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

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Spines are common morphological features found in almost all major biological groups offering an opportunity to explore large-scale evolutionary convergence across disparate clades. As an example, opuntioid cacti have spines with barbed ornamentation that is remarkably similar in form and scale to that found on porcupine quills, suggesting specific biomechanical convergence across the animal and plant kingdoms. While the mechanics of porcupine quills as defensive mechanisms has been previously tested, the mechanics of cactus spines (which have evolved to fill a number of functions including defence, climbing and dispersal) has not been characterized. Here we study the puncturing and anchoring ability of six species of cactus, including both barbed and non-barbed spines. We found that barbed spines require less work to puncture a variety of targets than non-barbed spines. Barbed spines also require more work than non-barbed spines to withdraw from biological materials, owing to their barbs engaging with tissue fibres. These results closely match those found previously for barbed versus non-barbed porcupine quills, implying biomechanical convergence. The variation in performance of barbed versus non-barbed spines, as well as between barbed spines from different species, is probably tied to the diversity of ecological functions of cactus spines.

1. Introduction

One of the core themes of biomechanics is understanding the extent to which morphology and function relate to each other, and how the degree to which one is attuned to the other varies based on evolutionary history, ecology, or functional trade-offs. As an example, spines and other pointed puncturing tools that occur outside the mouth, are found in a wide range of organisms. Animal spines are effective tools for deterring predation by either directly damaging the predator, effectively increasing prey body size to exceed predator gape, or preventing predators from gaining purchase [1–10]. In some cases, such as bee stingers or porcupine quills, spines are anchored into the target and left behind when the organism disengages. The spines of these taxa usually have ornamentation that helps to both puncture and anchor the spines in the tissue of the offending predator [6,11,12]. In plants, spines can serve to deter herbivory, but often have alternative functions as well [13–18]. Much like the animal examples above, certain plant spines are capable of being anchored in their targets, often aided by surface ornamentation. In particular, the barbed spines of the cactus subfamily Opuntioideae have surface ornamentation (figure 1) that is strikingly similar to that reported in porcupines [6]. This potentially represents a significant morphological/biomechanical convergence between animals and plants. However, while the function of the barbs on porcupine quills has been examined [6], no similar work has been done characterizing the function of cactus spines, which show a wide diversity of spine ornamentation. Here, we examine this potential cross kingdom

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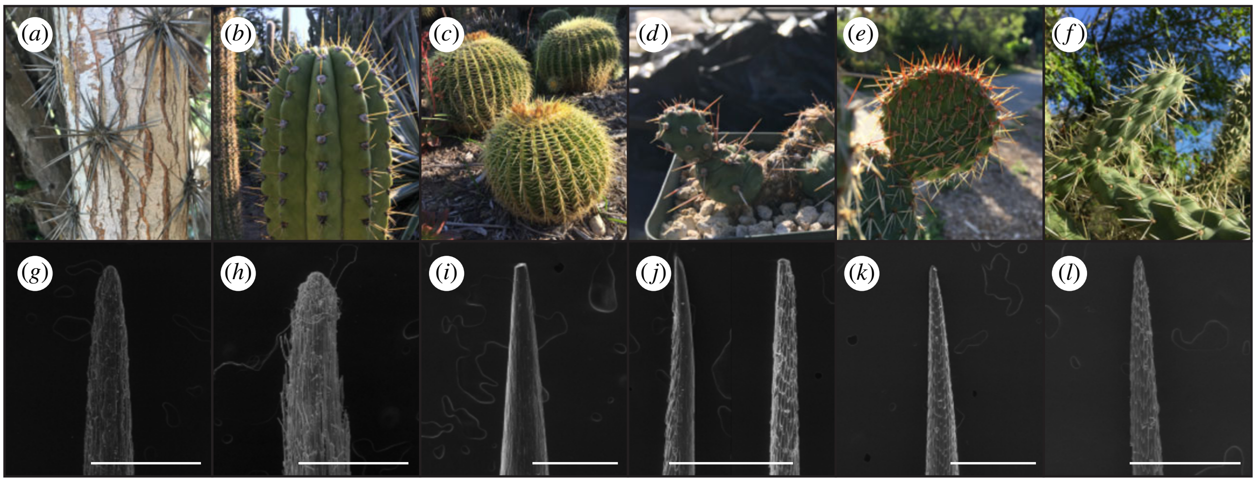

Figure 1. Species diversity and spine morphology. (*a*) *Pereskia grandiflora* and (*g*) non-barbed spine; (*b*) *Echinopsis terscheckii* and (*h*) non-barbed spine; (*c*) *Echinocactus grusonii* and (*i*) non-barbed spine; (*d*) *Opuntia fragilis* and (*j*) two partially barbed spines; (*e*) *Opuntia polyacantha* and (*k*) barbed spine; (*f*) *Cylindropuntia fulgida* and (*l*) barbed spine. All scale bars, 500 μm. Cactus photos credit: John N. Trager. (Online version in colour.)

biomechanical convergence by examining the biomechanical function of cactus spines with varying ornamentations.

