nature portfolio

Corresponding author(s): Detlef Weigel

Last updated by author(s): Dec 30, 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.

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	Short-read sequencing data for this study was generated in Illumina HiSeq 3000, Illumina HiSeqX and NovaSeq 6000 systems. Long-read sequencing data for this study was generated in a Sequel I system.
Data analysis	All publicly available software and their version used for data analysis in this study are specified in the Methods section. In addition, "Supplementary Table 2" lists all R-packages used for data manipulation and visualisation. Genome scaffolding with Hi-C data was performed by a third-party service provider (Dovetail Genomics, LLC.), and their methods are readily described in the corresponding Methods section. Relevant custom code for genome assembly, analysis of PacBio amplicons, probabilistic models and SLiM simulations is provided in a dedicated GitHub repository for this study: https://github.com/SonjaKersten/Herbicide_resistance_evolution_in_blackgrass_2022

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

Raw data for the genome assembly of Alopecurus myosuroides such as PacBio CLRs, Illumina PCR-free, Hi-C, Iso-Seq and Illumina RNA raw sequencing data can be accessed in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena/browser/home) under the project accession number PRJEB49257 (Accession codes

will be available before publication). Raw ddRAD-seq data for the population study, Illumina DNA-seq data for the bulked-segregant analysis, and PacBio CCS q20 reads can be downloaded from the ENA project accession number PRJEB49288 (Accession codes will be available before publication). Genome assembly, annotation files, the SNP matrices for both the ddRAD-seq and bulked-segregant experiments, and the fasta files with the haplotypes of ACCase and ALS can be found on: https://keeper.mpdl.mpg.de/d/520501d08acc4bd887f7

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

Life sciences study design

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

Sample size	47 populations from nine European countries with 22-24 individual plants each.
Data exclusions	We aimed to collect 8-weeks-old leaf tissue from 24 individuals per population, but due to insufficient germination in two populations, we were unable to collect material from two individuals and therefore finally obtained 1,126 samples for further processing. Further samples were excluded in the different sequencing experiments due to either low-coverage of other quality control metrics. In the methods section we have stated that for the RAD-seq experiment 1,123 individuals were finally analyzed, while for PacBio amplicons the final sample sizes were 1,046 individuals for ACCase and 842 individuals for ALS.
Replication	Since we analyzed populations of a highly heterozygous plant species, all individuals are unique genotypes. Therefore, biological replicates are not possible for this study.
Randomization	For phenotyping for the bulked-segregant experiment, 27 plants per population were divided into two treatment and two control groups, following a specific tray design to minimize spatial growth effects. Treatment 1: Atlantis WG® (Bayer Crop Science) + Synergist Atlantis WG® (10 plants per population). Control 1: Only Synergist Atlantis WG® (3 plants per population). Treatment 2: Axial® 50 (Syngenta) + Synergist Hasten (10 plants per population). Control 2: Only Synergist Hasten (4 plants per population).
Blinding	Not relevant for this study.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Leaf tissue from both the selected A. myosuroides plant and the reference standard Secale cereale cv. Daňkovské were simultaneously chopped with a razor blade in 250 µl of nuclei extraction buffer (CyStain PI Absolute P kit; P/N 05-5022). After the addition of 1 ml of staining solution (including 6 µl of propidium iodide (PI) and 3 µl of RNase from the same kit) the suspension was filtered through a 30 µm filter (CellTrics®; P/N 04-0042-2316). Five replicates of these samples were stored in darkness for 4 h at 4°C prior to flow cytometry analysis.
Instrument	BD FACSMelodyTM Cell Sorter (BD Biosciences) equipped with a yellow-green laser (561 nm) and 613/18BP filtering.
Software	BD FACSChorus™ Software.
Cell population abundance	A total of 25,000 events were recorded per replicate.
Gating strategy	The ratio of the mean PI-area values of each target sample and reference standard 2C peaks was used to estimate DNA content according to Dolezel & Bartos (2005).

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.