of the X chromosome [41]. In future research, it will be interesting to investigate whether outbred strains would also generate similar results to those shown in the present report, including a high correlation between the two sexes, a longer male lifespan and greater female variation.

Although the lifespan difference between the sexes varied in each strain, GLMM suggested that males collectively have a longer lifespan than females. Replication with more strains in future research might be helpful to further confirm these results; however, previous studies had already shown similar findings based on more than 100 strains [12,24]. In addition to lifespan differences between sexes, we also found greater variation in female lifespan, both within and across populations. Female life history is particularly influenced by a variety of internal and external factors. For example, reproductive investments, which impact females significantly more than males [42,43], may result in larger variation in female longevity than males.

Regarding starvation stress, it is worth noting that 5% agar was used in our starvation assay, in contrast to conventional 1% agar. Even so, our results still showed better resistance to starvation in females, which was consistent with most previous reports [26,27]. This sex difference is possibly due to the larger size or increased fat storage of females. On the other hand, better desiccation resistance in females has received less attention in previous studies. Cuticular hydrocarbons (CHCs) have been suggested to be critical for desiccation resistance. A recent study suggested that longer methyl-branched CHCs are correlated with higher desiccation resistance, but the difference between males and females was very minor [44]. Other factors, such as size, metabolic water stores or respiratory patterns [45,46], might also contribute to sex differences in desiccation resistance. Notably, female resistance to these two types of stressors was more variable than male resistance, which, similar to longevity, might also be due to the greater effect of reproduction on females than males.

Unlike starvation and desiccation stress, the difference between males and females in oxidative stress resistance varies for each strain. There was no sex difference in the coefficient of variation. In fact, the sex difference in oxidative stress resistance was quite inconsistent in previous studies [18,28,29,47,48]. The absence of a conclusion is likely due to many factors influencing the oxidative stress responses or results. For example, the feeding rate or the amount of paraquat ingested can substantially affect the response to oxidative stress. Different oxidizing agents or other environmental conditions could also lead to different results [49–52]. Specifically, in our assay, we administered daily supplements of paraquat solution to prevent dehydration, which could result in the accumulation of paraquat and an increased oxidative environment. Furthermore, we exposed the flies to a 10 mM paraquat solution with 5% sucrose [38,39,53], which was higher than most studies using 1% sucrose solution. Notably, such a high sucrose diet itself may induce additional stress conditions [54]. Consequently, despite our GLMM indicating better resistance in males than females, we maintain that the sex difference in oxidative stress resistance remains inconclusive and warrants further investigation.

It is generally believed that stress resistance shares the same mechanism and can potentially predict lifespan in flies [32,33]. Although there were some inconsistent results [34,35], many studies have applied artificial selection or screening to generate flies with longer lifespans along with better stress resistance [18,47,55,56]. It is therefore surprising to find no correlations between longevity and resistance to three types of stressors among these inbred strains in our experiments. The positive relationships among these three stress assays were not particularly striking either. More strains might be needed to reveal the positive relationship among these phenotypes. Regardless, our results did not exclude the possibility that some manipulations, such as dietary restriction, can regulate longevity and stress resistance together [57,58]. The social environment can also modulate fly longevity and stress resistance in a similar direction, as shown in our previous study [15]. However, the failure to detect significant correlations among these strains implied that the pathways for these phenotypes are still very different. Notably, since males generally showed a longer lifespan but females showed better starvation and desiccation resistance, there are likely some sex-specific factors regulating lifespan and stress resistance differently, which await future investigation.