Ancestrally, cactus spines probably first evolved as a defence from herbivores [13–18]. However, spines have been co-opted for a wide range of secondary uses, including but not limited to: anchoring climbing forms onto substrates, thermoregulation, shading and ultraviolet protection, and reducing evaporative water loss by changing the boundary layer around the plant [19–21]. There is a great deal of variation in cactus spine size and morphology (e.g. straight versus curved, round versus flattened in cross section) and this probably affects both puncture ability as well as secondary functionality [19,22]. The surface of spines also varies, ranging from smooth to covered with various tubercle morphologies or barbs [23,24]. Opuntioids are easily distinguished from other subfamilies by the microscopic barbs on their spines and glochids (small easily detached barbed spines that form clumps at the base of the larger fixed spines) [19,24]. Opuntioid spines, like cactus spines in general, are multi-functional and aid in dispersal of vegetative propagules and to collect water from ambient fog [19,25–29]. Based on their morphological similarity to the barbs found on porcupine quills, the barbs of some opuntioid cacti should make puncturing more efficient by concentrating applied stresses in the target material. Puncture efficiency is important since cacti puncture passively, depending on force applied by the target to successfully penetrate [6]. As previously shown with the porcupine quills as well as other examples of barbed spines, these surface barbs should also effectively anchor the cactus spines into target tissues, which will aid in the dispersal of vegetative propagules [6,11]. However, the degree to which opuntioid cacti depend on this active dispersal varies, and the number of spines, barb covering, and cladode attachment strength all vary [30,31]. This range of diversity offers an opportunity to delve deeper into the relationship between ornamentation and biomechanical function.

Our main goal for this study is to characterize the puncture performance of cactus spines and how barbs affect the ability of cactus spines to both penetrate tissues, and remain anchored in said tissues. As cactus spines depend on external forces to drive puncture events, we expect them to be particularly sharp, minimizing the surface area over which force is applied, to efficiently initiate puncture. At the same time, species that depend heavily on vegetative propagation will need spines that can both puncture a target and hold on sufficiently to detach the cladode and support it as the host travels some distance away from the parent plant. Species that do not reproduce via vegetative propagation will not need to anchor to the target, but should still readily puncture targets. The specific aims of this project are threefold: (i) to quantify differences in spine tip sharpness and how this affects fracture initiation; (ii) to determine if the presence of barbs on spines reduces the energy required to puncture compared to non-barbed spines, as would be predicted based on their similarity to porcupine quills; and (iii) to test whether the barbs anchor cactus spines in a target in a manner similar to porcupine quills.

2. Methods

Cactus specimens were collected from The Huntington Botanical Gardens in San Marino, CA, USA during the early spring of 2018. A total of six species (figure 1) were used in this study including: three live specimens: *Echinocactus grusonii*, *Opuntia fragilis* and *Cylindropuntia fulgida*; two cuttings of live specimens: *Pereskia grandiflora*, *Echinopsis terscheckii*; and one dead, desiccated specimen: *Opuntia polyacantha*. Cactus species were selected to represent a diversity of spine morphologies, both barbed and un-barbed.

Prior to our experiments, we used pilot data from barbed and non-barbed spines in a power analysis to help determine the sample size required for our tests. The effect size we calculated based on these preliminary data was large enough that the power analysis indicated a sample size of $n = 5$ would be sufficient. Therefore, we selected five representative spines from each species to quantify morphology. For each spine, we measured total length, from the tip to the end of the removed spine, and width at both 30% and 50% of the total spine length as measured from the tip. We took additional measurements from environmental scanning electron microscope (ESEM) images (FEI Quanta FEG 450 ESEM; FEI Company) to quantify tip sharpness and to compare ornamentation. To quantify tip sharpness, we took three measurements: the radius of curvature (RoC) at the tip [32–37], inclusive angle of the tip [34,36,38], and the width of the tip 1 μm from the tip, with which we can estimate tip surface area [37].

To test spine performance on targets with a homogeneous structure, and known, reproducible material properties, we used

