Data from: Meunier et al, 2017. Multilevel selection in a filamentous ascomycete, N. tetrasperma

Files are labelled according of being R scripts, or Data. In each script is indicated which data file to use.

1. **Analyses of conditions impacting nuclear ratios**

NuclearRatios\_Data.csv and NuclearRatios\_Rscripts

To derive main figures 2 and 3.

Columns of the data file are labelled ad follows :

-Exp: type of inoculum, either conidia (105 in inoculum) or spores (1 per plate)

-Obs.Ratio: the observed ratio as a proportion of mat A nuclei, determined by qPCR, either on DNA extracted from growing mycelium, or on DNA extracted for conidia mixes.

-Lineage: lineage used in the experiment. 3 levels : L1, L6 or L10.

-Initial.Cndn : initial nuclear ratio in the inoculum. 4 levels : R1 is 90% matA, R2 is 50% matA and R3 is 10% matA. For spores inoculum, it reads R2-spores because spores pass through a 50-50% stage.

-Type: 2 levels : rep or inoc. To distinguish between qPCR performed on growing mycelium (rep) or on conidia inoculum (inoc).

-Medium: growth medium (see Methods in MS). 2 different growth media, code for inocula is Zinoc (for plotting purposes)

-Time.Point : harvesting time point, 2 different duration of growth T1 and T2 (see main text). T0 are inocula.

-Strain: details on the strains used in the experiment

The Rscript gives the code to get the figures and the statistical analyses.

1. **Comparison of nuclear ratios in conidia and mycelium** NR\_Conidia\_Mycelium\_Data.csv and NRConidiaVsMycelium\_Rscript

To derive Fig.4.

See Methods for details on the experiment. Nuclear ratios were compared between mycelium and conidia growing on the same plate.

Columns of the datafile are labeled as follows:

-Sample: sample code

-Obs.Ratio: estimated nuclear ratio (proportion of matA) in the growing mycelium (no conidia) or in the conidia (no mycelium).

-Con.Myc: type of saple, 2 levels, con=conidia and myc=mycelium.

-Plate: code to distinguish all plates.

-Lineage: lineages used in the experiment, 3 levels: L1, L6, L10

> summary(data)

Sample Obs.Ratio Con.Myc Plate Lineage

L1 con1.1: 1 Min. :0.4000 con:45 L6P3 :10 L1 :27

L1 con1.2: 1 1st Qu.:0.4700 myc:37 L10P1 : 9 L10:27

L1 con1.3: 1 Median :0.5400 L10P2 : 9 L6 :28

L1 con1.4: 1 Mean :0.5593 L10P3 : 9

L1 con1.5: 1 3rd Qu.:0.6300 L1P1 : 9

L1 con2.1: 1 Max. :0.8300 L1P2 : 9

(Other) :76 (Other):27

The Rscript gives the code to get the figure (Fig4) and the statistical analyses.

1. **Vegetative fitness: conidia yield**

Fitness\_Conidia\_Data.csv and Fitness\_Conidia\_Rscript

To derive Fig.5. See main text for details on the methods.

Columns of the datafile are labeled as follows:

-Lineage: lineages used in the experiment, 3 levels: L1, L6, L10

-Het.Hom: heterokaryon (Het) or homokaryons (Hom)

-Category: 5 levels: BA=homokaryons matA ,R1=heterokaryon with initial matA of 90%, R2= heterokaryon with initial matA of 50%, R3=heterokaryon with initial matA of 10%, sa=homokaryons mat a

-Conid.Count: raw count of conidia

-Dil=dilution at which counts were done

-factor accounting for further dilution

-Log.Total.Count=log(Conid.Count\*dilution\*times): the variable to work with.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Lineage | Het. Hom | Category | Rep | Conid. Count | Dil | times | Log.Total.Count |
| L1 :100 | Het: 150 | BA:50 | A.10:3 | Min.: 2.760 | Min.: 2.0 | Min. :1e+05 | Min. :5.742 |
| L10: 90 | Hom: 100 | R1:50 | A.11: 3 | 1st Qu.: 7.503 | 1st Qu.: 2.0 | 1st Qu.:1e+05 | 1st Qu.:6.422 |
| L6 : 60 |  | R2:50 | A.12: 3 | Median: 10.700 | Median :20.0 | Median :1e+05 | Median :7.227 |
|  |  | R3:50 | A.3: 3 | Mean: 10.835 | Mean :13.4 | Mean :1e+05 | Mean :6.932 |
|  |  | sa:50 | A.4: 3 | 3rd Qu.: 13.400 | 3rd Qu: 20.0 | 3rd Qu.:1e+05 | 3rd Qu.:7.368 |
|  |  |  | A.5: 3 | Max.: 32.400 | Max. :20.0 | Max. :1e+05 | Max. :7.640 |
|  |  |  | (Other):232 |  |  |  |  |

The Rscript gives the code to get the figure (Fig5) and the statistical analyses.

1. **Reproductive Fitness**

FitnessReproAscopores\_Data.csv / FitnessReproPeritheciaSize\_Data.csv and FitnessRepro\_Rscript

Underlying Fig 6A and 6B.

* 1. Ascospores

Datafile FitnessReproAscospores\_Data.csv

Guide through the column labels:

-Lineage: lineages used in the experiment, 3 levels: L1, L6, L10

-Ratio: 5 levels: BA=homokaryons matA ,R1=heterokaryon with initial matA of 90%, R2= heterokaryon with initial matA of 50%, R3=heterokaryon with initial matA of 10%, sa=homokaryons mat a

-Het.Hom: 2 levels, Hom=homokaryons, Het=heterokaryon

-Tube: code for tube number (one growing individual)

-Ech: code for ascospore sample (5 samples per tube)

-Count: raw count of ascospore number

-Dil:dilution before counting

-Conc: concentration=Count/Dil

The Rscript (part1) gives the code to get the figure (Fig6B and I) and the statistical analyses.

* 1. Protoperithecia size

Datafile FitnessReproPeritheciaSize\_Data.csv

Guide through the column labels:

-Lineage: lineages used in the experiment, 3 levels: L1, L6, L10

-Ratio: 5 levels: BA=homokaryons matA ,R1=heterokaryon with initial matA of 90%, R2= heterokaryon with initial matA of 50%, R3=heterokaryon with initial matA of 10%, sa=homokaryons mat a

-Tube: code for tube number (one growing individual, 10 measures per tube)

-Size of protoperithecia: measures in arbitrarian units

-Date: date of measures

The Rscript (part2) gives the code to get the figure (Fig6A) and the statistical analyses.

1. Sectoring experiment

Sectoring\_Data and Sectoring\_Rscript

Underlying Table1, Fig D1 and F2. See main text for explanations of the experiment.

Guide through the column labels:

-Exp: experiment, either aimed at measuring the technical variance(level techvar) or the sector variance (level sectoring)

-Lineage: lineages used in the experiment, 3 levels: L1, L6, L10

-Ratio: 3 levels: R1=heterokaryon with initial matA of 90%, R2= heterokaryon with initial matA of 50%, R3=heterokaryon with initial matA of 10

-Obs.Ratio : Proportion of matA in the mycelium

-Unic.Sect=code for plates and sectors of the plate (as well as ratio and lineage). Ex L10R1P1s1=sector1 of plate 1 of Ratio 1 of lineage10

The Rscript gives the code to get the figures in appendix and the statistical analyses underlying Table 1.