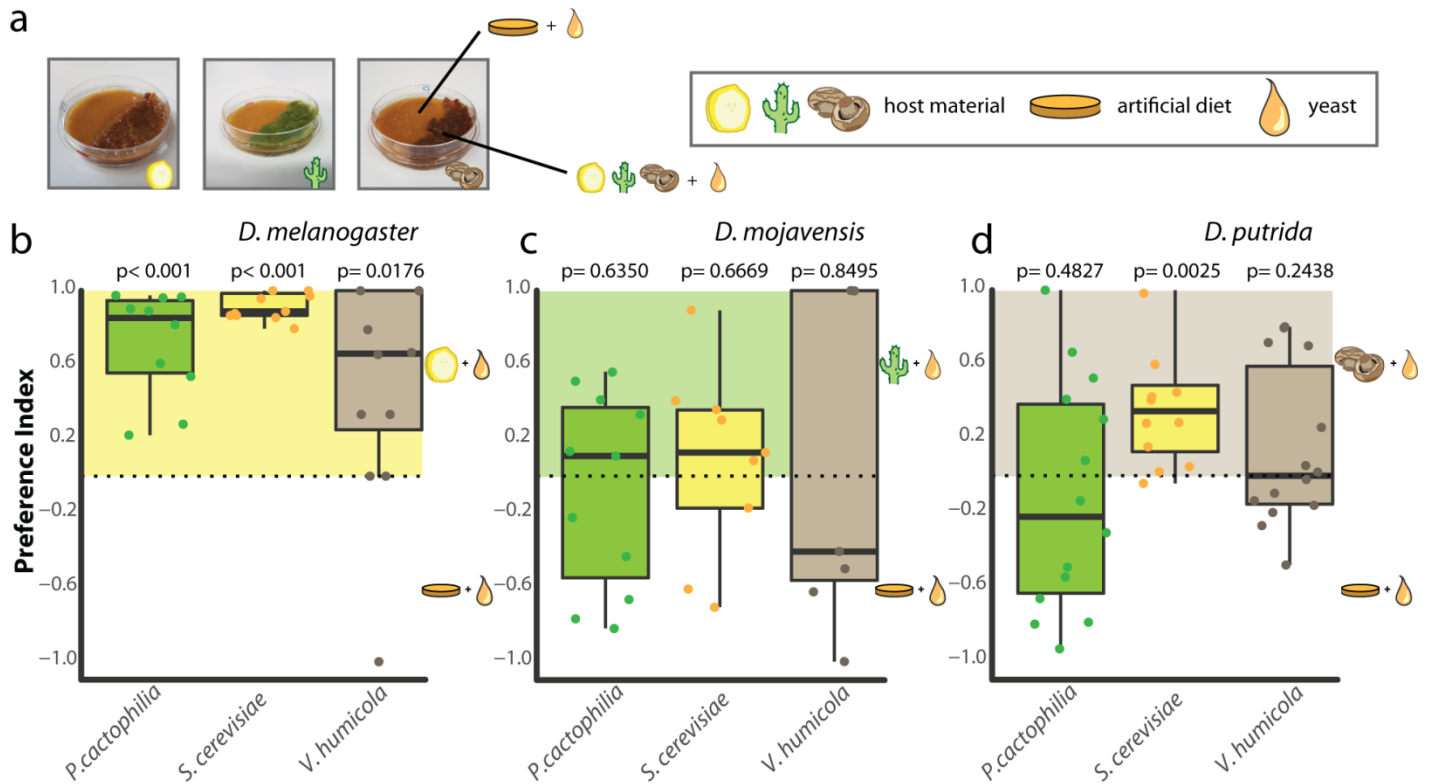


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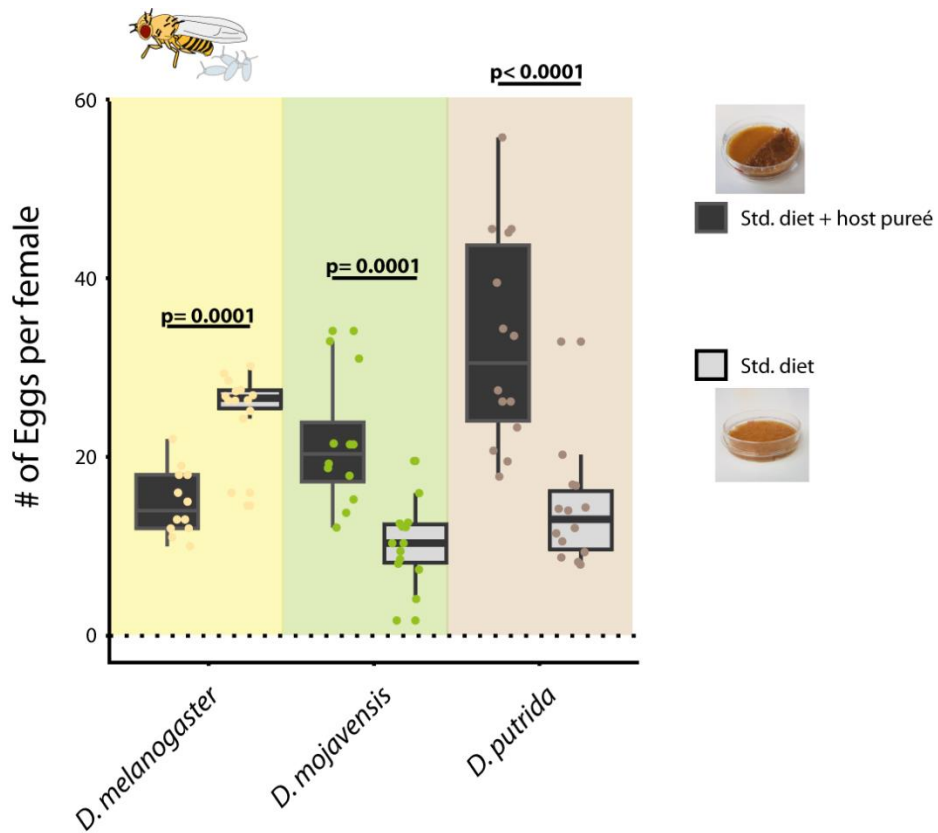
**OIK-07180**

Koerte, S., Keeseey, I. W., Easson, M. L. A. E., Gershenzon, J., Hansson, B. S. and Knaden, M. 2020. Variable dependency on associated yeast communities influences host range in *Drosophila* species. – Oikos doi: 10.1111/oik.07180