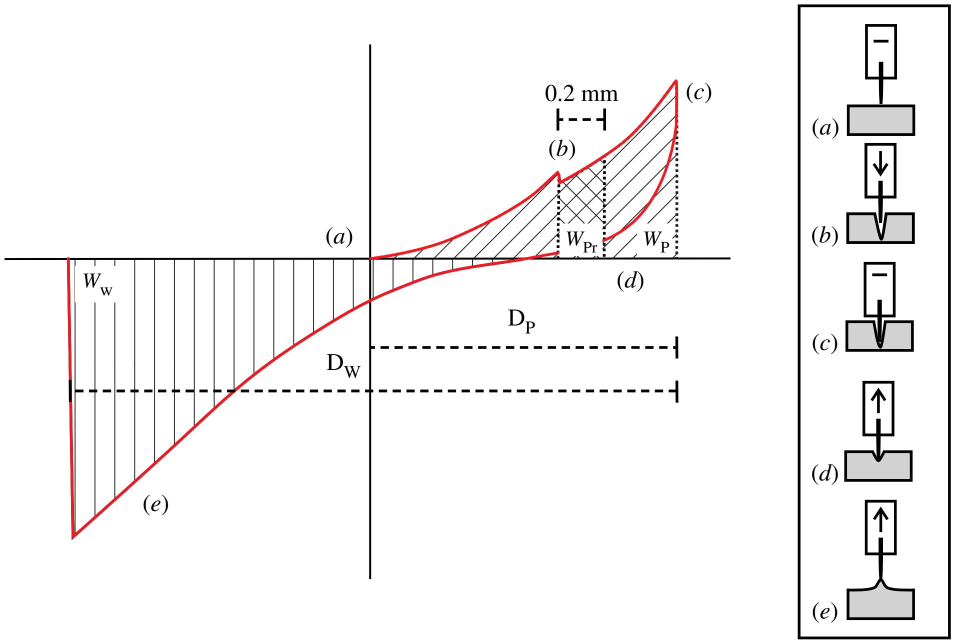

Figure 2. Example of a force–displacement trace and measurements for an idealized puncture event. (*a*) Puncture event (diagrammed in inset) begins with the spine at zero displacement, such that the tip of the spine is resting on the surface of the target (grey rectangle). (*b*) An increase in spine displacement (mm; *x*-axis), where the spine is lowered to fracture the target. (*c*) The spine is further lowered to propagate puncture to a pre-defined depth. (*d*) The spine is then raised (displacement decreases) and the spine withdrawn. (*e*) In some instances the spine may be raised above the surface of the target before the spine is disengaged. Measured metrics are labelled on the figure. (Online version in colour.)

two batches of polydimethylsiloxane (PDMS), one prepared by mixing Sylgard 184 silicone elastomer base and Sylgard 184 silicone elastomer curing agent in a 10 : 1 ratio by weight, the second by mixing the two components in a 25 : 1 ratio. The fully cured 10 : 1 PDMS has an elastic modulus of 2.66 MPa (s.d. 0.0797 MPa) and is overall less deformable than the 25:1 PDMS which has an elastic modulus of 0.98 MPa (s.d. 0.0368 MPa) [39]. To test spine performance on biologically relevant targets, we measured spine performance when puncturing unfrozen, skinless chicken breast, bought the same day as testing, and samples of fresh pig tissue provided by the University of Illinois at Urbana-Champaign (UIUC) Meat Sciences Department. Pig tissue was taken from the shoulder and included a 2 mm layer of skin, a 1.3 mm layer of underlying fat, and a 1–2 mm layer of muscle. For most taxa we were able to test five individual spines on each target. However owing to the limited number of *Echinopsis terscheckii* spines, we were only able to test four spines from that taxon on the 10 : 1 PDMS and were unable to test any on the chicken breast.

Individual spine performance was quantified by measuring the force (N) and displacement (mm) required to puncture and withdraw from a target material as shown in figure 2. Force/displacement data were used to calculate work (mJ) required to puncture and withdraw from the target. During testing, spines were glued to a holster attached to the 500 N load cell of an Instron 5944 (Norwood, MA, USA). During the test, spines were lowered at a rate of 30 mm min^{-1} until a predefined depth of penetration was achieved, held in place for 1–3 s, then raised at a rate of 30 mm min^{-1} until the tip of the spine was no longer embedded in the target.

For all tests, spines punctured the target material well before the predefined depth of penetration and we recorded this as the force to initiate fracture (F_I in N). For both the 10 : 1 and 25 : 1 PDMS targets, spines were lowered to 30% of the measured spine length. The only exception to this was *O. polyacantha*, the only species whose spines buckled during testing. In order to avoid the confounding variable of buckling effects, we lowered those spines to a set depth of 7 mm regardless of individual spine length. For the chicken and pig tissues, spines were lowered to 50% of the measured spine length, except *O. polyacantha* spines which were lowered to a depth of 6 mm to prevent buckling. To take the varying spine penetration depths into account, we standardized work to puncture by dividing the calculated work by the estimated fracture surface area at full puncture, based on puncture depth and the maximum spine width for that portion of the spine. This resulted in a measure of the work required to create a unit of fracture surface in the material (W_P in mJ mm^{-2}). We also calculated the work to propagate puncture immediately beyond initial fracture (W_{Pr} in mJ), as the area under the force–displacement curve between the displacement at initial fracture and 0.2 mm beyond that.

All tests were set up to continue data collection until spines were raised a known distance, greater than the depth of penetration, but if spines disengaged prior to reaching this point, we ended the test and data collection before the programmed endpoint. As before, we standardized the work of withdrawal by the known puncture depth, to account for friction (W_W in mJ mm^{-1}). We also calculated the relative withdrawal distance (pD_W) as the ratio between the distance the spine travelled before disengaging from the target material and the predefined depth of penetration (figure 2). For both types of PDMS, data collection continued until spines were retracted 30% of the total spine length past the starting position, or until the spines had disengaged from the elastomer. When testing the pig tissue, we initially set data collection to continue until spines were retracted 70% of the total spine length past the starting position, however this was not far enough for the spine to fully disengage from the tissue and we changed this to 300% of the total spine length for the remaining pig tests and all tests on the chicken breast. In the early instances when automated data collection stopped after the spines had retracted 70% but before the spine was fully withdrawn, the spine was raised at an unknown rate until released and the distance to withdrawal and F_W were recorded from videos of the digital handset. In two instances while testing the pig tissue, retraction of 300% of the total spine length was not far enough (see Results), and we were unable to collect displacement data but were able to measure the maximum force required to release the spine from the tissue, which we recorded for qualitative comparisons with the other data.

