Supplemental Materials for: The influence of cactus spine surface structure on puncture performance and anchoring ability is tuned for ecology.

Authors: S. B. Crofts<sup>1\*</sup> and P. S. L. Anderson<sup>1</sup>

Author affiliation:

 Department of Animal Biology, University of Illinois, Urbana Champaign, 515 Morrill Hall, 505 S Goodwin Ave, Urbana, IL 61801.

\*Corresponding Author:

Stephanie B. Crofts

scrofts@illinois.edu

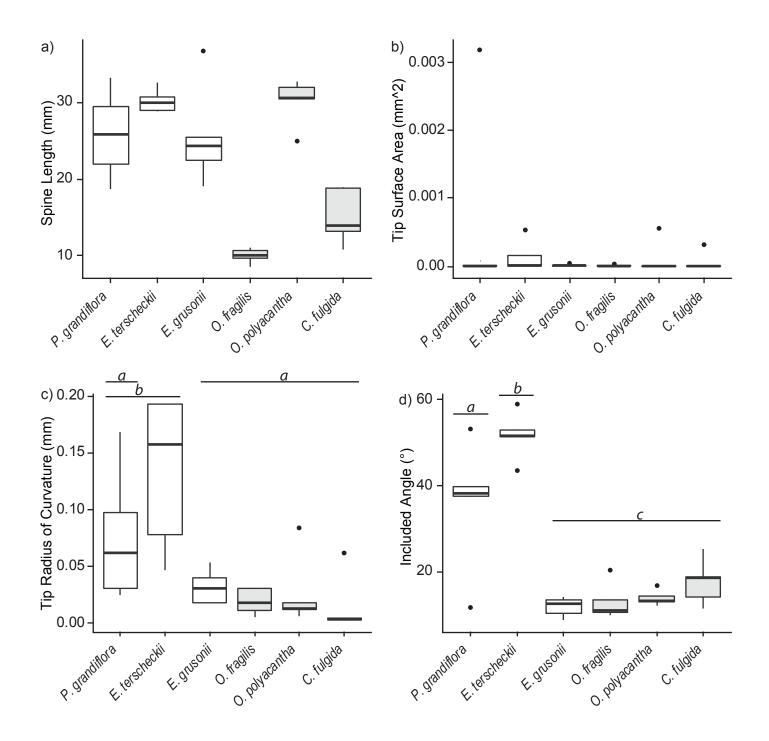
Supplemental Materials include: Power analysis data; Supplemental Fig 1: Spine length and tip sharpness compared between species; Supplemental Fig 2: Figures and more detailed discussion of Force to initial fracture and spine tip morphology; Supplemental Fig. 3: Work to initial fracture; Supplemental Fig. 4: A species level comparison of work to puncture and work to propagate fracture; Supplemental Fig 5: Relative distances required for spine withdrawal; Supplemental Fig. 6: An example series of force-displacement graphs showing the progressive decline in performance associated with repeated testing in barbed spines; Supplemental Fig. 7: ESEM image showing the undulating edges of *Echinocactus grusonii* spines.

Power Analysis prior to experiments: # groups: 6; f ~ 0.7; sig. level: 0.05; power: 0.8  $\rightarrow$  n = 5