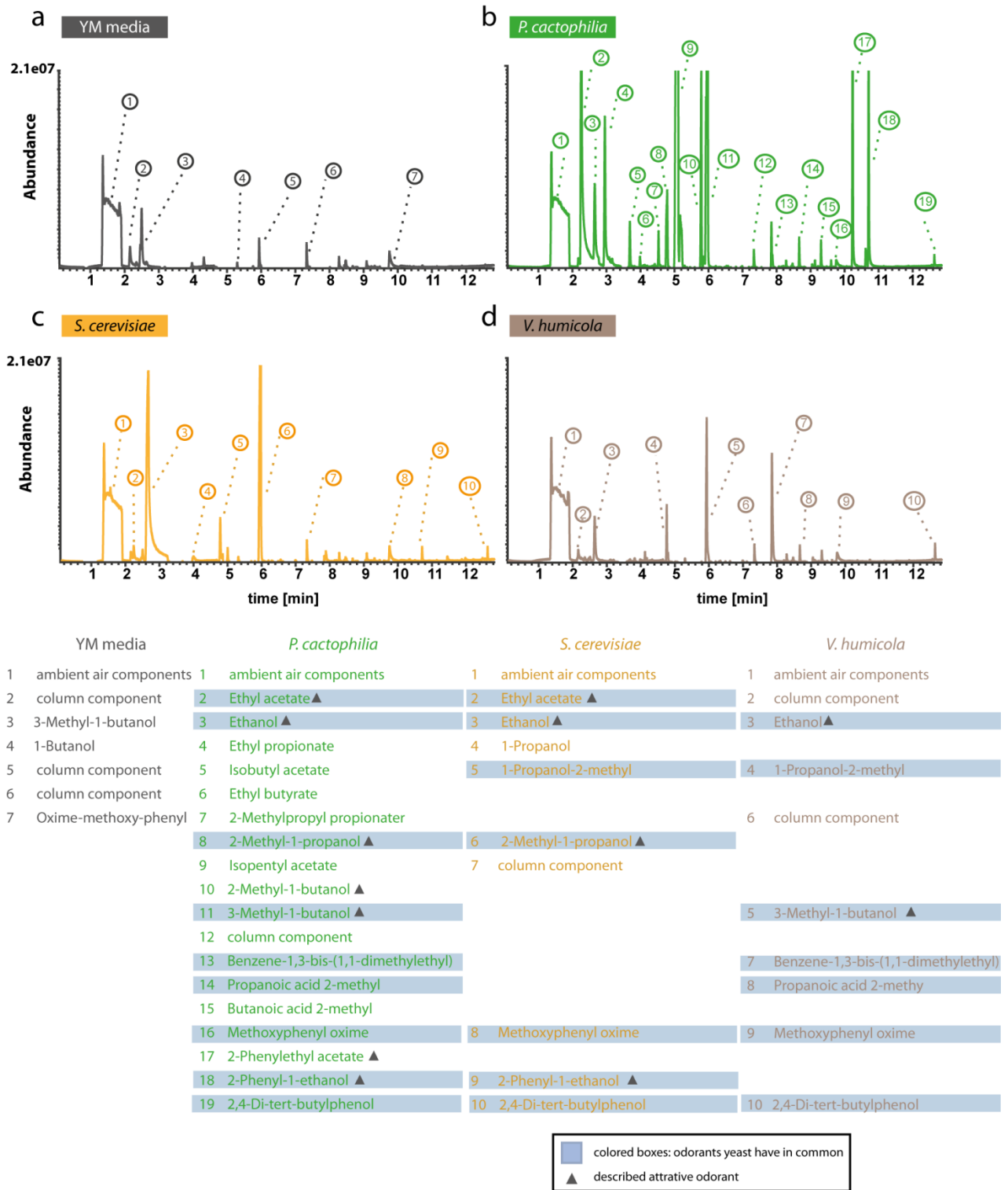
Appendix 1



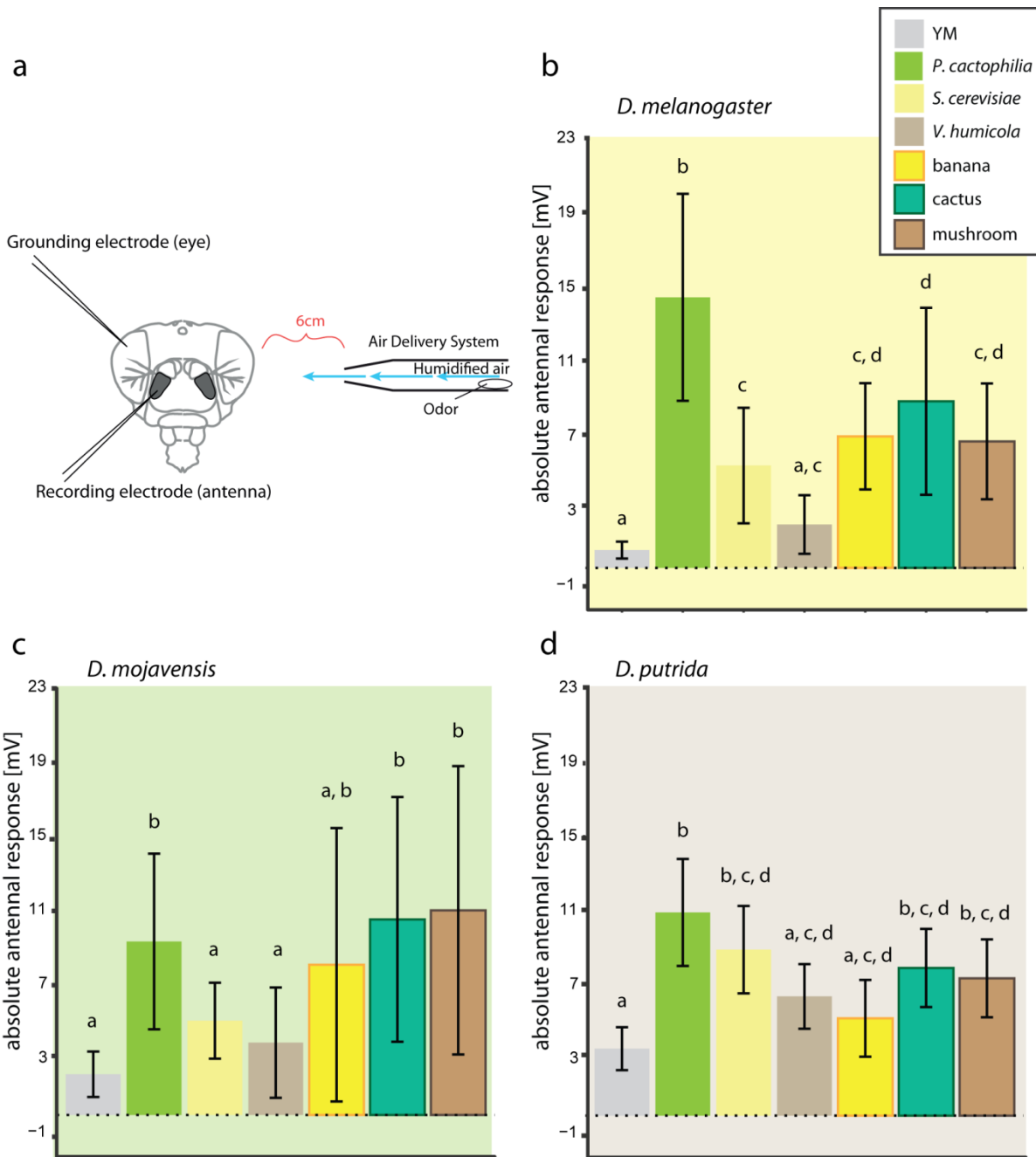
**Figure A1. Preference of *Drosophila* species to oviposit on artificial diet or host material inoculated with different yeast species.** Distribution of eggs on oviposition plates from oviposition assays with host material vs artificial diet (Preference Index=(Number of eggs on host material-number of eggs on artificial diet)/number of eggs total). For each *Drosophila* species the PI of six to 14 oviposition plates per yeast species was calculated. p-values for the statistical analyses of the data are stated above the individual box plots (two tailed One-sample t-test; ns  $p > 0.05$ ). Box plots show the median (bold horizontal lines) and whiskers the interquartile range.



