Effect of deletions of the *CBS*, *CSE* and *MST* hydrogen sulfide biosynthesis genes under restricted intake of sulfur-containing amino acids (methionine and cysteine) on the viability of *Drosophila melanogaster*.

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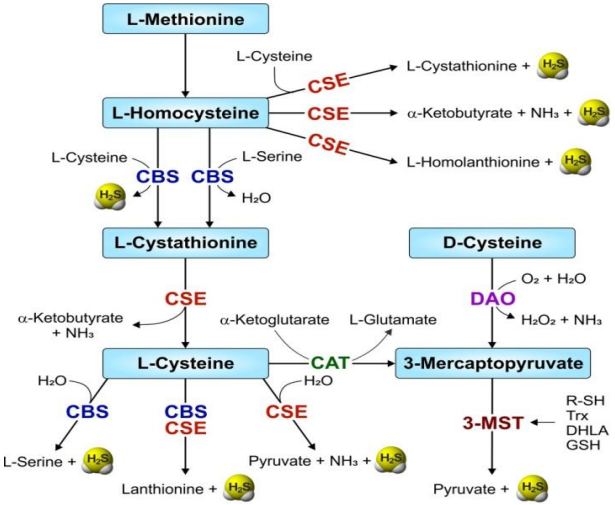

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H_2S is an endogenously produced gaseous signaling molecule that plays a critical role in various physiological and pathological processes. The synthesis of H_2S is primarily mediated by three enzymes: CBS, CSE, and MST, each of which is subject to complex regulation by various factors.

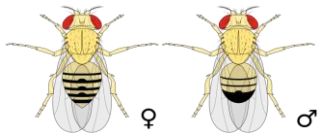
The common substrate for all three enzymes is cysteine, either directly or indirectly through cystathionine or 3-mercaptopyruvate. The enzymes then catalyze reactions that ultimately produce the gasotransmitter hydrogen sulfide from cysteine. Methionine and cysteine are metabolically related. Methionine serves as a precursor to cysteine through the transsulfuration pathway. Both amino acids are vital for maintaining cellular health, antioxidant defense, and metabolic regulation in *Drosophila*.

The aim of the present study was to investigate the effect of deletion of *CBS, CSE* and *MST* genes in different combinations on longevity, stress tolerance and locomotor activity of *D. melanogaster* while controlling methionine and cysteine content in their diet.

Laboratory lines of *D. melanogaster* were obtained from the Engelhardt Institute of Molecular Biology collection. Homozygous for deletions of the CBS (designated as *CBS-/-*), 3-MST (*3-MST-/-)* genes, as well as a hybrid line with deletions of two genes *CBS* and *CSE* (*CBS-/-; CSE-/-*) were used. Line 58492 (BDSC_58492, Bloomington, USA, designated as (CBS+/+, CSE+/+)) was used as a control. The deletions of *CBS* and *CSE* genes were confirmed through real-time quantitative reverse transcription PCR (qRT-PCR) analysis (Zatsepina et al., personal communication).

Control and experimental flies were kept on synthetic nutrient medium devoid of yeast, with a precisely controlled composition of carbohydrates, amino acids, lipids, vitamins, and microelements (mixture TD.210411, ENVIGO, USA) differing in the content of the sulfur-containing amino acids methionine (Met) and cysteine (Cys):

Variants of nutrient media on which flies lived:
Met+, Cys+ – control medium containing methionine and cysteine
Met-, Cys- – medium free of methionine and cysteine
Met+, Cys- – medium containing only methionine
Met-, Cys+ – medium containing only cysteine



