

Supplemental Material

Abbreviations

DF	degrees of freedom
EPS	equal protein sugar ratio
GP	glycerol phosphate
HPS	high protein sugar ratio
LPS	low protein sugar ratio
m/z	mass to charge ratio
MetS	metabolic syndrome
MS	mass spectrometry
OOB	out of bag error
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PHC	phosphocholine
PHI	cyclic phosphate inositol
PI	phosphatidylinositol
PS	phosphatidylserine
RF	random forest
RT	retention time
SS	sum of squares
UPLC	ultra-performance liquid chromatography

Table S1 Description of the diets used (Matzkin *et. al.* 2011)

	HPS	EPS	LPS
Sucrose, g	8	20	32
Active dry yeast, g	32	20	8
Yellow cornmeal, g	9	9	9
Distilled H ₂ O, mL	200	200	200
Granulated agar, g	1	1	1
Absolute ethanol, mL	4.5	4.5	4.5
Methyl paraben, g	0.45	0.45	0.45
Total protein:carbohydrate	0.43	0.20	0.10
Calories, kJ/100g	452	456	469

EPS equal protein sugar ratio
HPS high protein sugar ratio
LPS low protein sugar ratio

Table S2 ANOVA development time, n=10 (HPS, EPS and LPS) for *D. melanogaster*. n=10 (HPS, EPS) and n=9 (LPS) for *D. arizonae*. n=10 (HPS and EPS) for *D. mojavensis*.

Species	Source	DF	SS	F value ¹
<i>D. melanogaster</i>	diet	2	0.46667	2.52
	residuals	27	2.500	
<i>D. arizonae</i>	diet	2	25.940	9.387***
	residuals	26	35.92	
<i>D. mojavensis</i>	diet	1	3.2	5.434*
	residuals	18	10.6	

DF degrees of freedom

SS sum of squares

¹ *** $P < 0.001$, * $P < 0.05$

Table S3 Tukey multiple comparisons of *D. arizonae* development time means with diet as dependent variable.

Comparison	Mean difference	Lower bound	Upper bound	Significance ¹
HPS-EPS	-0.5	-1.8062	0.8062	0.6135
LPS-EPS	1.7444	0.4024	3.0865	0.0091*
LPS-HPS	2.2444	0.9024	3.5865	0.0009***

¹ *** $P < 0.001$, * $P < 0.05$

Table S4 ANOVA dry weight, n=5 (HPS, EPS and LPS) for *D. melanogaster* females and males. n=5 (HPS, EPS and LPS) for *D. arizonae* females, n=5 (HPS), n=6 (EPS) and n=2 (LPS) for *D. arizonae* males. n=5 (HPS and EPS) for *D. mojavensis* females and males.

Species	Source	DF	SS	F value ¹
<i>D. melanogaster</i>	Diet	2	0.001647	3.1667
	Sex	1	0.05547	213.3462***
	Diet:Sex	2	0.00254	4.8846*
	Residuals	24	0.00624	
<i>D. arizonae</i>	Diet	2	0.029679	12.9653***
	Sex	1	0.065722	57.4207***
	Diet:Sex	2	0.002063	0.9012
	Residuals	22	0.02518	
<i>D. mojavensis</i>	Diet	1	0.0095703	20.5038***
	Sex	1	0.0200933	43.0487***
	Diet:Sex	1	0.0013203	2.8287
	Residuals	16	0.0074681	

¹ *** $P < 0.001$, * $P < 0.05$

Table S5 Tukey multiple comparisons of *D. arizonae* dry weight means with diet as dependent variable.

Comparison	Mean difference	Lower bound	Upper bound	Significance ¹
HPS-EPS	-0.0573	-0.0944	-0.0202	0.0023*
LPS-EPS	-0.0761	-0.1171	-0.0350	0.0004***
LPS-HPS	-0.0188	-0.0606	0.0231	0.5091

¹ *** $P < 0.001$, * $P < 0.05$

Table S6 Data sets summary of *Drosophila* species and RF models targeted to Diet with complete data sets for variable importance estimation.