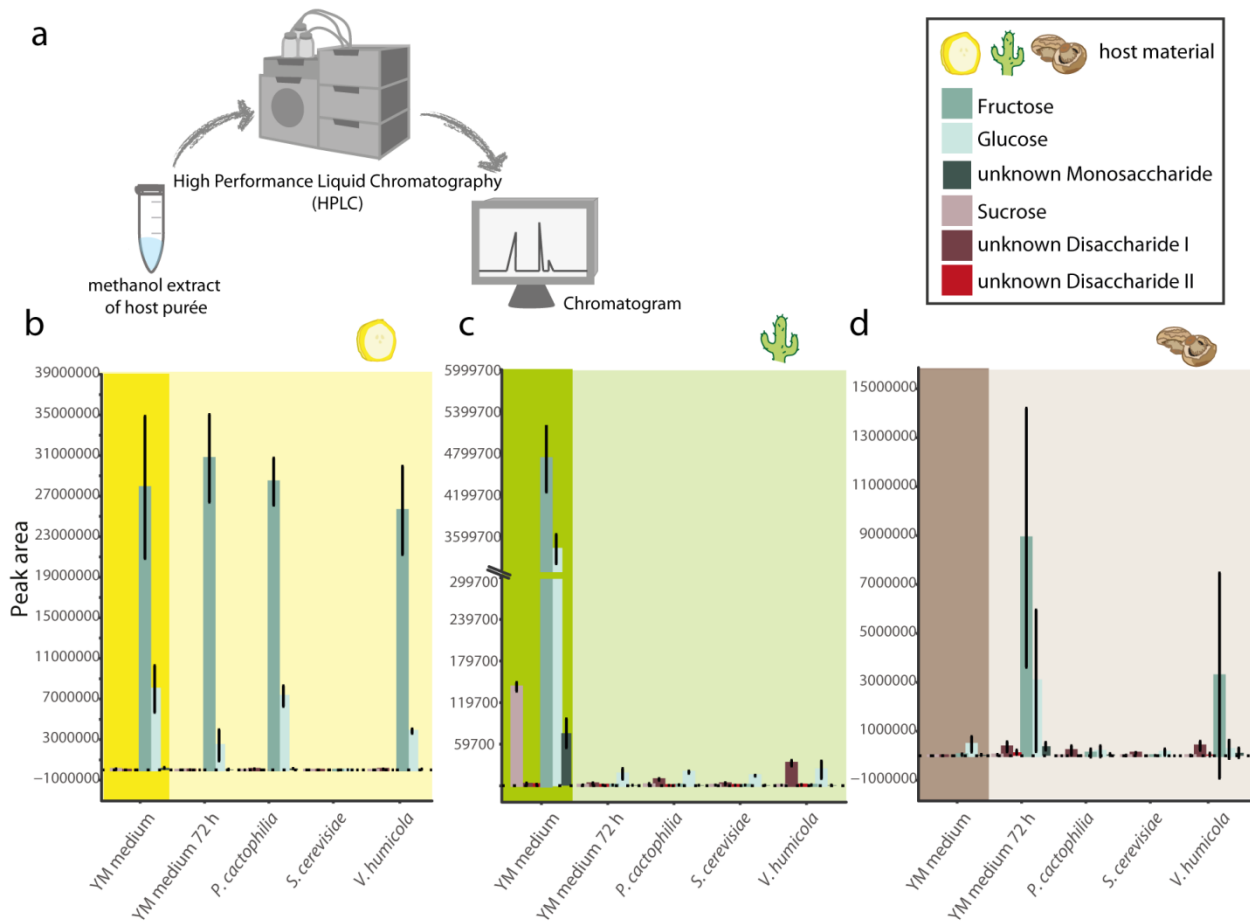
**Figure A2. Comparison of egg numbers on either artificial diet or host material both inoculated with yeast.** For each *Drosophila* species the data from the oviposition assays in Fig. 2 and 3 was compared for the numbers of eggs per female. Box plots show the median (bold horizontal lines) and whiskers the interquartile range. p-values for the statistical analyses of the data are stated above the individual box plots (*D. melanogaster* & *D. mojavensis*: two-tailed Mann-Whitney test, *D. putrida*: unpaired t-test with Welch correction; n=12-14).



**Figure A3. Analysis of the volatile compounds found in the headspace of the three target yeast species.** (A-D) Representative GC-MS chromatograms of the headspace collected over 30 min from glass vials containing either 5 mL of pure yeast malt (YM) liquid medium or 5 ml of indicated yeast cultures grown in YM medium. A color-coded identity of numbered peaks is given under the chromatograms for each treatment. Colored boxes highlight odorants found in all or minimum two yeast species. Triangles highlight described attractive odorants for *D. melanogaster*.



