

Letter to the Editor

Spider odors induce stoichiometric changes in fruit fly *Drosophila melanogaster***Ronalds KRAMS^{a,b}, Tatjana KRAMA^{a,b,c}, Māris MUNKEVICS^{b,d}, Sarah EICHLER^e, David M. BUTLER^f, Linda DOBKEVIČA^g, Priit JÕERS^h, Jorge CONTRERAS-GARDUÑOⁱ, Janīna DAUKŠTE^j, and Indrikis A. KRAMS^{c,d,j,k,*}**

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Animal senses and signals are amazingly diverse, and the major modalities by which animals acquire sensory input from their environments are sound, light, vibration, and chemical signals. Insects mainly rely on visual, nociceptive, and olfactory cues to discriminate between rewards and risks. It has been shown that the visual and olfactory cues of predators substantially affect the adult phenotype in *Drosophila melanogaster* (Krams et al. 2016), a prominent animal model for biological research. A recent study has found that fruit flies can rely solely on vision in predator detection (de la Flor et al. 2017). However, the olfactory system of *D. melanogaster* is also highly developed and can be efficiently used in parasitoid (Ebrahim et al. 2015) and predator detection. Relying on olfaction as an additional sensory modality is adaptive because fruit flies may be active under conditions of low light when vision is limited as an antipredator tool (Izutsu et al. 2016). In this study, we investigated whether the larvae of *D. melanogaster* respond to chemical cues of spiders by changing adult body mass and stoichiometry of body nitrogen (N) and carbon (C) in a similar way as it occurs in the presence of a real spider (Krams et al. 2016). The general stress paradigm (GSP) states that predator exposure generally increases the production of glucocorticosteroids in prey. Predator-induced stress generally causes oxidative stress and induces glucogenesis, which in turn increases metabolic rate, raising the overall demand for carbohydrate-based fuel and shifting the metabolic balance away from the anabolism

that produces the nitrogen-rich (N) proteins necessary for growth (Hawlena and Schmitz 2010; Trakimas et al. 2019). These complex processes generally increase the C/N ratio (Hawlena and Schmitz 2010). However, *D. melanogaster* fruit flies reared together with spiders have a high concentration of body N and lower body mass, while their body C remains the same as fruit flies in the control group. Based on the results of Krams et al. (2016), we predicted higher N in flies reared with spiders and in flies grown in the presence of olfactory cues from spiders (but in absence of actual spiders), relative to flies in the control group. Methodological details can be found in the [Supplementary materials](#).

We observed a significant effect for the treatment group (two-way analysis of variance (ANOVA): $F_{2,64} = 56.05$, $P < 0.001$) and sex ($F_{1,64} = 627.824$, $P < 0.001$) on the body mass of the fruit flies. There was no significant interaction effect between the treatment group and sex on the flies' body mass ($F_{2,64} = 1.86$, $P = 0.165$). Male fruit flies reared with spider odors (0.15 ± 0.01 mg; mean \pm SD) and real spiders (0.15 ± 0.01 mg) were significantly lighter (Tukey's honestly significant difference test (Tukey HSD): $P_s < 0.001$) than male flies in the control group (0.20 ± 0.01 mg) (Figure 1A). Females that were reared with spider odors (0.27 ± 0.03 mg) and with real spiders (0.27 ± 0.03 mg) were also significantly lighter (both $P_s < 0.001$) than females in the control group (0.33 ± 0.03 mg) (Figure 1A). The body mass of flies in the