Species	EPS/HPS replicates	Female/male replicates	OOB [%]
<i>D. melanogaster</i>	6/5	5/6	45.5
<i>D. arizonae</i>	6/7	8/5	30.8
<i>D. mojavensis</i>	6/8	7/7	7.1

OOB out of bag error

Table S7 Random forest models targeted to sex with left-one-out cross validation for *Drosophila* species.

Species	OOB [%]	Accuracy
<i>D. melanogaster</i>	16.4 ± 3.1	81.8
<i>D. arizonae</i>	42.3 ± 4.5	38.5
<i>D. mojavensis</i>	44.0 ± 2.3	57.1

Table S8 MS2 data dependent analysis. Fragments and neutral losses in positive and negative ion mode of *Drosophila* species extracts. MS-Dial parameters: The retention time range was from evaluated from 0.1 to 54 minutes and the mass range between 50 and 900 Da. MS1 and MS2 tolerance were set to 0.35 Da. Peak detection parameters were: linear weighted moving average for smoothing, 1 scan for the smoothing level, 5 scans for the minimum peak width, and 100 as minimum peak height. 0.32 Da for-mass slice width was set as peak spotting parameter. Alignment results were exported to a matrix, with MS/MS included. Fragments were revised manually in Excel (Tsugawa *et. al.*, 2015).

<i>m/z</i>	RT [min]	Aduct	[X-141] ⁺	[X-59] ⁺	[PHC] ⁺	[PHC-H ₂ O] ⁻	[GP-H ₂ O-H] ⁻	[PHI] ⁻	Class
340.31	11.18	[X+NH ₄] ⁺	Yes	No	No	No	No	No	PE
491.03	24.08	[X+H] ⁺	No	Yes	No	No	No	No	PC
426.86	24.31	[X+H] ⁺	Yes	No	No	No	No	No	PE
569.67	24.85	[X-H] ⁻	No	No	No	No	No	Yes	PI
468.49	25.03	[X+H] ⁺	No	No	Yes	No	No	No	PC
517.07	25.36	[X+H] ⁺	No	Yes	No	No	No	No	PC
495.22	25.36	[X+H] ⁺	No	No	Yes	No	No	No	PC
451.60	25.52	[X+H] ⁺	Yes	No	No	No	No	No	PE
*452.08	26.22	[X+H] ⁺	Yes	No	No	No	No	No	PE
517.30	26.30	[X+H] ⁺	No	Yes	No	No	No	No	PC
495.40	26.30	[X+H] ⁺	No	No	Yes	No	No	No	PC
*453.82	26.32	[X+H] ⁺	Yes	No	No	No	No	No	PE
520.33	26.33	[X-H] ⁻	No	No	No	No	Yes	No	PE, PI, PS, PC
516.93	26.35	[X+H] ⁺	No	Yes	No	No	No	No	PC
494.89	26.40	[X+H] ⁺	No	No	Yes	No	No	No	PC
595.56	26.67	[X-H] ⁻	No	No	No	No	No	Yes	PI
521.11	27.16	[X+H] ⁺	No	No	Yes	No	No	No	PC
543.11	27.46	[X+H] ⁺	No	Yes	No	No	No	No	PC
521.43	28.12	[X+H] ⁺	No	No	Yes	No	No	No	PC
543.40	28.14	[X+H] ⁺	No	Yes	No	No	No	No	PC
571.53	28.16	[X-H] ⁻	No	No	No	No	No	Yes	PI
454.80	28.53	[X+H] ⁺	Yes	No	No	No	No	No	PE
496.66	28.62	[X+H] ⁺	No	No	Yes	No	No	No	PC
497.71	29.30	[X+H] ⁺	No	No	Yes	No	No	No	PC