**Figure A4. Electroantennogram recordings from *Drosophila* species towards the headspace of their host material or different yeast cultures.** (A) Schematic drawing of the experimental setup for the recording of electroantennograms (EAGs). (B-D) EAG responses to the different odors normalized to the smallest response. Bar plots represent mean antennal response in mV towards different stimuli (n=7-8), while error bars indicate standard deviation. Letters above the box plots indicate significant differences (Repeated measures ANOVA followed by Tukey-Kramer multiple comparison post hoc test).



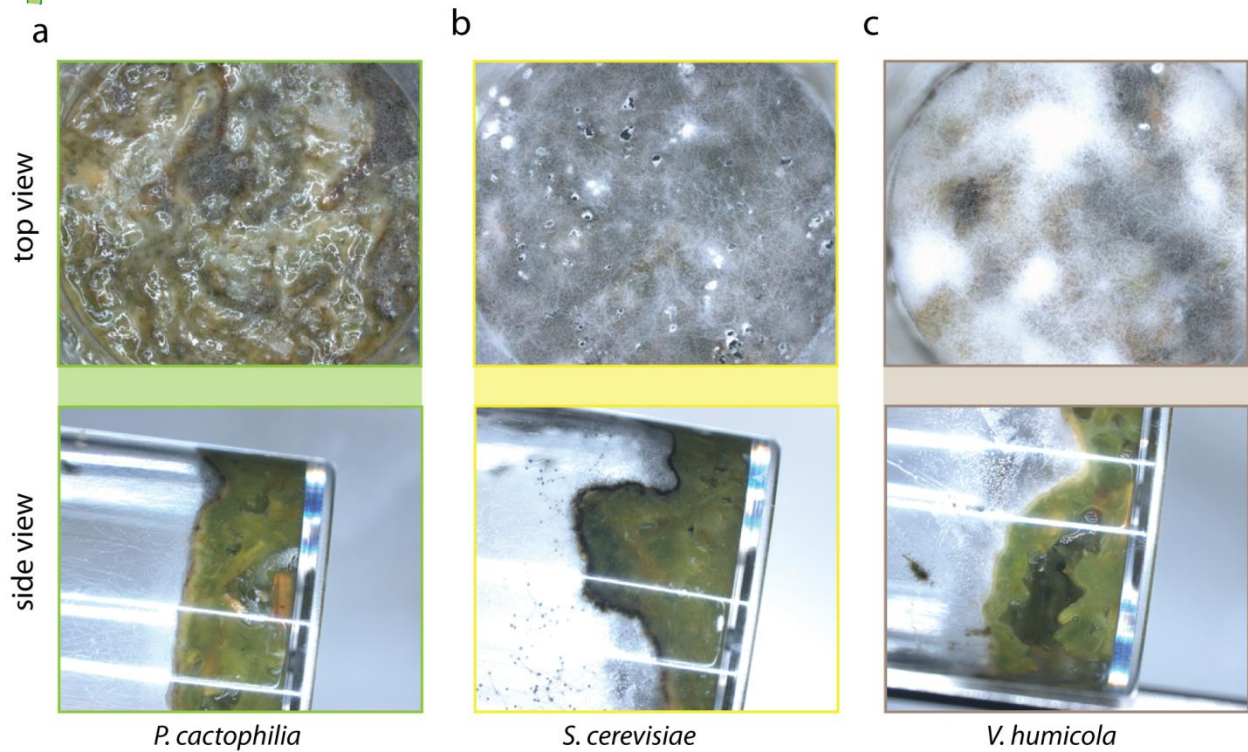
**Figure A5. Performance of yeast species on the host material of different *Drosophila* species** (A-D) Abundance of selected sugars found in *Drosophila* host material after inoculation with different yeast species. Homogenized host material of different *Drosophila* species was inoculated with target yeast species or mixed with YM medium and incubated for 72 h at 25°C. Bar plots show the mean peak area of indicated sugars in methanol extracts from host purée inoculated with depicted yeast species or supplemented with growth medium run as HPLC. All samples were run in triplicate. The error bars represent the standard deviation from the mean peak area of the corresponding sugar.



