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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect empirical data. Simulated data were collected using a custom Python script, which is available at https://github.com/bgitschlag/MiSelf. This code is also included as a supplementary file with this study.
Data analysis	Data were analyzed using a custom Python script, which is available at https://github.com/bgitschlag/MiSelf. This code is also included as a supplementary file with this study.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All source data needed to reproduce our results are publicly available and provided in this study. Data from experiments measuring uaDf5 frequency and selection on uaDf5 (Fig. 3a-c) were previously published (ref. 26). These and the experimental data encompassing the remaining four genotypes (Fig. 4) are provided in the

Source Data. These data can also be downloaded in a code-readable format at https://github.com/bgitschlag/MiSelf. The modeling parameters generated from the maximum-likelihood inference on the empirical and bootstrap data are provided in Supplementary Data Files 1-5.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	This study did not involve human subjects.
Reporting on race, ethnicity, or other socially relevant groupings	This study did not involve human subjects.
Population characteristics	This study did not involve human subjects.
Recruitment	This study did not involve human subjects.
Ethics oversight	This study did not involve human subjects.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This study focused on the relationship between genotype (in the form of mutant frequency) and phenotype (in the form of fitness), for five heteroplasmic mitochondrial mutations. Mutant mtDNA fitness effects can manifest at different levels of selection. Intra-organismal (within-host) selection was measured by comparing mutant frequency between parents and progeny. Inter-organismal (between-host) selection was measured by competing heteroplasmic individuals (mutant mtDNA carriers) against their wildtype counterparts. Mutant frequency was also sampled randomly in adult heteroplasmic individuals for each mtDNA genotype.
Research sample	Six strains of Caenorhabditis elegans were used in this study: five mitochondrial mutant strains, each carrying a unique heteroplasmy in which mutant and wildtype mtDNA coexist and are stably co-transmitted within the same host organism. A sixth strain was used as a wildtype control (the Bristol strain, N2). All mutant mtDNA frequencies measured in individual organisms (intra-organismal selection and mutant frequency distribution measurements) were measured in day-2 adult C. elegans, representing the period of peak fecundity. Population-wide mutant mtDNA frequency (inter-organismal selection) was measured in individuals of varying ages.
Sampling strategy	To measure mutant mtDNA frequency in individual organisms (intra-organismal selection and mutant frequency distribution measurements), individuals at the fourth and final larval stage were picked and transferred at random from a laboratory stock population onto isolated fresh food plates. After 48 hours of incubation, these day-2 adults were re-transferred to fresh food plates for 4 hrs to allow for the production of an age-synchronized progeny cohort. Adults were then lysed. Progeny were likewise subsequently lysed upon reaching day 2 of adulthood to control for age-dependent effects on mutant mtDNA frequency. Three progeny were lysed together per parent, hence mutant mtDNA frequency in progeny was measured as the average frequency across three progeny. Population-wide mutant mtDNA frequency (inter-organismal selection) was measured in C. elegans of varying ages, by randomly sequestering a bulk sub-set of the population for lysis.
Data collection	Mutant mtDNA frequency was measured using a multiplex quantitative PCR method, namely Droplet DigitalTM PCR, in which mutant and wildtype mtDNA copy numbers were measured within the same reaction using mutant- and wildtype-specific PCR primers.
Timing and spatial scale	Mutant mtDNA was sampled in day-2 adults of the parent generation, and day-2 adults of the progeny generation, in multiple isolated parent-progeny lineages, to control for the confounding effects of inter-organismal selection between lineages. In the inter-organismal selection experiments, mutant mtDNA frequency was measured on a bulk sample of each replicate population every 3 days (the lower end of the generational life cycle in C. elegans).
Data exclusions	For the intra-organismal selection experiment, parents that failed to produce viable progeny were omitted, to minimize the confounding influence of inter-organismal selection. No data were omitted on the basis of statistical testing for outliers.
Reproducibility	All empirical data in this study are included in the Source Data file. These data, together with the code used in this study, is also publicly available at https://github.com/bgitschlag/MiSelf.
Randomization	All individual C. elegans featured in this study were sampled randomly from laboratory stock populations for age-synchronization and

Randomization	selection experiments. For inter-organismal selection experiments, populations were washed off of food plates into collection tubes, and mutant mtDNA frequency was measured in bulk using a pooled lysate obtained from these population-scale collection tubes.
Blinding	Blinding was not relevant to this study. All individual organisms were subjected to the same experimental protocols and conditions.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Laboratory populations of Caenorhabditis elegans (Bristol N2 strain plus mutant mtDNA genotypes uaDf5, mptDf2, mpt4, mpt2, and mptDf3).
Wild animals	This study did not involve wild animals.
Reporting on sex	C. elegans is a hermaphroditic species, in which mature adults self-fertilize by producing both sperm and oocytes. All individuals featured in this study are hermaphrodites (with the possible exception of <1% males in the bulk populations used for measuring inter-organismal selection).
Field-collected samples	This study did not involve samples collected in the field.
Ethics oversight	This study did not involve animals apart from C. elegans. No vertebrates were used, hence no ethical approval was required.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	This study did not involve plants.
Novel plant genotypes	This study did not involve plants.
Authentication	This study did not involve plants.