519.73	29.40	[X+H]+	No	Yes	No	No	No	No	PC
597.73	29.49	[X-H]-	No	No	No	No	No	Yes	PI
519.28	29.59	[X+H]+	No	Yes	No	No	No	No	PC
545.23	29.86	[X+H]+	No	Yes	No	No	No	No	PC
523.11	29.87	[X+H]+	No	No	Yes	No	No	No	PC
524.97	29.89	[X+H]+	No	No	Yes	No	No	No	PC
502.88	29.90	[X+H]+	Yes	No	No	No	No	No	PE
503.12	29.91	[X+H]+	Yes	No	No	No	No	No	PE
522.44	30.14	[X-H]-	No	No	No	No	Yes	No	PE, PI, PS, PC
524.26	30.23	[X+H]+	No	No	Yes	No	No	No	PC
480.23	30.28	[X+H]+	Yes	No	No	No	No	No	PE
523.50	30.65	[X+H]+	No	No	Yes	No	No	No	PC
482.11	30.65	[X+H]+	Yes	No	No	No	No	No	PE
525.27	30.68	[X+H]+	No	No	Yes	No	No	No	PC
545.67	30.83	[X+H]+	No	Yes	No	No	No	No	PC
392.55	35.10	[X+H]+	No	No	No	Yes	No	No	PC
655.47	44.26	[X+H]+	Yes	No	No	No	No	No	PE
701.90	44.63	[X+H]+	No	Yes	No	No	No	No	PC
685.46	44.73	[X+H]+	Yes	No	No	No	No	No	PE
685.01	44.96	[X+H]+	Yes	No	No	No	No	No	PE
728.53	44.99	[X+H]+	No	Yes	No	No	No	No	PC
711.10	45.22	[X+H]+	Yes	No	No	No	No	No	PE
737.21	45.74	[X+H]+	Yes	No	No	No	No	No	PE
779.14	45.99	[X+H]+	No	Yes	No	No	No	No	PC
692.14	46.43	[X+H]+	Yes	No	No	No	No	No	PE
713.15	46.48	[X+H]+	Yes	No	No	No	No	No	PE
755.18	46.77	[X+H]+	No	Yes	No	No	No	No	PC
*717.68	46.93	[X+H]+	Yes	No	No	No	No	No	PE
*716.09	47.03	[X+H]+	Yes	No	No	No	No	No	PE
781.30	47.22	[X+H]+	No	Yes	No	No	No	No	PC

*782.68	47.23	[X+H] ⁺	No	Yes	No	No	No	No	PC
742.10	47.26	[X+H] ⁺	Yes	No	No	No	No	No	PE
742.43	47.43	[X+H] ⁺	Yes	No	No	No	No	No	PE
743.67	47.66	[X+H] ⁺	Yes	No	No	No	No	No	PE
689.05	47.82	[X+H] ⁺	Yes	No	No	No	No	No	PE
689.91	47.93	[X+H] ⁺	Yes	No	No	No	No	No	PE
690.54	47.93	[X+H] ⁺	Yes	No	No	No	No	No	PE
741.92	48.09	[X+H] ⁺	Yes	No	No	No	No	No	PE
719.95	48.10	[X+H] ⁺	Yes	No	No	No	No	No	PE
745.84	48.25	[X+H] ⁺	Yes	No	No	No	No	No	PE
745.51	48.26	[X+H] ⁺	Yes	No	No	No	No	No	PE
746.45	48.34	[X+H] ⁺	Yes	No	No	No	No	No	PE
783.17	48.36	[X+H] ⁺	No	Yes	No	No	No	No	PC
809.34	48.68	[X+H] ⁺	No	Yes	No	No	No	No	PC

GP glycerol phosphate

PC phosphatidylcholine

PE phosphatidylethanolamine

PHC phosphocholine

PHI cyclic phosphate inositol

PI phosphatidylinositol

PS phosphatidylserine

(Brügger *et al.*, 1997; Milne *et al.*, 2006; Ivanova *et al.*, 2009)

References for the Supplemental section

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