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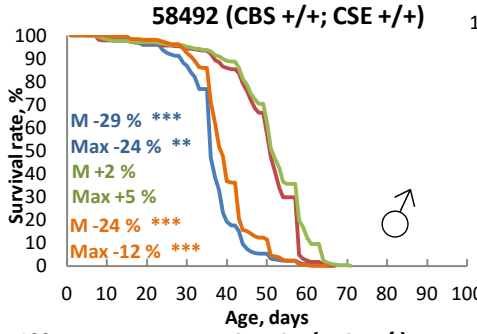
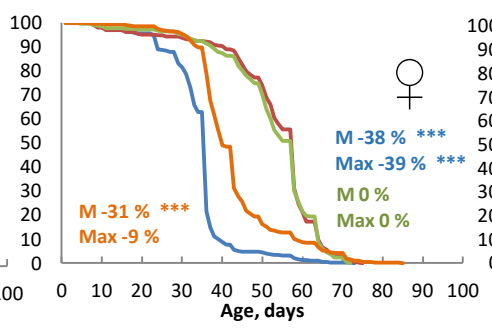
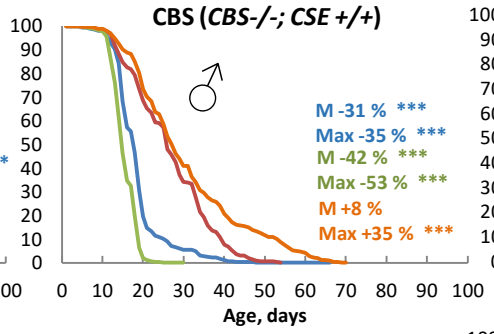
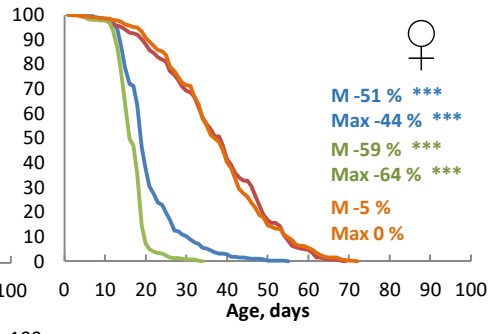
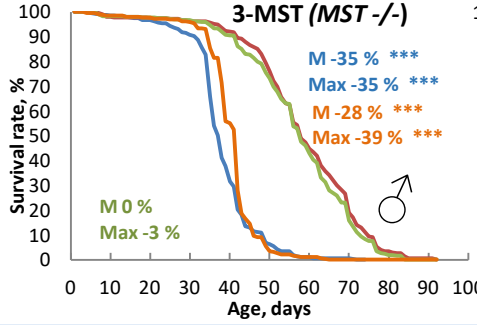
Effect of *CBS*, *CSE* and *MST* deletions on lifespan

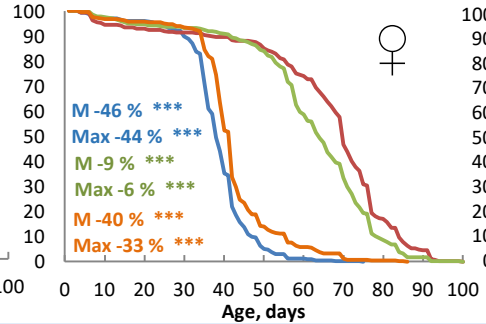


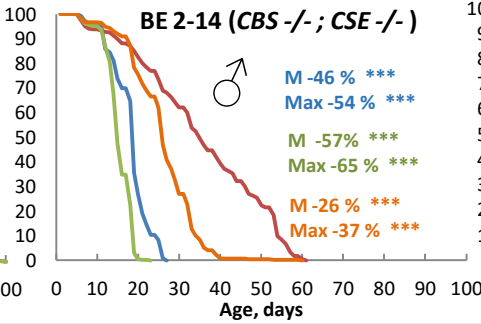


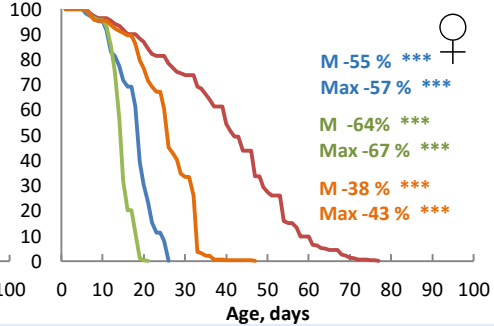












35 flies in 3 test tubes for each variant of the experiment. Flies were maintained at 25°C and a 12-hour light regime. Males and females lived separately.