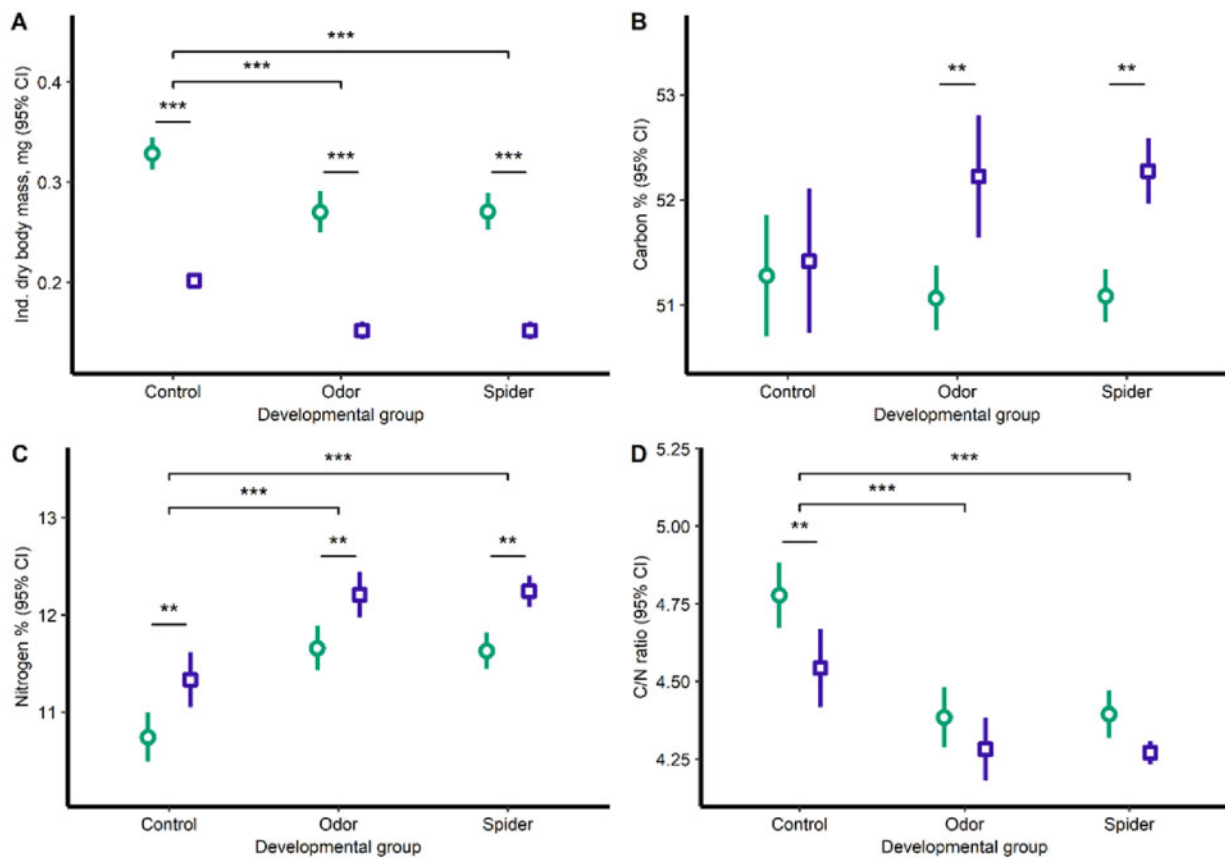


Figure 1. Dry body mass (A), average body C percentage (B), average body nitrogen percentage (C), and C/N ratio (D) in female (green circles) and male (blue squares) fruit flies reared in the control group, the predator odor stress group, and the real predator group. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

odor and spider groups did not differ significantly within each sex (both $P_s > 0.9$). Female fruit flies were heavier than males in each treatment group (all $P_s < 0.001$). There was no significant main effect for the treatment group (two-way ANOVA: $F_{2,64} = 1.34$, $P = 0.269$) on body C concentration in fruit flies. There was a significant effect for sex on body C ($F_{1,64} = 20.15$, $P < 0.001$) and for the interaction between both factors ($F_{2,64} = 3.62$, $P = 0.032$). Male fruit flies had significantly higher (all $P_s < 0.01$) body C concentration in both treatment groups (odor: $52.22 \pm 0.91\%$, spider: $52.28 \pm 0.46\%$; mean \pm SD) (Figure 1B) when compared to female fruit flies of the respective treatment group (odor: $51.07 \pm 0.48\%$, spider: $51.09 \pm 0.38\%$). However, body C did not differ significantly ($P = 0.999$) between sexes in the control group (control males: $51.42 \pm 1.08\%$, control females: $51.28 \pm 0.91\%$) (Figure 1B). There was a significant main effect for the treatment group (two-way ANOVA: $F_{2,64} = 48.90$, $P < 0.001$) and sex ($F_{1,64} = 45.82$, $P < 0.001$) on body N. We did not find significant influence for interaction between the treatment group and sex ($F_{2,64} = 0.05$, $P = 0.953$) on body N. We observed significantly increased (Tukey HSD: $P_s < 0.001$) body N in male fruit flies grown with spider odors ($12.21 \pm 0.37\%$; mean \pm SD) and with real spiders ($12.24 \pm 0.24\%$) when compared with control group male flies ($11.33 \pm 0.45\%$) (Figure 1C). Females grown with spider odors ($11.63 \pm 0.36\%$) and with real spiders ($11.63 \pm 0.28\%$) also had significantly higher body N (both $P_s < 0.001$) compared to the control group females ($10.74 \pm 0.40\%$) (Figure 1C). Body N in both males and females in

