

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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
☒	☐ 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 [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 in data collection.
Data analysis	Software used in data analysis are described in detail, with associated references, in the Methods section. No custom algorithms or software were used in the research. Sequence data were processed using a combination of Cutadapt 4.2, Stacks v2.52, and BWA 0.7.17 to generate SNP genotypes. SNP genotypes were filtered for quality using VCFTools. Environmental data were processed in R 4.1, and QGIS 3.16.14 with GRASS 7.8.5. The analyses DAPC, ResistanceGA, sPCA, pRDA, GDM, and LFMM were conducted in R 4.1 using packages described in the Methods. Figures were created using ggplot2 in R 4.1 and Inkscape 1.1. The analysis FastStructure used version 1.0, and the analysis EEMS used version 1.0. Code underlying analyses has been deposited in a GitHub repository (https://github.com/marcabeer/stquoll_landscape_genomics).

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](#)

Sequence data and sample metadata necessary for reproducing the study have been deposited at NCBI under BioProject PRJNA922561 (<https://dataview.ncbi.nlm.nih.gov/object/PRJNA922561?reviewer=k665j7vuqj8hum6io4jklkki48>) and BioSamples SAMN32664143—32664814. Note that this link is a temporary reviewer link and full public access will be granted following publication. Any other relevant data can be found within the article and its supplementary information.

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

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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 concerns the population genetics of the spotted-tailed quoll (*Dasyurus maculatus*) across Tasmania, Australia. Our data are quantitative and analyses typically used regression modeling methods to understand how environmental factors impact genetic variation in the study species.

Research sample

The initial dataset consisted of 548 unique samples of the spotted tailed quoll (*Dasyurus maculatus*), which were collected 2004—2019 across Tasmania, Australia. Samples were geographically and temporally widespread to capture the distribution of abiotic and biotic environmental conditions encountered by the study species. The final dataset consisted of 345 samples that were similarly geographically widespread as the initial dataset and spanned 2004—2017.

Sampling strategy

Sample size was not predetermined, as samples were collected opportunistically several years prior to conception of the study. Samples best conform to a random spatial sampling procedure, as samples are geographically widespread and represent a range of

environmental conditions. The final dataset containing 345 geographically widespread samples exceeds recommendations based on landscape genomics simulations, which show that ≥ 200 samples are typically sufficient to achieve high statistical power (Selmoni et al., 2020; <https://doi.org/10.1111/1755-0998.13095>).

Data collection	Tissue samples were collected by the University of Tasmania and the Department of Natural Resources and Environment Tasmania. Individuals were caught in baited pipe traps. All individuals caught were implanted with a subcutaneous microchip to allow individual identification and had a 3mm biopsy (Kai Medical TM) taken from the lower edge of their right ear. Staff involved in sample collection were blind to the study hypothesis because this study was conceived several years after sample collection.
Timing and spatial scale	Sample collection dates spanned 2004–2019, with the final dataset spanning 2004–2017. All years except for 2015, which was not sampled, were represented by ≥ 2 samples; the mean number of samples for a given year was 24.6 samples (S.D. = 17.8 samples; max = 60 samples in 2012). Samples spanned the island of Tasmania, Australia, as depicted in Figure 2A. The mean distance between samples was 121km (S.D. = 91km; max = 373km).
Data exclusions	Genotype data were filtered to remove single nucleotide polymorphisms (SNPs) with high missing data across samples, as well as to remove samples with high missing data across SNPs. Samples lacking specific geographic coordinates or collection dates were also excluded following genotyping. This procedure, including missing data thresholds, is described in Extended Data Table 1. Missing data thresholds were predetermined such that they resulted in a final, nominal missing data rate of $<50\%$ on a per-SNP and per-sample basis, which is typical in the field of landscape genomics.
Reproducibility	Analyses characterized by stochasticity were repeated multiple times to ensure reliability, as described in the Methods.
Randomization	Randomization was not relevant to the study, as we applied analyses uniformly to the entire sample set.
Blinding	Blinding was not relevant to the study, as we applied analyses uniformly to the entire sample set.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Field conditions were not recorded during field work. General location and when possible, specific geographic coordinates were recorded.
Location	Sampling was conducted across the island of Tasmania, Australia. There were numerous (>300) unique geographic coordinates for samples, which are provided in the repository to which our data have been deposited.
Access & import/export	Field collections occurred across the island of Tasmania, Australia, at numerous locations. We complied with University of Tasmania Animal Ethics Permits (A0008588, A0010296, A0011696, A0013326, A0015835, A0018223, A0016789), as described in the Methods.
Disturbance	Individuals were captured in baited pipe traps that did not cause physical harm. Tissue samples were collected as 3mm ear biopsies to cause minimal damage to the individual. Individuals were released following implantation of a subcutaneous microchip and tissue collection. Handling procedures took 5-10min.

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
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

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	The study did not involve laboratory animals.
Wild animals	The study involved temporary capture of individual spotted-tailed quolls (<i>Dasyurus maculatus</i>) across Tasmania, Australia. Individuals were released following microchipping and tissue sample collection on-site.
Reporting on sex	Findings apply to both sexes, as they were analysed together throughout the study. The initial 548 individuals included 330 males, 160 females, and 182 individuals of unknown sex. The final dataset of 345 individuals included 213 males, 101 females, and 31 individuals of unknown sex. Population genetic analyses do not typically stratify samples by sex.
Field-collected samples	Individuals were not taken into the laboratory. Samples were collected in the form of preserved tissue biopsies.
Ethics oversight	Ethics oversight was provided by the University of Tasmania Animal Ethics and the Tasmania Department of Natural Resources and Environment Ethics Committees.

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