Metabolomics

PLOS Genetics

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Cage Studies

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Aw WC, Towarnicki SG, Melvin RG, Youngson NA, Garvin MR, Hu Y, Nielsen S, Thomas T, Pickford R, Bustamante S, Vila-Sanjurjo A, Smyth GK, Ballard JWO (2018) Genotype to phenotype: Diet-by-mitochondrial DNA haplotype interactions drive metabolic flexibility and organismal fitness. PLoS Genet 14(11): e1007735. doi: 10.1371/journal.pgen.1007735

PROTOCOL STATUS

Working

1 Female third instar wandering larvae were weighed, and methanol was added to make up each sample to 20 mg/ ml.

2 Metabolites were extracted using an ultrasonic probe (30 s), 1 h incubation at 4Â° C and then centrifugation to remove particulates

3 100 Âµl aliquots of the supernatant were derivatised [200] before mass spectrometric interrogation with an Agilent GC/MSD system (Agilent Technologies, CA, USA) controlled by Chemstation software.

4 The GC inlet temperature was set to 230Â° C. 1.0 Âµl of derivatised sample was injected in splitless mode, using helium as a carrier gas at constant-flow of 1.0 ml/ min.

5 Chromatographic separation was performed on a 30 m SH-RXi-5Sil MS column (Shimadzu, NSW, Australia) with 0.25 mm internal diameter and 0.25 Âµm film thickness.

6 The oven temperature was programmed at 70Â° C for 2 min, then ramped at 15Â° C/ min to 320Â° C, and held 8 min.

7 Electron ionisation mass spectra were recorded at 1.4 scans/ s over the range *m/z* 50â€“700. The MSD auxiliary temperature, source temperature, and quadrupole temperature were set to 280Â° C, 230Â° C, and 150Â° C, respectively.

8 Analytes were identified using the NIST 2011-Wiley Mass Spectra Library. GC peaks containing mass spectra with a match quality (spectral purity) of more than 70% were considered to be tentatively identified.

9 No internal standard was employed. Normalisation was performed by maintaining a constant sample mass per volume.

10 Peak areas were log-transformed and statistical analysis used the limma package. Benjamini-Hochbergâ€™s correction was used to control the FDR.