To determine if there is a degradation in performance with repeated spine use, we tested each individual spine an additional four times or until spines were no longer able to puncture the target material. This resulted in up to five tests per spine. Each additional test followed the same protocol as the described above. Performance metrics and Force–Displacement traces were qualitatively compared between subsequent tests of a single spine to determine if there was a noticeable change, and patterns of change were qualitatively compared between target materials. In the instance where a change in performance was observed, post-test ESEM images were collected for comparison to pre-test surface morphology.

Data were analysed with R for statistical computing v. 2.15.3 (2013-03-01– 'Security Blanket') [40]. Using the means and variance of the collected data, we calculated new effect sizes and re-ran our power analyses for each of our subsequent statistical tests (electronic supplementary material). We tested for species-level differences in tip sharpness using ANOVA and *post hoc* Tukey honest significant difference (HSD) tests. To determine how sharpness affects force to initial fracture (F_I), we used a Student's *t*-test to look for differences in F_I between sharp and blunt spines. Similarly, we used Student's *t*-tests to detect differences in W_P, W_{Pr}, W_W, and pD_W between barbed and non-barbed spines, and ANOVA and *post hoc* Tukey HSD tests to compare between species. For all of these analyses, we only looked at the first trial (of five) for each spine tested.

3. Results

Gross morphological spine measurements are summarized in table 1 and comparisons shown in the electronic supplementary material, figure S1. Variation in spine surface structure can be seen in figure 1. For both included angle and radius of curvature there is a general trend where *P. grandiflora* and *Echinopsis terscheckii* have blunter spine tips than *Echinocactus grusonii*, *O. fragilis*, *O. polyacantha* and *C. fulgida* (electronic supplementary material, figure S1). These morphological differences had an effect on fracture initiation with blunt spines requiring more force (N) and work (mJ) than sharp spines, though effect sizes for PDMS gels were too small for statistical comparison (electronic supplementary material, figures S2 and S3). Further emphasizing this difference, only spines from the taxa categorized as sharp were able to puncture pig tissue.

As expected, non-barbed spines take significantly more work to puncture than barbed spines in the 10 : 1 PDMS ($p \ll 0.01$), the 25 : 1 PDMS ($p \ll 0.01$), and the chicken breast ($p \ll 0.01$), but there is no significant difference in the work to puncture the pig skin, probably owing to the greater degree of deformation relative to the other target materials (figure 3). To account for this, we compared the work to propagate fracture (W_{Pr}) for both biological materials. This allows for a more direct comparison between spines, as we are comparing the work to expand the fracture a set amount. We found that W_{Pr} is significantly greater for non-barbed spines than for barbed spines for the pig tissue ($p \ll 0.01$), with a similar trend for the chicken (figure 3). Species level comparisons for W_P and W_{Pr} can be found in the electronic supplementary material, figure S4.

For both PDMS elastomers, non-barbed spines took significantly more work to withdraw from the elastomers than the barbed spines (10 : 1 PDMS $p \ll 0.01$, 25 : 1 PDMS $p \ll 0.01$; figure 4*a*,*b*). However in the biological tissues, barbed spines took more work to withdraw from target tissues,

Table 1. Mean cactus spine morphological measurements ± s.d. (Species highlighted in bold are considered to have 'blunt' spines, unbolded species have 'sharp' spines; all distributions are normal.)

species	mean spine length (mm)	mean tool width (mm)[a]		mean RoC (mm)	mean angle (°)	mean surface area (mm^2)	ornamentation
		PDMS	biological tissues				
***Pereskia grandiflora* (*n* = 5)**	**25.8 ± 5.82**	**0.58 ± 0.084**	**0.6 ± 0.139**	**0.077 ± 0.059**	**36.1 ± 15.1**	**$6.41 \times 10^{-4} \pm 1.42 \times 10^{-3}$**	**none**
***Echinopsis terscheckii* (*n* = 5)**	**30.2 ± 1.55**	**0.68 ± 0.612**	**n.a.**	**0.13 ± 0.067**	**51.7 ± 6.31**	**$1.39 \times 10^{-4} \pm 2.3 \times 10^{-4}$**	**none**
Echinocactus grusonii (*n* = 5)	25.6 ± 6.7	0.89 ± .154	1.2 ± 0.381	0.032 ± 0.015	11.9 ± 2.28	$1.14 \times 10^{-5} \pm 1.56 \times 10^{-5}$	reduced tubercles create undulating edge starting about 3 mm from tip of spine
Opuntia fragilis (*n* = 5)	10.0 ± 0.972	0.3 ± 0.036	0.3 ± 0.011	0.019 ± 0.011	13.1 ± 4.31	$9.05 \times 10^{-6} \pm 1.43 \times 10^{-5}$	partially barbed for the first 1.167 mm of the spine
Opuntia polyacantha (*n* = 5)	30.2 ± 3.06	0.25 ± 0.032	0.35 ± 0.035	0.027 ± 0.032	14.0 ± 1.82	$1.13 \times 10^{-4} \pm 2.51 \times 10^{-4}$	barbed for first 1.26 mm of the spine
Cylindropuntia fulgida (*n* = 5)	15.1 ± 3.62	0.34 ± 0.06	0.35 ± 0.014	0.015 ± 0.026	17.7 ± 5.31	$6.24 \times 10^{-5} \pm 1.39 \times 10^{-4}$	barbed for first 3.166 mm of the spine

[a] Maximum width for portion of spine used to puncture target material.

