natureresearch

Corresponding author(s): Nicholas A. Levis

Reporting Summary

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Соі	nfirmed			
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement			
	\boxtimes	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly			
	\boxtimes	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.			
	\boxtimes	A description of all covariates tested			
	\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons			
	\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (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>			
\ge		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings			
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes			
	\boxtimes	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated			
	\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)			
Our web collection on statistics for biologists may be useful.					

Software and code

Policy information about availability of computer code

,	No software was used to collect data.
Data analysis	Data were analyzed using JMP Pro and R. Specific packages are indicated in the text.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data is included as supplementary material and uploaded to the Dryad Digital Repository. We declare no conflicts of interest.

Field-specific reporting

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

Life sciences Behavioural & social sciences Kelogical, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Ecological, evolutionary & environmental sciences study design

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

Study description	We manipulated the diet of 120 tadpoles from a non-polyphenic species and 302 tadpoles from a polyphenic species and determined if there were diet-induced differences in 1) various trophic morphological characters, 2) growth, and 3) expression level of diet-related genes. In addition, we compared trophic morphology among wild-collected tadpoles from a non-polyphenic species (n = 50), two morphs of a polyphenic species (total n = 156), and a secondarily non-polyphenic species (n = 82).
Research sample	For the focal lineage that possesses the novel trait (resource-use polyphenism), we chose Spea multiplicata. For our ancestor-proxy (non-polyphenic) lineage, we chose Scaphiopus holbrookii. This species is an appropriate proxy for ancestral Spea (prior to the evolution of the carnivore-omnivore polyphenism), because Scaphiopus: 1) is the closest extant outgroup to Spea ; 2) produces omnivores only, the reconstructed state for the ancestor of Scaphiopus and Spea; and 3) is ecologically similar to Spea. We reared each species in both the novel environment (i.e., shrimp diet, the diet of the derived carnivore morph) and the ancestral environment (i.e., detritus diet, the diet of the ancestral omnivore morph). To evaluate if frequency of trait expression predicts the extent to which it is refined, we then contrasted production of carnivore features in wild-caught tadpoles Sc. couchii, Sp. multiplicata, and Sp. bombifrons. The first species never produces carnivores, the second sometimes produces carnivores through diet-induced plasticity, and the third often produces carnivores regardless of diet.
Sampling strategy	Sample sizes were maximized using available laboratory space and were comparable with similar studies/systems.
Data collection	Data were collected by N.A.L. and A.J.I. by measuring tadpole morphology with digital calipers and measuring gene expression level using RT-qPCR.
Timing and spatial scale	Experimental morphological data were collected for Sp. multiplicata were collected in the summer 2015, and experimental morphological data for Sc. holbrookii were collected in the summer 2016. The later date for Sc. holbrookii was due to insufficient number of breeding pairs at the initial time point and limitations on space for experimental procedures. Gene expression data were collected in summer 2017 using samples that had been stored at -80C in RNAlater from the time of collection (2013 for Sp. multiplicata and 2016 for Sc. holbrookii). Wild-caught morphological data were collected in summer 2017 using previously collected specimens stored in ethanol. These specimens were collected from the field in 2006, 2008, and 2016.
Data exclusions	Data were only excluded if a molecular technique (e.g. qPCR reaction) failed for all genes assessed. In these cases, the individual was omitted from analysis. Full detail is given in the methods of the manuscript.
Reproducibility	We had numerous replicates for each experiment performed, but have not taken steps to formally reproduce the entire study. Sample sizes ranged from 10 to14 replicates (for molecular assays) and 60 to >100 replicates for morphological measurements.
Randomization	As described in our methods, tadpoles from each family were randomly, and evenly, divided among treatment groups where applicable.
Blinding	Where possible, the investigators did not know the treatment group from which an individual was derived during morphological

Did the study involve field work?

Field work, collection and transport

Field conditions	Field work only consisted of collecting tadpoles from naturally occurring ponds in the southwestern United States during the summer and following heavy monsoon rains.
Location	Ponds were located near Portal, AZ (lat: 31°54'49.14"N, long: 109° 8'29.34"W)
Access and import/export	These ponds have studied extensively for 20+ years. Access to ponds is annually coordinated with property owners and collection permits are obtained annually from the Arizona Game and Fish Department.
Disturbance	These tadpoles often develop in ponds used as a water source for cattle so they are used to frequent disturbance. However, we try to minimize our impact by not over-collecting at a given pond and only spending as much time in the water as necessary to collect samples.

Reporting for specific materials, systems and methods

Materials & experimental systems Methods n/a Involved in the study n/a Involved in the study \boxtimes \boxtimes Unique biological materials ChIP-seq Γ \boxtimes \boxtimes Antibodies Flow cytometry \square \boxtimes Eukaryotic cell lines MRI-based neuroimaging Palaeontology Animals and other organisms Human research participants \boxtimes

Animals and other organisms

Policy information about <u>stu</u>	dies involving animals; ARRIVE guidelines recommended for reporting animal research
Laboratory animals	Laboratory animals were tadpoles of Scaphiopus holbrookii and Spea multiplicata.
Wild animals	Wild animals (Spea multiplicata, Spea bombifrons, and Scaphiopus couchii) were obtained from ponds near Portal, AZ 10-14 days after hatching using hand-held dip nets. Tadpoles were immediately euthanized in MS-222 and ultimately transported back to the University of North Carolina for measurements.
Field-collected samples	Field-collected samples were euthanized prior to any laboratory work performed on them. Samples were stored in 95% ethanol prior to use.