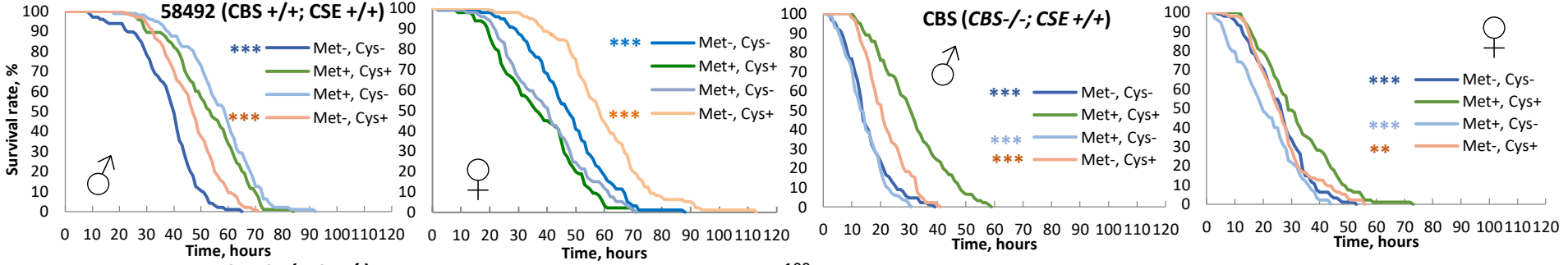
The longest life span was observed in flies living on control medium (Met+, Cys+). Interestingly, the life expectancy on methionine medium (Met+, Cys-) sharply decreased in CBS and BE 2-14 lines. This is probably due to the accumulation of homocysteine, an intermediate product of methionine metabolism. Its accumulation negatively affects the vital functions of the organism. The 3-MST line lived longest on the control medium (Met+, Cys+) and on the medium containing only methionine (Met+, Cys-). Probably deletion of this gene does not have serious consequences in contrast to deletion in the *CBS* gene. The CBS line had the longest lifespan in variants maintained on nutrient medium with cysteine, regardless of methionine supplementation (Met-, Cys+) and (Met+, Cys+).

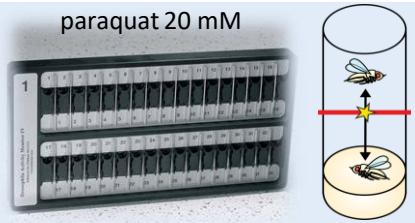
M – median lifespan;
Max – maximum lifespan (age of 90% mortality)

Met-, Cys-
Met+, Cys+
Met+, Cys-
Met-, Cys+

Comparison of control and experimental flies.
*p<0.05, **p<0.01, ***p<0.001 – Fisher's Exact Test.

Effects of deletions on resistance to oxidative stress





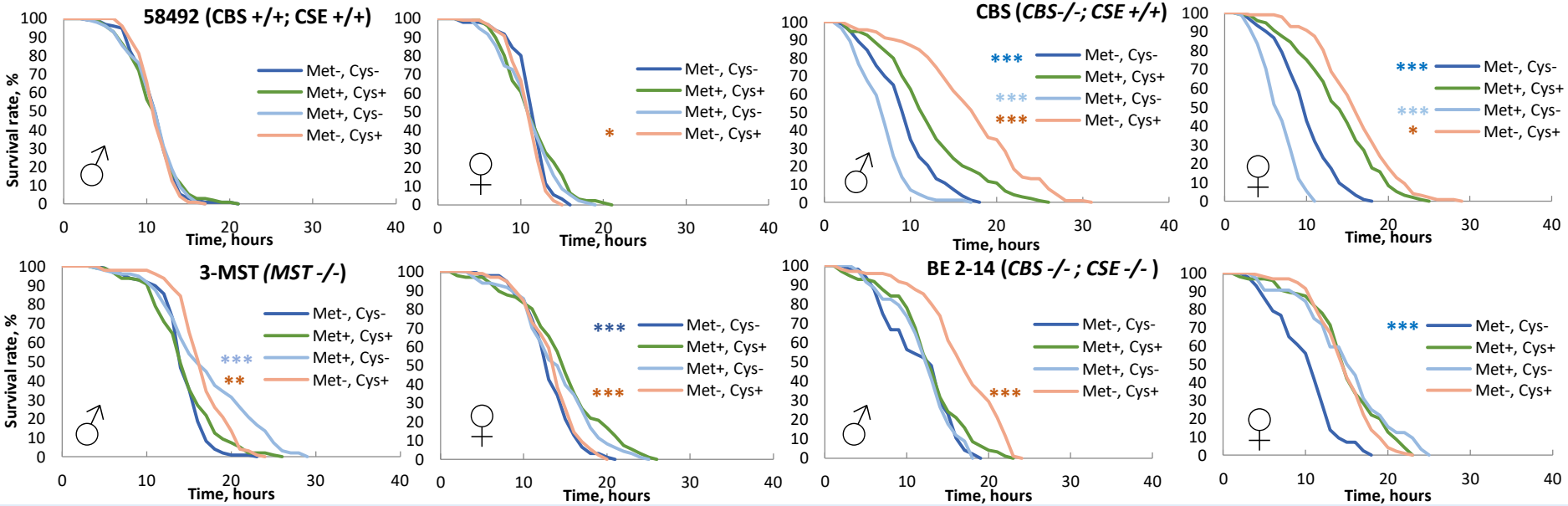
Drosophila Activity Monitor (Trikinetics, USA)
1 fly x 32 capillaries
Activity detection every half hour
Detection of time of death by absence of activity