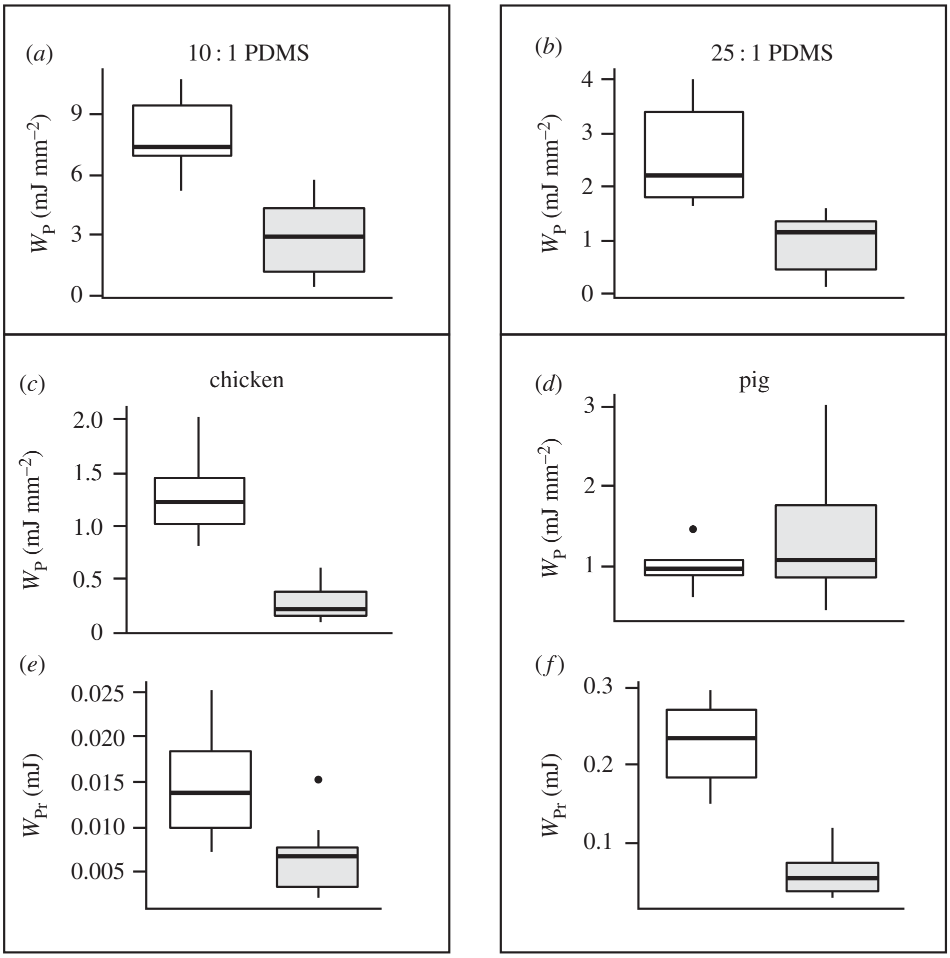

Figure 3. Comparison of work to puncture and work to propagate fracture. Non-barbed spines (white) require significantly more work to puncture (in mJ mm^{-2}) than barbed spines (grey) in (*a*) 10 : 1 PDMS, (*b*) 25 : 1 PDMS, and (*c*) chicken breast, but no significant difference can be determined in (*d*) porcine tissue. Non-barbed spines require more work to propagate fracture 2 mm beyond initial fracture in the (*e*) chicken breast and significantly more in the (*f*) porcine tissue.

though our effect sizes were too small for statistical comparison (figure 4*c*,*d*). When individual taxa are compared with an ANOVA, we did have enough power to show a significant difference in performance. *Opuntia polyacantha* spines required significantly more work to withdraw from the chicken than any other species ($p \ll 0.01$), and *C. fulgida* required significantly more work in the pig tissue trials ($p < 0.01$; figure 4*g*,*h*). Finally, the W_W for barbed spines was overall greater when being withdrawn from the porcine tissue than from chicken breast. Comparing ESEM images of post-puncture spines, those used to puncture chicken breast are covered with a layer of tissue, and those used to puncture pig skin retain no tissue and are missing barbs (figure 5).

Our effect size was not large enough to run a statistical analysis for the pD_W in the PDMS samples. For the biological tissues, pD_W of barbed spines was significantly greater than the pD_W of non-barbed spines (chicken $p \ll 0.01$, pig $p \ll 0.01$; electronic supplementary material, figure S5). This indicates that the barbed spines were required to withdraw further than the predefined puncture depth before disengaging from the biological targets. For the two instances where spines did not disengage even after 300% retraction, pig tissue hung suspended from *C. fulgida* spines, indicating that the weight of the test specimen was not sufficient to remove the spines. For these tests no withdrawal distance could be recorded.

We tested the puncture ability of each spine five times and observed no change or degradation in performance in either PDMS elastomer or in the chicken breast. When testing spines puncturing pig skin, we observed no progressive change in performance for *Echinocactus grusonii* but noticeable differences for species with barbed spines. *Opuntia polyacantha* spines were able to puncture the pig skin 2–5 times, with no observable difference in puncture performance and a decrease in W_W and pD_W. There was a stronger pattern of decreasing performance in *C. fulgida* spines, which were unable to puncture the pig skin after only one or two tests. W_P increased in the second test, when a second test was possible, and W_W and pD_W both decreased (electronic supplementary material, figure S6).

