Population Cage studies including four mitotype

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 Placing about 210 eggs from each fly line onto instant *Drosophila* media (Carolina Biological Supply Company, NC, USA) in bottles.

2 Bottles, each with a different mitotype, were placed into population cages (22 cm x 21 cm x 36 cm) such that there were ~850 flies/cage.

3 To establish a homogeneous gut flora each generation, four males from each mitotype, raised on instant food unless otherwise stated, were ground in distilled water and 130 ÂµL of the homogenate aliquoted into each bottle.

4 On the first day of eclosion (adult emerging from a pupal case), plugs were removed from bottles and flies were released into population cages for 3 d.

5 Bottles were removed and oviposition resources (yeast placed on top of the solidified agar-based medium containing 4% agar and 10% treacle) were put in cages and eggs were collected from 3-5 d old adult females.

6 Surface sterilisation of eggs was achieved by washing in dilute bleach, and ~200 eggs were then placed on each diet.

7 This protocol was then repeated for each generation. Following oviposition, adult flies were frozen.Â

8 The frequency of adult females harbouring the distinct mitotypes was individually determined by sequencing and allele-specific PCR of 95 individual females from each cage.

9 For the initial studies, DNA was extracted, and a ~900bp region of mtDNA amplified using the ND4L forward 5â€™-TAAACAAACTAATCTAACTAATA-3â€™ and reverse 3â€™-GGTTGTGATATATTATCTTATGG-5â€™ primer and Sanger sequenced.

10 Chromatograms were imported into Sequencher 4.5 (Gene Codes, MI, USA) and the proportion of each mitotype/ diet/ generation determined.