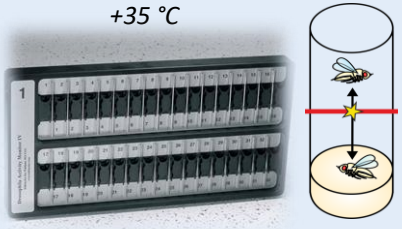
Paraquat-induced oxidative stress continuously produces reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (H_2O_2). Glutathione is a crucial antioxidant in the body, playing several key roles in cellular function and protection against oxidative stress.

Exposure to paraquat strongly reduces glutathione levels, probably by impairing its synthesis through impaired cysteine metabolism or an increased requirement for it (doi.org/10.1039/C8FO01457D). Glutathione is synthesized from cysteine, glutamate, and glycine. Lines with deletions in the *CBS* and *CSE* genes, as well as with a single deletion in the *CBS* gene, showed worse survival under cysteine deficiency in the nutrient medium. The best survival was on control medium (Met+, Cys+).

Comparison of control and experimental flies.
*p<0.05, **p<0.01, ***p<0.001 – Log-rank Test.

Effects of deletions on resistance to hyperthermia





Hyperthermia has been shown to increase oxidative stress (doi.org/10.1007/978-981-10-0719-4_2), suggesting that cysteine availability may be vital for survival in the absence of the *CBS* gene. Surprisingly, in double-knockout females (*CBS -/- ; CSE -/-*), the presence of cysteine or methionine has an equivalent effect on survival. It is possible that methionine indirectly promotes survival under these conditions. In contrast, the presence of methionine at *CBS* gene deletion has a deleterious effect on survival.

Notably, flies of the CBS line maintained on cysteine-containing medium (Met-, Cys+) showed longer survival under hyperthermia than the control line and other lines.

Drosophila Activity Monitor (Trikinetics, USA)