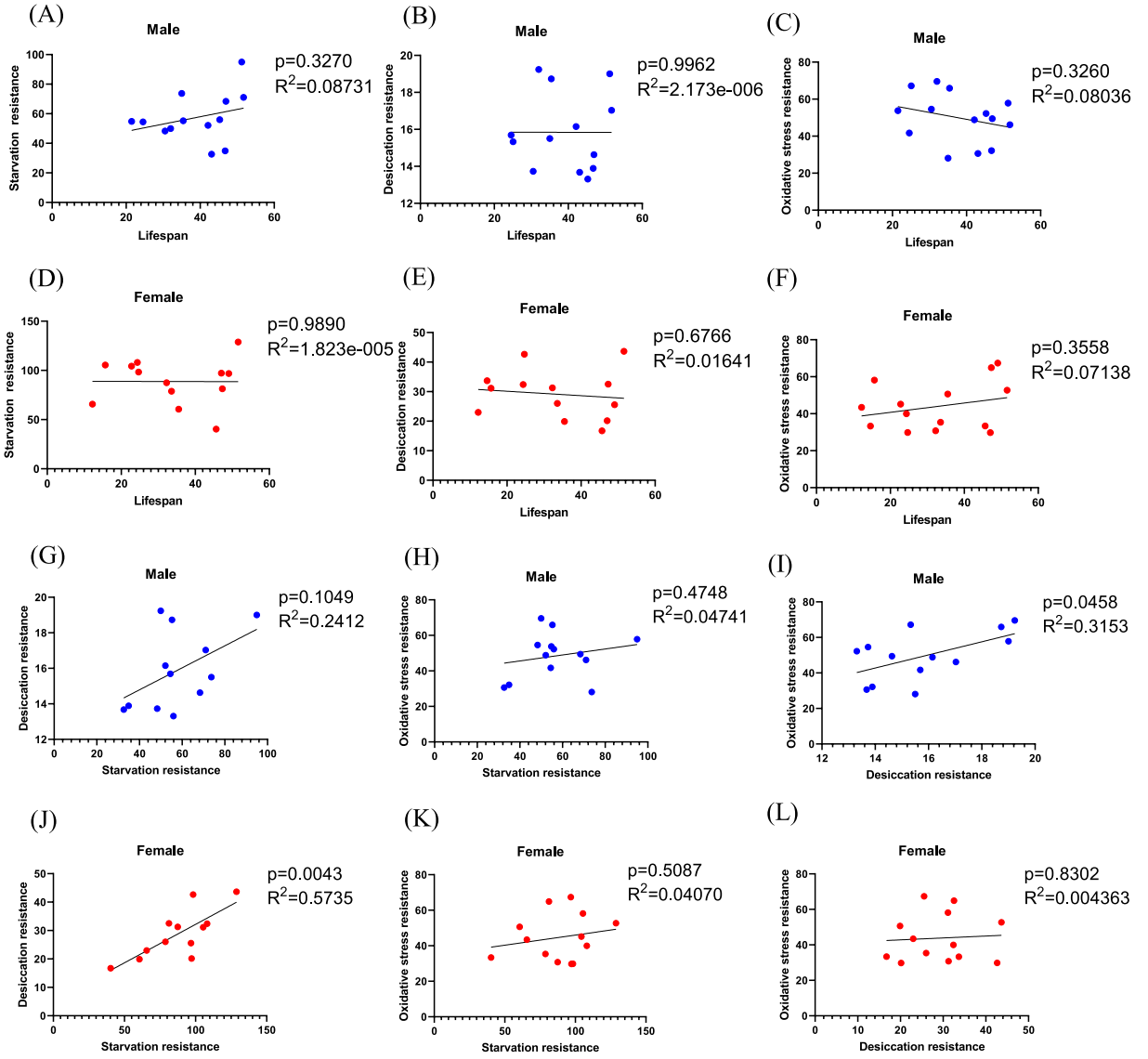

Fig. 1. There was no significant correlation between lifespan and stress resistance of multiple strains. (A) Correlation between average lifespan and starvation resistance in males. (n = 13) (B) Correlation between average lifespan and desiccation resistance in males. (n = 13) (C) Correlation between average lifespan and oxidative stress resistance in males. (n = 14) (D) Correlation between average lifespan and starvation resistance in females. (n = 13) (E) Correlation between average lifespan and desiccation resistance in females. (n = 13) (F) Correlation between average lifespan and oxidative stress resistance in females. (n = 14) (G) Correlation between starvation resistance and desiccation resistance in males. (n = 12) (H) Correlation between starvation resistance and oxidative stress resistance in males. (n = 13) (I) Correlation between desiccation resistance and oxidative stress resistance in males. (n = 13) (J) Correlation between starvation resistance and desiccation resistance in females. (n = 12) (K) Correlation between starvation resistance and oxidative stress resistance in females. (n = 13) (L) Correlation between desiccation resistance and oxidative stress resistance in females. (n = 13) Pearson correlation was used to examine the correlation between lifespan and stress resistance tests.

To respond to the questions proposed in our introduction, our results based on GLMM analyses suggested a longer lifespan and better resistance to oxidative stress in males but better resistance to starvation and desiccation stress in females. Greater female variations were shown in lifespan, starvation and desiccation resistance. However, we failed to detect a correlation between lifespan and stress resistance. By examining these old questions, we provided new data about the sex differences and pose many questions for this field: Why do male fruit flies have a longer lifespan in general? Why do females exhibit greater variation in these phenotypes? Why do females show a shorter lifespan but better stress resistance? Future studies focusing on these questions would potentially provide important information regarding sexual dimorphism and help us better understand similarities or differences between longevity and stress resistance.

Author contribution

Yu-Chiao Lin: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. MingYang Zhang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data. Yu-Jen Chang: Analyzed and interpreted the data. Tsung-Han Kuo: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data access statement

Data for this research work is available at Mendeley Data: https://doi.org/10.17632/csm8zkywy7.1.

Declaration of competing interest

The authors declare that they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e18178.

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