Spider odors induce stoichiometric changes in fruit fly

*Drosophila melanogaster*

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Materials and Methods

Stock flies and experimental groups

Fruit flies were maintained in the lab at 22 ± 1 °C, under a constant 12:12 h light-dark cycle. In this study, we used the wild strain Oregon-R-modENCODE (#25211) of *D. melanogaster* obtained from the Bloomington Drosophila Stock Centre (IN, U.S.A.). The flies were isolated under carbon dioxide anesthesia.

To obtain the fruit flies for this study, we placed 20 females and 6 male fruit flies together to copulate and oviposit for 24 hours. Flies were placed in Flystuff 8oz round bottom bottles (Ø 55 mm; height = 78 mm) and we subsequently removed the adult flies from the bottles. Each bottle contained 18 ml of food. The food was cooked as a mixture of 500 ml water, 20 g dextrose, 15 g sucrose, 10 g brewer’s yeast, 35 g cornmeal, 4.5 g agar, and 12.5 ml of a Tegosept (methyl-p-hydroxybenzoate) stock solution. Fifty g of Tegosept was dissolved in 500 ml of 95% ethanol for the Tegosept stock solution. This recipe was adapted from that of the Cold Spring Harbor Protocols (doi:10.1101/pdb.rec081414). The density of larvae was controlled, and we removed the first-instar larvae from the vials so that each bottle contained c. 140 larvae in either the predator odor group or the mechanical stress group. This larval density can be considered as being between low and average density (Bierbaum 1989).

The bottles with eggs were placed horizontally on the floor of net-covered plastic jars (10 cm height × 12 cm diameter). In the predator odor group, each jar contained a filter paper disc with the odors of *Phidippus apacheanus* jumping spider. To prepare the odor discs, we placed one spider in one closed Petri dish (Ø 37 mm) containing a filter paper disc (Ø 35 mm) for 7 h. This represents a reliable method of collecting insect odors (e.g. Pölkki et al. 2012; Krams et al. 2015). To ensure the effect of spider odors, we always used two odor discs per each net-covered plastic jar. The discs were replaced by unused ones every 7.5 hours and removed when larvae started to pupate. In the predator group, each jar contained a jumping spider. The spiders often walked into the bottles, and all of them were observed consuming the *D. melanogaster* larvae. The predators were removed from the jars
at the beginning of the pupation phase. In total, we had 10 experimental jars where *D. melanogaster* larvae were reared with spiders, 12 jars containing filtering paper disc with spider odors, and 12 control jars containing no spiders and no spider odors. The spiders were collected in Florida, USA, and were received from the phids.net supplier.

The adult flies were collected within 5-7 hours after imaginal eclosion. The flies were frozen at -80 °C and dried at 75 °C for 72 hours on the next day after being frozen. We weighed the flies as groups of 10 individuals (Krams et al. 2016, 2020) using Sartorius MC5 (accuracy ±1μg) microanalytical balances. The females and males were collected, dried, and weighed separately. The dry body mass was calculated for individual flies as the dry mass of each replicate, divided by the number of flies assigned in each replicate.

**Fruit fly body C and N content**
The percentage of C and N content was measured from the mass of whole flies using an element analyzer EuroVector EA3000 (Eurovector Srl, Pavia, Italy). Samples of C and N concentrations were measured as groups of 10 fruit flies, representing each vial equally. In total, we measured 100 males and 100 females in the odor stress group, 100 males and 100 females in the predator stress group, and 120 males and 120 females in the control group. In total, there were 640 flies measured across all four groups.

**Statistics**
We used two-factor analysis of variance (two-way ANOVA) to assess the influence of treatment and sex of the fruit flies on the fly body mass, body C and N composition, and the ratio of C and N. We reported only the main effects if no treatment or interaction effect among treatment and sex were found. Otherwise, we also reported Tukey’s honest significance (Tukey HSD) results to compare differences between groups. We used Levene’s test to assess heterogeneity of variances. We applied natural log transformation to individual body mass data to reduce heteroscedasticity. We considered the differences as statistically significant at *P* < 0.05 in all tests. We performed the analyses in R, version 3.6.2 (R Core Team, 2019) and used dplyr (Wickham et al. 2019) and Rmisc (Hope 2013) packages to filter and summarize data. We also used ggplot2 (Wickham 2016) and ggsignif (Ahlmann-Eltze 2019) for data visualization.
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