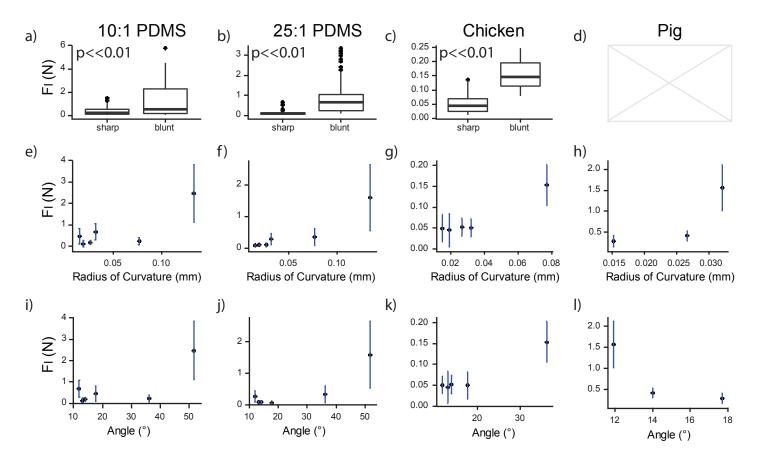
Power analysis based on experimental data:												
Test	Target	Measurement	# groups	f	sig. level	power	calculated n					
ANOVA by species	NA	Spine length	6	1.8283	0.05	0.8	1					
ANOVA by species	NA	Tip Area	6	0.9033	0.05	0.8	4					
ANOVA by species	NA	Included Angle	6	3.04	0.05	0.8	1					
ANOVA by species	NA	RoC	6	0.9772	0.05	0.8	3					
t-test: sharp-blunt	10:1 PDMS	Fi	2	0.0018	0.05	0.8	NA					
t-test: sharp-blunt	25:1 PDMS	Fi	2	0.5094	0.05	0.8	16					
t-test: sharp-blunt	Chicken	Fi	2	1.6795	0.05	0.8	3					
t-test: barbed- unbarbed	10:1 PDMS	Wp	2	1.48	0.05	0.8	3					
t-test: barbed- unbarbed	25:1 PDMS	Wp	2	1.1531	0.05	0.8	4					
t-test: barbed- unbarbed	Chicken	Wp	2	1.714	0.05	0.8	2					
t-test: barbed- unbarbed	Pig	Wp	2	0.2655	0.05	0.8	56					
t-test: barbed- unbarbed	Chicken	Wpr	2	0.8303	0.05	0.8	6					
t-test: barbed- unbarbed	Pig	Wpr	2	1.1142	0.05	0.8	4					
ANOVA by species	10:1 PDMS	Wp	6	2.8462	0.05	0.8	1					
ANOVA by species	25:1 PDMS	Wp	6	1.7852	0.05	0.8	1					
ANOVA by species	Chicken	Wp	5	2.2838	0.05	0.8	1					
ANOVA by species	Pig	Wp	3	0.309	0.05	0.8	34					
ANOVA by species	Chicken	Wpr	5	0.0597	0.05	0.8	670					
ANOVA by species	Pig	Wpr	3	1.3206	0.05	0.8	3					
t-test: barbed- unbarbed	10:1 PDMS	Ww	2	1.8045	0.05	0.8	3					
t-test: barbed- unbarbed	25:1 PDMS	Ww	2	1.0043	0.05	0.8	5					

Power analysis based on experimental data:

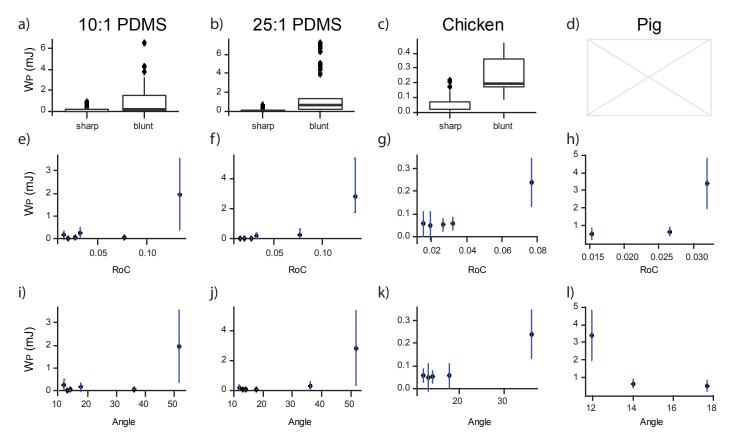
t-test: barbed- unbarbed	Chicken	Ww	2	0.5651	0.05	0.8	13
t-test: barbed- unbarbed	Pig	Ww	2	0.5054	0.05	0.8	16
ANOVA by species	10:1 PDMS	Ww	6	3.379	0.05	0.8	1
ANOVA by species	25:1 PDMS	Ww	6	1.1051	0.05	0.8	3
ANOVA by species	Chicken	Ww	5	3.8229	0.05	0.8	1