4. Discussion

The purpose of this study was to characterize how cactus spine shape and surface structure influences puncture and anchoring performance in a variety of materials. Cactus spines puncture passively, relying on the target to provide the force driving puncture. Therefore, we expected spines to be sharp in order to more efficiently initiate fracture. We expected barbs to serve as a means for reducing the work to puncture, as in porcupine quills, as well as to anchor the spine. When comparing the tip sharpness between different species of cacti, we found that spines can be broadly categorized as blunt or sharp based on either included angle or radius of curvature at the tip, and blunt spines generally

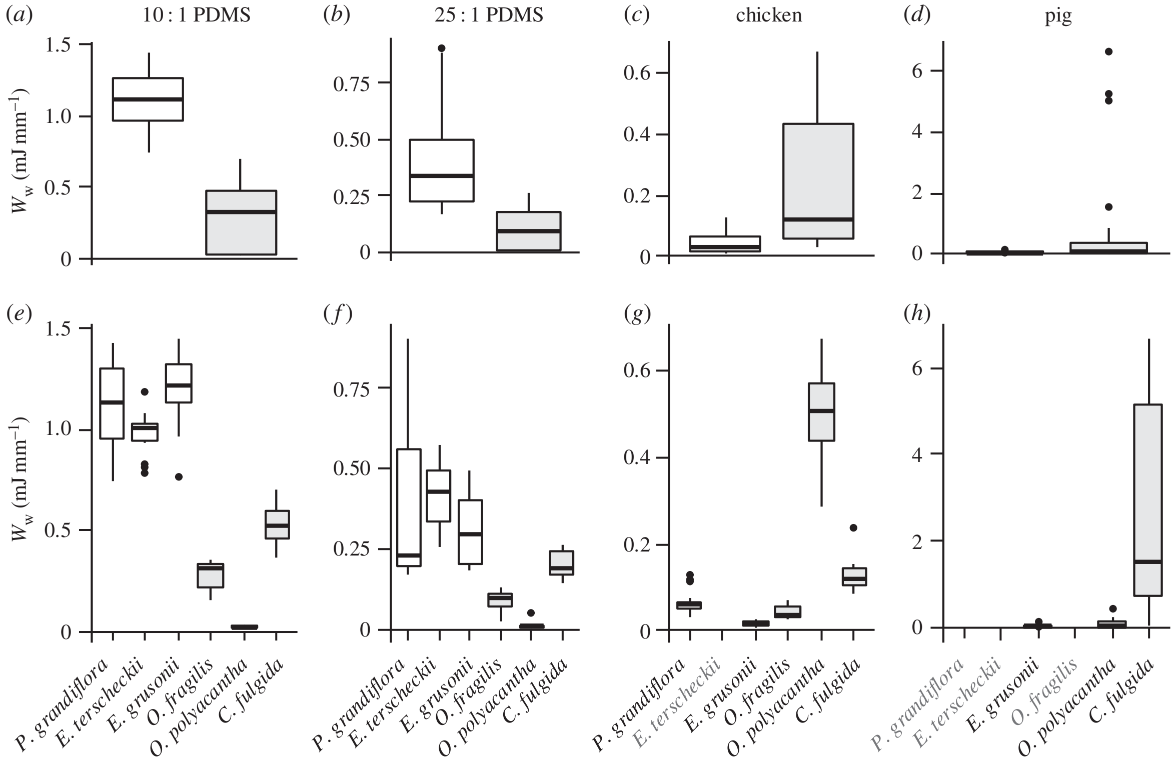

Figure 4. Work required to withdraw spine from target. Significantly more work is required to withdraw non-barbed spines (white) from 10 : 1 and 25 : 1 PDMS elastomers than barbed spines (grey; *a*,*b*,*e*,*f*). In biological materials, barbed spines require more work to withdraw from target material when examined together (*c* and *d*), but effect sizes were too small to determine significance. In chicken breast tests (*g*), *C. fulgida* required significantly more force to withdraw than any other species, followed by *O. polyacantha*, which required more force to withdraw than the remaining barbed and non-barbed spines. In the porcine tissue tests (*h*), *C. fulgida* required significantly more force to withdraw than either of the other species tested, barbed or non-barbed.

require more force to initiate fracture than sharp spines. We determined that barbed spines required less work to puncture than non-barbed spines, showing functional convergence with porcupine quills. Finally, barbed spines anchored very effectively in fibrous target materials, but not at all in homogeneous elastomers, again behaving similarly to porcupine quills.

(a) Mechanics of barbed spines

All opuntioid cacti studied here have barbed spines, though the proportion of the spine that is barbed and the extent to which the barbs encircle the surface of the spine varies between species. The presence of barbs leads to a significant decrease in the work required to propagate fracture and to puncture to a predefined depth, even when comparing only sharp spines. This is probably because the barbs act as stress-concentrators, localizing the applied strain in the target tissue and reducing the required force to penetrate, similar to what has been found in porcupine quills [6]. In addition to creating stress concentration, Cho *et al.* propose that quill barbs reduce force required to puncture muscle and reduce damage to the tissue by stretching muscle fibres, which are of a similar size as the barbs on a porcupine quill, rather than cutting them [6]. The barbs of the opuntioid cacti studied here are slightly shorter (50–105 μm) than the porcupine quill barbs (100–120 μm), but like the porcupine quill barb are on the same scale as muscle fibres, so may be acting in the same way [6].