**Figure A6. Comparison between host decay of samples without and with *Drosophila* males**  
*Drosophila* males were fed 24 h prior to the start of the assay with our selected yeast species. Since we did not find a visible difference in the rate of decay between the three yeast species, we generally compared the progress of host decay from samples with flies to controls without flies. (A-C) Representative images of the decomposition progress of banana (A), cactus (B) and mushroom (C) samples without and with 20 *Drosophila* males which were fed with our target yeast species. The *Drosophila* species used for the assays were as follows: Banana- *D. melanogaster*, cactus- *D. mojavensis* and mushroom- *D. putrida*.



cactus purée after 7d of incubation



**Figure A7. Effects of yeast presence on the growth of filamentous fungi on cactus purée.** Cactus purée was inoculated with 400  $\mu$ l of yeast pre-culture at an  $OD_{600}$  of 2 and incubated at room temperature. (A-C) Representative images of cactus purée seven days after inoculation with three different yeast species; yeast species are indicated at the bottom of the images.

**Table A1.** Recipe for standard diet.

Ingredient	per 500 ml
Molasses	59 g in 101 ml hot water
Brewer's yeast	5.4 g
Agar	2.1 g in 135 ml cold water
Polenta	47 g in 169 ml hot water
Propionic acid	1.2 mL in 54 ml cold water
Nipagain (30 %)	1.65 ml

**Table A2.** Tritrophic interaction partners.

<i>Drosophila</i> species	associated yeast	host
<i>D. melanogaster</i>	<i>S. cerevisiae</i>	Fermenting fruits
<i>D. mojavensis</i>	<i>P. cactophila</i>	Cactus (pitaya agria, organ pipe cactus and at times cina cactus as well as barrel and prickly pear cactus)
<i>D. putrida</i>	<i>V. humicola</i>	Mushrooms (Agaricaceae, Amantiaceae, Baletaceae, Russulaceae and Tricholmataceae)

**Table A3.** Outline of the schedule for the multiple reaction monitoring (MRM) detection of sugars in host material with and without yeast inoculation; DP (de-clustering potential), EP (entrance potential), CE (collision energy), CXP (cell exit potential).

Sugar type	Retention time [min]	Precursor Ion [m/z]	Product Ion [m/z]	DP [V]	EP [V]	CE [V]	CXP [V]
Fructose	5.3	178.80	89.00	-50	-9.5	-10	0
Glucose	6.2	178.80	89.00	-50	-9.5	-10	0
Unknown Monosaccharide	6.15	148.98	88.92	-50	-8.5	-10	-2
Sucrose	7.55	340.90	59.00	-65	-10	-46	0
Unknown Disaccharide I	8.28	340.90	59.00	-65	-10	-46	0
Unknown Disaccharide II	8.7	340.90	59.00	-65	-10	-46	0

**Table A4.** Comparison of total egg numbers laid on diet containing *V. humicola*.

Diet	<i>Drosophila species</i>				Statistical test
		<i>D. melanogaster</i>	<i>D. mojavensis</i>	<i>D. putrida</i>	
Modified Std. diet plus yeast (Figure 2 C-D)	<i>D. melanogaster</i>	x	Not significant	p<0.01	Kruskal-Wallis-test
	<i>D. mojavensis</i>	Not significant	x	p<0.001	
	<i>D. putrida</i>	p<0.01	p<0.001	x	
Modified Std. diet, host purée and yeast (Figure 3)	<i>D. melanogaster</i>	x	Not significant	p<0.0001	Kruskal-Wallis-test
	<i>D. mojavensis</i>	Not significant	x	p<0.001	
	<i>D. putrida</i>	p<0.0001	p<0.001	x	