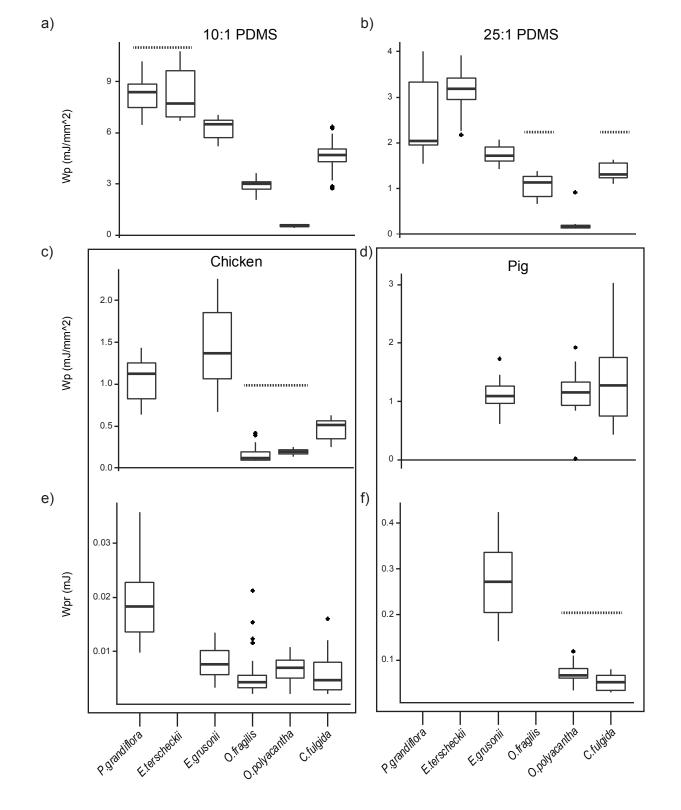
Supplemental Fig. 1 Spine length (a) and sharpness as measured by b) tip surface area (mm<sup>2</sup>), c) tip radius of curvature S(mm), and d) included angle of the spine tip. Species shown in white (*P. grandiflora*, *E. terscheckii*, and *E grusonii*) have non-barbed spines and species in grey (*O. fragilis*, *O. polyacantha*, and *C. fulgida*) have barbed spines. Bars show significant groupings.



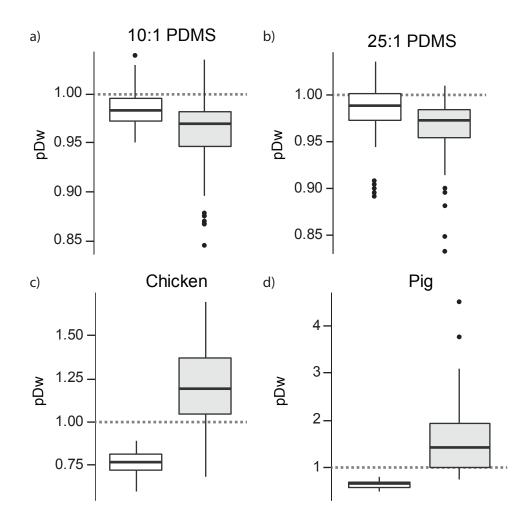
Supplemental Figure 2 - The relationship between spine morphology and force to initiate fracture in different targets. Top row shows comparisons between sharp and blunt spines for a) 10:1 PDMS, b) 25:1 PDMS, and c) chicken breast. Only sharp spines were able to penetrate pig skin, so no comparison is possible. There is an overall trend where 'blunt' spines, those with significantly larger included angles and radii of curvature, require more force to initiate fracture all targets, though we only have the statistical power to determine that this relationship is significant for the chicken tests. Middle row shows relationships between spine tip radius of curvature and the force to initiate fracture in e) 10:1 PDMS, f) 25:1 PDMS, g) chicken breast, and h) pig tissue. The bottom row shows relationships between tip included angle and the force to initiate fracture in i) 10:1 PDMS, j) 25:1 PDMS, k) chicken breast, and l) pig tissue.