odor group did not differ significantly from the respective sex in the spider group (both $P_s = 0.999$). We found a significant main effect for the treatment group (two-way ANOVA: $F_{2,64} = 37.82$, $P < 0.001$) and sex ($F_{1,64} = 18.65$, $P < 0.001$) on the body C/N ratio in fruit flies. There was no significant interaction between treatment group and sex on fruit fly body C/N ratio ($F_{2,64} = 1.34$, $P = 0.270$). In male fruit flies, C/N ratio was significantly lower ($P_s < 0.001$) in both treatment groups (odor: 4.28 ± 0.16 , spider: 4.27 ± 0.05 ; mean \pm SD) (Figure 1D) when compared to control group males (4.54 ± 0.20). In female fruit flies, we also observed significantly lower (both $P_s < 0.001$) C/N in the odor (4.38 ± 0.15) and the spider (4.39 ± 0.11) groups, relative to the female flies in the control group (4.78 ± 0.17) (Figure 1D). There were no differences in C/N ratio between odor and spider groups within both sexes (both $P_s = 0.999$).

The data presented in this study show that rearing larvae in the presence of spider odors affects fruit fly stoichiometry (i.e., body C, N, and the C/N ratio) similarly as rearing larvae together with real spiders (Krams et al. 2016). Contrary to the predictions of the GSP (Hawlena and Schmitz 2010), body N was found to rise and the C/N ratio to decline in both real predator and odor groups. It is likely that predator odors stimulate *D. melanogaster* to grow and leave the area as fast as they can. The results of this study indicate that the effects of predator presence on fruit fly stoichiometry are equal to the effects of predator odors on developing larvae, or when predators affect visual and olfactory modalities simultaneously (Krams

et al. 2016). Thus, we show that olfaction alone may be sufficient to detect predators in the environment during larval development. This is important because visual detection of predators is not possible in the dark, and fruit fly activity is not restricted by the absence of light (Izutsu et al. 2016). We also found that male flies had a higher concentration of body C than females in the treatment groups, which may indicate a somewhat higher stress sensitivity in males. However, a sizable fraction of the body of a mated female is made up of developing eggs, and eggs have a significantly different metabolism than the female soma. This might decrease the degree of plasticity of stoichiometric responses of females. An alternative explanation has been recently suggested by Adamo and McKee (2017). They found that repeated flight-or-flight responses induce reproductive responses which decrease body mass and have the potential to increase body N and to reduce the C/N ratio as observed in male fruit flies in this study. Adamo and McKee (2017) suggested that even short-lived insects may alter their reproductive efforts based on the level of predation risk in their environment because the induction of reproductive investment might be an adaptive strategy under higher uncertainty of survival. It is important to note that sex differences in body C between the odor stress and the predator stress groups still await an explanation from the point of view of the GSP, as do differences in stoichiometry between individuals of both stress groups. As shown earlier, developmental speed is an important determinant of the concentration of biogenic elements in the body (Trakimas et al. 2019; Krams et al. 2020). Therefore, developmental speed and/or species-specific features of metamorphosis are the most likely factors to be taken into account to explain the observed differences between *D. melanogaster* and grasshoppers (Hawlena and Schmitz 2010), crickets (Trakimas et al. 2019), and other arthropods (Van Dievel et al., 2020) in their stoichiometry and other responses to biotic and abiotic stressors in the environment. Finally, (Rinehart and Hawlena, 2020) suggest that predator hunting mode may also have profound effects on prey stoichiometric responses to predation risk. Overall, our results show that prey individuals can use different modalities to acquire sensory input about their predators and these main sensory modalities should be taken into account in future studies in the field of ecological stoichiometry.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material can be found at <https://academic.oup.com/cz>.

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