In addition to decreased W_P, barbed spines also had significantly higher W_W and greater pD_W in biological tissues than non-barbed spines. Cho *et al.* determined that it was the action of tissue fibres being caught by the barbs during withdrawal that led to this increase in work, and found that porcupine quill barbs ‘deploy’, the barb tips are pulled away from the shaft of the quill, during withdrawal [6]. When post-test cactus spines were examined using ESEM, spines tested on chicken breast show fewer than 10 ‘deployed’ spines per spine compared with untested spines. However, these spines are covered in retained muscle tissue (figure 5). By contrast, spines that were tested in porcine tissue have less apparent tissue adhering to the spine, but have lost all terminal barbs, with intermittent barbs immediately proximal to the bare spine tip slightly deployed (figure 5). This is probably owing to the toughness of skin relative to muscle. Skeletal muscle fibres have a Young’s modulus that varies between about 2–4 Pa, and increases with the amount of cross-linked collagen present, though a relatively small proportion (1–2%) of skeletal muscle is composed of collagen [41,42]. Pig skin, composed of a complex interlocking network of predominantly collagen fibres, has similar mechanical properties to human skin, with a Young’s modulus of about 2 MPa [43].

In contrast to these fibrous biological tissues, barbed spines require less retraction distance and less work to disengage from the PDMS targets than non-barbed spines. PDMS is a gel that lacks any fibrous material, leaving nothing for the barbs to engage with. For porcupine quills, it was found that work to withdraw increased as fibre density increased. We find a similar result with barbed cactus spines when moving from a non-fibrous gel to chicken muscle and pig skin [6]. Without any material to engage with, the barbs may actually work to concentrate stress during spine retraction, as they do during penetration, allowing the barbed

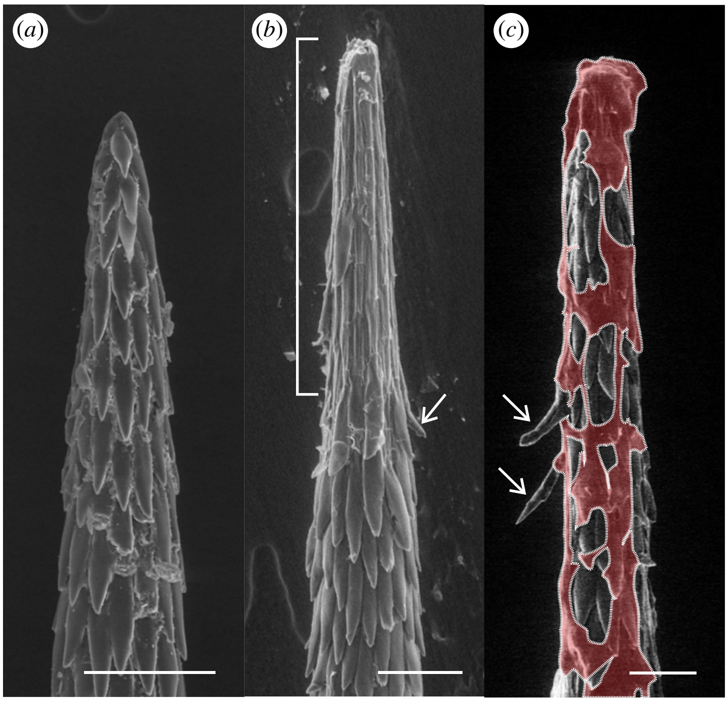

Figure 5. Comparison of spines before and after testing in biological tissues. *Cylindropuntia fulgida* spine (*a*) prior to testing, (*b*) the same spine after puncturing porcine tissue, missing terminal barbs (bracket), and (*c*) a separate spine after puncturing chicken breast with remaining chicken tissue outlined in white and shaded in red. White arrows in (*b*) and (*c*) indicate 'deployed' barbs. All scale bars are 100 μm. (Online version in colour.)

spines to cut their way out of the elastomers more effectively than the non-barbed spines [6]. The stark differences seen in cactus spine performance when different target materials are tested illustrates how the effect of barbed spines on puncturing and anchoring is contingent on the material being tested. This is not surprising, but should serve as a caution: that experimental outcomes can depend on the target properties as well as the morphology being tested.