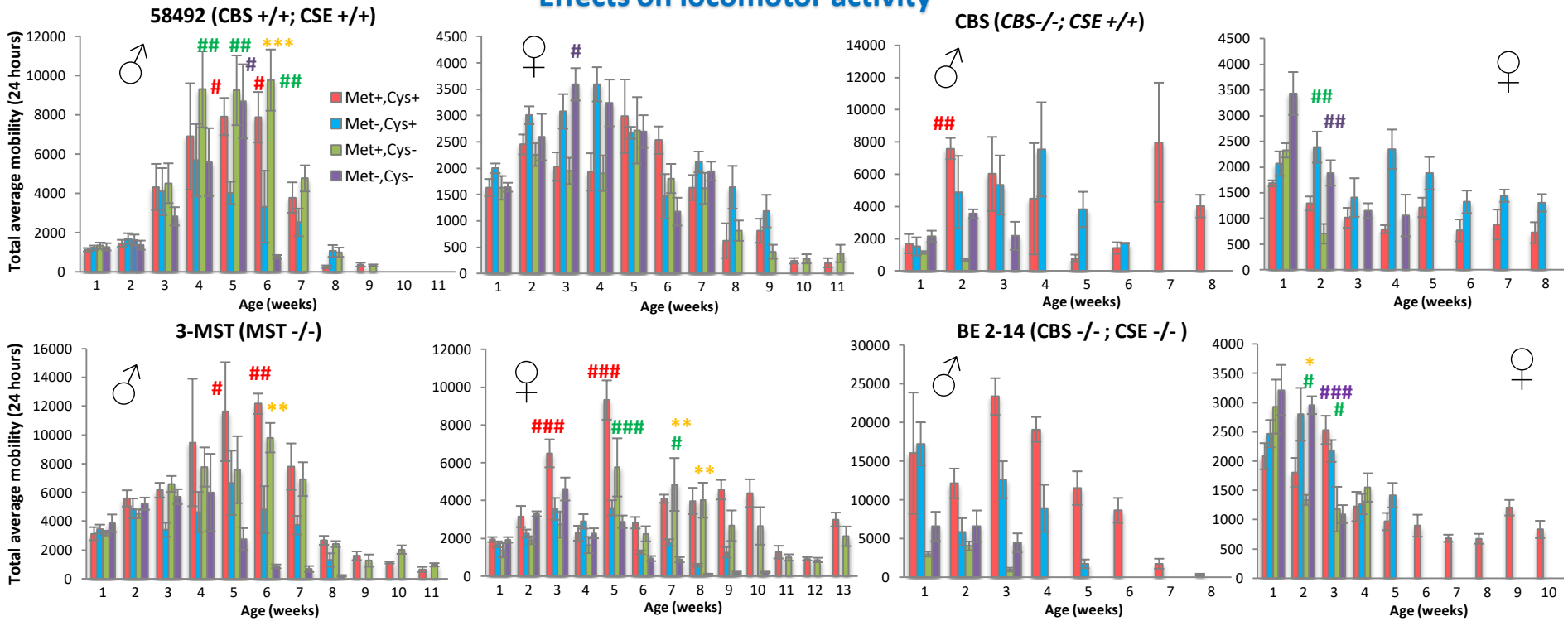
1 fly x 32 capillaries

Activity detection every half hour

Detection of time of death by absence of activity

Comparison of control and experimental flies.
*p<0.05, **p<0.01, ***p<0.001 – Log-rank Test.

Effects on locomotor activity



Using two-factor analysis of variance and Tukey's HSD posterior test, it was found that the sulfur-containing amino acid methionine and age significantly affected the locomotor activity of *Drosophila*. It was observed that methionine supplementation decreased locomotor activity in females of three lines (58492, CBS and BE 2-14), but increased activity in the MST line. In contrast, in males, the presence of methionine consistently increased locomotor activity. In addition, motor activity was found to initially increase with age but then subsequently decrease.

Comparison of control and experimental flies.
Two-way analysis of variance (ANOVA) along with post-hoc Tukey's HSD (Honestly Significant Difference)
*p<0.05, **p<0.01, ***p<0.001 - methionine contribution
#p<0.05, ##p<0.01, ###p<0.001 - contribution of age

Conclusion

1. Our study revealed that the longest lifespan in *Drosophila* was observed in flies maintained on control medium containing both methionine and cysteine (Met+, Cys+). However, CBS and BE 2-14 lines showed a dramatic decrease in longevity on methionine medium (Met+, Cys-), likely due to the accumulation of homocysteine, an intermediate metabolite of methionine, which negatively affects vital functions. The 3-MST line lived longest on control and methionine-only media, suggesting limited consequences of this gene deletion. In contrast, the CBS line had the longest lifespan on cysteine-supplemented nutrient medium, regardless of methionine supplementation.
2. Lines with deletions in the *CBS* and *CSE* genes, as well as a single deletion in the *CBS* gene, showed worse survival under cysteine deficiency in the nutrient medium. This effect may be attributed to paraquat-induced depletion of glutathione levels, which is likely to occur through impairment of its synthesis due to disturbances in cysteine metabolism or an increase in its demand. The best survival was observed on control medium (Met+, Cys+).
3. The presence of cysteine may be critical for survival in the absence of the *CBS* gene under hyperthermic conditions. Flies of the CBS line maintained on cysteine medium (Met-, Cys+) showed longer survival than controls and other lines. In contrast, the presence of methionine in the nutrient medium decreased survival. In double-knockout females (*CBS -/- ; CSE -/-*), the presence of cysteine and methionine had similar effects on survival.
4. Two-factor analysis of variance and Tukey's HSD post-hoc test revealed that methionine and age significantly affected locomotor activity in Drosophila. Methionine supplementation decreased locomotor activity in females of three lines (58492, CBS, and BE 2-14), but increased activity in the MST line. In contrast, in males, the presence of methionine consistently increased locomotor activity. Motor activity initially increased with age but then subsequently decreased.

Overall, our findings suggest that the availability of specific sulfur-containing amino acids differentially affects lifespan, survival under oxidative stress conditions, and locomotor activity in various gene deletion lines. Further studies are needed to elucidate the molecular mechanisms underlying these effects.