nature portfolio

Corresponding author(s):	Fay-Wei Li
Last updated by author(s):	Oct 5 2021

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.

\sim				
ζ.	ta	١Ť١	ıst	100

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
	$oxed{x}$ 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.
X	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about <u>availability of computer code</u>

Data collection

Nanopore sequencing and basecalling were done on the GridION platform using the flip-flop version of Guppy (v3.1). Illumina sequencing was carried out on the NovaSeq6000 platform with 2x150 bp paired-ends.

Data analysis

We have used a large collection of bioinformatic programs for this study, and they are detailed in the Methods (including program names, version number, citation, and any non-default parameters).

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 <u>availability of data</u>

 $All\ manuscripts\ must include\ a\ \underline{data\ availability\ statement}.\ This\ statement\ should\ provide\ the\ following\ information,\ where\ applicable:$

- 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 the raw sequences were deposited in the NCBI Sequence Read Archive under the BioProject PRJNA735564. Genome assembly and annotation are available at https://genomevolution.org/coge/GenomeInfo.pl?gid=61511. Sequence alignments and tree files can be found at https://github.com/dawickell/Isoetes_CAM.

	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 o	f the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf
Life scie	nces study design
All studies must d	isclose on these points even when the disclosure is negative.
Sample size	NA
Data exclusions	A single outlier sample from each of six time points (1hr, 4hrs, 7hrs, 10hrs, 13hrs and 19hrs) was removed from the final dataset based on multidimensional scaling plot.
Replication	Five biological replicates for titratable acidity measurements, and three biological replicates for RNA-seq.
Replication Randomization	Five biological replicates for titratable acidity measurements, and three biological replicates for RNA-seq. Individual plants were randomly selected for diel measurements of titratable acidity and RNA-seq.

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	Methods	
n/a Involved in the study	n/a Involved in the study	
X Antibodies	ChIP-seq	
Eukaryotic cell lines	Flow cytometry	
Palaeontology and archaeology	MRI-based neuroimaging	
Animals and other organisms	'	
Human research participants		
Clinical data		
Dual use research of concern		