(b) Functional morphology of cactus spines

The relative performance of both barbed and non-barbed spines in different biological materials is probably reflective of the different ecologies of these species. Spines probably first evolved as a form of defence from large to medium herbivores ([14,16–18] for an overall review of non-cactacea spinescence and [44,45] for a Cactaceae specific review). In keeping with this, all spines tested here were able to puncture skinless chicken breast, the target most similar to the inside of a vertebrate mouth. Spines that serve to deter herbivores by puncturing soft tissues are probably repeatedly subject to puncturing loads as new herbivores try their luck. This is supported by our observation that no species show a decline in performance when puncturing chicken breast, and that the non-barbed *Echinocactus grusonii* spines show no decline in performance when puncturing the pig tissue. The relatively long spines of species like *P. grandiflora*, *Echinopsis terscheckii*, *Echinocactus grusonii*, *O. polyacantha* and even *C. fulgida* may serve to dissuade herbivores not only via damage, but by increasing size and complexity of the non-edible portion of the plant [14,17]. Alternately, spines may serve to direct herbivores to eat only the cactus fruits as a hold-over from seed dispersing megafauna [45]. In South Africa, *Opuntia* cacti are an invasive species, but large mammals such as elephants and giraffes frequently consume their fruits [44]. With this in mind, it would be interesting to compare spine morphology and puncture ability between the New World cacti, especially non-barbed species, and Old World plants, such as the *Acacia* or the *Euphorbia*.

Only spines from three species, all with sharp spine tips, were able to puncture the pig tissue target. Of these three, *Echinocactus grusonii* was the only species with non-barbed spines, however the spines are not without ornamentation. Instead of barbs, as on the opuntioid cactus spines, the sides of *Echinocactus grusonii* spines undulate, which may be a result of secondarily reduced tubercles, starting approximately 3 mm from the tip of the spine ([23]; electronic supplementary material, figure S7). The change in width may act similarly to the barbs to help concentrate stress during crack propagation, but with less anchoring and without losing performance from barbs being deployed or broken after withdrawal. Spines of barrel cacti, such as *Echinocactus grusonii*, are robust puncturing tools that have been compared to steel needles, with recorded instances of a spine passing through a phalangeal bone without blunting [46].

Spines, especially barbed spines, can aid in asexual reproduction via vegetative propagation, a common strategy for opuntioid cacti. Some species depend more heavily on this method than others, and species that are more likely to reproduce via vegetative propagation will have more spines per areole and their spines will be more strongly barbed to better adhere to passing vertebrate fauna [30,31]. For dispersal, spines need only anchor once, which probably accounts for the decline in *C. fulgida* performance with repeated puncture events and the variable number of successful repeated puncture tests in *O. polyacantha*. Some species, the 'jumping' cacti, have easily detached terminal cladodes that will attach to passing vertebrates for active dispersal [30,31,47]. While *C. fulgida* is the only species studied here that the literature describes as a 'jumping' cactus [19,30,31], it is suggested that the wide ranges of both *O. fragilis* and *O. polyacantha* may in part be owing to vertebrate mediated dispersal, since the fruits of both are largely sterile and all three reproduce mainly asexually [27,29].

While spines from all three species with barbed spines were able to puncture the chicken breast and required significantly more work to withdraw than non-barbed spines, only *C. fulgida* and *O. polyacantha* were able to puncture the pig skin, and only *C. fulgida* required significantly more work to withdraw the spine from the pig skin than the non-barbed *Echinocactus grusonii* spine. This is of note, since spines adhering to passing vertebrates will be more likely to puncture skin than muscle. This firmer attachment will increase the range of dispersal, which is beneficial so long as the passing vertebrate travels only through areas where the cactus can live [26,48]. Not only does a single *C. fulgida* spine anchor firmly, but the recoil experienced by cladodes detaching from the parent plant will drive additional spines into the passing vertebrate, which may be driven further into the tissue with handling [46].

It is worth noting, however, that dispersal may not require spines to puncture skin. In the case of *O. fragilis*, which was unable to puncture pig skin, cladodes are small and easily detachable so dispersal may instead occur when spines become entangled in fur, clothing, or shoes [27–29]. Alternately, dispersal may not require vertebrate vectors, or may be less effective having evolved in tandem with now extinct fruit eating/seed dispersing megafauna [27,44,45,48]. Passive dispersal, where seeds, fruits, or stems fall to the ground and are not moved by animals, accounts for some proportion of cactus distribution and in some cases may be a more successful strategy [47,48]. *Opuntia fragilis* will form mats if left undisturbed, and it has been observed that only about 7% of cladodes will be dispersed annually [26,28,48]. *Opuntia polyacantha* has been observed to spread via root-sprouts rather than vegetative dispersal. In the case of passive dispersal, spines may instead serve to protect the cladode from damage or increase the odds of moving from the spot where they fell [26,48].

5. Conclusion

Opuntioid cacti and porcupine quills show a striking degree of morphological and biomechanical convergence across kingdoms. We have shown that the barbs found on cactus spines, like those on porcupine quills, aid in puncturing as well as anchoring spines in fibrous tissue, though the proportion of the spine covered with barbs and the type of material being punctured are important factors to consider. This is in no way an exhaustive look at cactus spine ornamentation, and much work remains to be done comparing between barbed taxa, comparing function of glochids to spines of the same individual and between taxa, as well as comparing performance of different types of ornamentation such as tubercles [23,49]. Moreover, the striking convergence seen here suggests that examples of similar ornamentation on spines may exist in other disparate groups that follow analogous patterns of form and function.

Data accessibility. Additional figures and results have been uploaded as part of the electronic supplementary material. The raw data from puncture experiments are archived in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5878v18 [50].

Authors' contributions. S.B.C. helped design the study, carried out morphology data collection and analysis, experimental testing of spine function, analysis of puncture performance data, and drafted the manuscript. P.S.L.A. conceived and designed the study, carried out experimental testing of spine function and helped draft the manuscript.

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