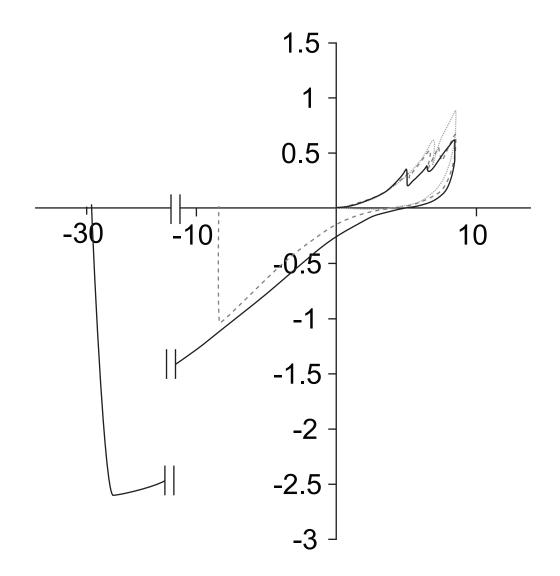
Supplemental Figure 3 - The relationship between spine morphology and work to initiate fracture in different targets. Top row shows comparisons between sharp and blunt spines for a) 10:1 PDMS, b) 25:1 PDMS, and c) chicken breast. Only sharp spines were able to penetrate pig skin, so no comparison is possible. Middle row shows relationships between spine tip radius of curvature and the work to initiate fracture in e) 10:1 PDMS, f) 25:1 PDMS, g) chicken breast, and h) pig tissue. The bottom row shows relationships between tip included angle and the work to initiate fracture in i) 10:1 PDMS, j) 25:1 PDMS, k) chicken breast, and l) pig tissue.



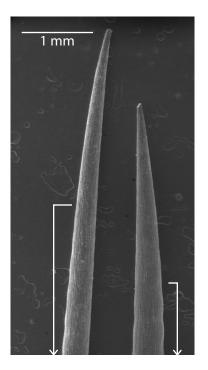
Supplemental Figure 4 - Work to puncture and propagate fracture by cactus species. Statistically equivalent species indicated by dotted line. a) Work to full puncture (mJ/mm^2) in 10: 1 PDMS elastomer by species. *P. grandiflora* and *E. terscheckii* are not significantly different from one another, all other speceis are significantly different. b) Work to puncture in 25: 1 PDMS elastomer by species. *O. fragilis* and *C. fulgida* are not significantly different from one another, all other speceis are significantly different from one another, all other speceis are significantly different. c) Work to puncture chicken breast by species. *O. fragilis* and *O. polyacantha* are not significantly different from one another, all other speceis are significantly different from one another, all other speceis are significantly different from one another, all other speceis are significantly different from one another, all other speceis are significantly different from one another, all other speceis are significantly different. c) Work to puncture chicken breast by species. *O. fragilis* and *O. polyacantha* are not significantly different from one another, all other speceis are significantly different. d) Work to puncture pig tissue by species, sample size was too small to determine significance. e) Work to propagate fracture in chicken breast by species, neither *E. tercheckii* nor *O. fragilis* were able to puncture pig skin. *E. grusonii* took significantly more work to propagate than *O. polyacantha* and *C. fulgida*, which were statistically indistinguishable.



Supplemental Fig. 5. Relative distance required for spine withdrawal. In 10:1 and 25:1 PDMS elastomers (a and b) non-barbed spines (white) travelled greater distances for withdrawal than barbed spines (grey), but neither type of spine travelled further than the puncture depth as indicated by the dotted line at 1. Values less than 1 indicate that target material deflected prior to puncture. In the biological tissues (c and d) the barbed spines travelled significantly further than the non-barbed spines to full withdraw from the target, and both travelled further than the depth of puncture ( values > 1).



Supplemental Fig. 6 - Example of progressive decline in performance. First run (solid black line) of testing *C. fulgida* spine in pig tissue. Second run (dashed dark grey line) results in higher force to initial fracture and lower mangitude force to withdraw and distance required for spine to disengage from tissue. The spine does not anchor into tissue during the third run (light grey dotted line) which results in possible higher force to fracture and force to full puncture, or a broken spine tip.



Supplemental Fig. 7 ESEM image of *E. grusonii* spines showing undulating sides (white brackets). Scale bar is 1 mm.