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PRIMER

Model systems for regeneration: the spiny mouse, *Acomys cahirinus*

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ABSTRACT

The spiny mouse, *Acomys* spp., is a recently described model organism for regeneration studies. For a mammal, it displays surprising powers of regeneration because it does not fibrose (i.e. scar) in response to tissue injury as most other mammals, including humans, do. In this Primer article, we review these regenerative abilities, highlighting the phylogenetic position of the spiny mouse relative to other rodents. We also briefly describe the *Acomys* tissues that have been used for regeneration studies and the common features of their regeneration compared with the typical mammalian response. Finally, we discuss the contribution that *Acomys* has made in understanding the general principles of regeneration and elaborate hypotheses as to why this mammal is successful at regenerating.

KEY WORDS: Spiny mouse, *Acomys*, Tissue regeneration, Fibrosis, Scarring

Introduction

The field of regenerative medicine aims to identify strategies to either engineer or repair human tissues, organs and body parts that cannot naturally be replaced when damaged by trauma or disease. Who would not want to take a drug to stimulate the proliferation of cardiomyocytes and recover from a heart attack? Or, stimulate axonal regrowth across a site of contusion in the spinal cord and recover function of the lower body and extremities? To solve this problem of inducing regeneration in humans, conventional wisdom suggests that we must identify the molecular and cellular signals that guide regeneration in regeneration-competent organisms, such as axolotls and zebrafish, and extrapolate what we learn to induce it in regeneration-incompetent organisms such as rats and mice, and eventually humans.

But is it true that mammals really are regeneration-incompetent organisms? The general impression would surely be yes, mammals cannot regenerate, but a deeper look across the remarkably few species that have been investigated reveals some surprises. For example, human children and mice can regenerate their digit tips (Illingworth, 1974; Yu et al., 2019); mammalian fetuses can heal skin wounds in a regenerative manner (Yates et al., 2012); male deer can annually regenerate their antlers, which are initially covered in skin or velvet which itself has regenerated (Goss, 1983); young C57BL/6J mice can regenerate hair follicles via wound-induced hair follicle neogenesis following large skin wounds (Ito et al., 2007); the neonatal mouse heart can regenerate until postnatal day 7 (Porrello et al., 2011); and some species can regenerate large holes punched through their ears, including rabbits (Gawriluk et al., 2016;

Voronstova and Liosner, 1960) and perhaps also chinchillas, cows and pigs (Williams-Boyce and Daniel, 1986). It is also known that several individual mammalian tissues can regenerate, such as skeletal muscle after myotoxin administration (Musarò, 2014) and the liver, which displays prodigious powers of proliferation during compensatory hypertrophy (Fausto et al., 2012). This compensatory hypertrophy is a process which the lungs can also undergo (Hsia, 2017), whereby the tissue remaining after removal of part of the organ expands to compensate for the missing part. Many mammalian epithelial tissues, such as the epidermis or the intestinal lining, also exhibit continuous replacement, although this is a property of all animals and so is not considered an unusual regenerative process. Admittedly, all of these regenerative abilities observed in mammals are limited compared with those seen in axolotls (Joven et al., 2019) or zebrafish (Marques et al., 2019), but this only represents a very narrow sampling of extant mammalian species and there may be some truly regeneration-competent mammals out there that remain undiscovered.

Spiny mice, *Acomys* spp., are one such example of a regeneration-competent mammal, regenerating several tissues of their body to full functionality after injury – rather than the reduced functionality normally observed after scarring or fibrosis. Here, we provide an overview of the history and regenerative abilities of spiny mice. We propose that if fibrosis can be prevented in humans – as it is in spiny mice – then we may be able to regenerate a surprising array of tissues. Moreover, we highlight how the study of spiny mice can allow us to begin to compare regeneration-competent mammals with regeneration-incompetent mammals to discover the cellular and molecular signals governing regeneration.

General background on *Acomys*

There are 2050 living species of rodents (which are defined by having upper and lower pairs of continually growing incisors) and two thirds of these belong to the family Muroidea. There are likely 16 subfamilies of Muroidea including the old world rats (*Rattus*)

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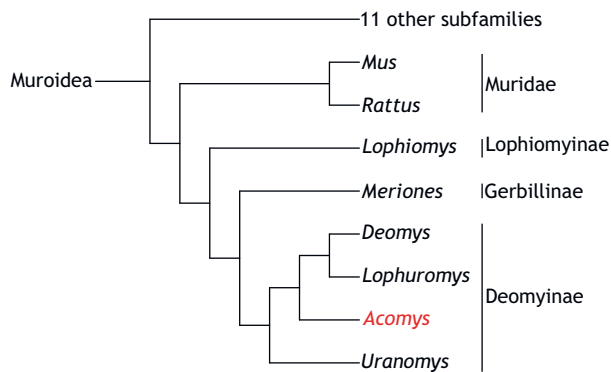*Author for correspondence (malcmaden@ufl.edu)

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Model systems for regeneration

This article is part of a series entitled 'Model systems for regeneration'. This series of articles aims to highlight key model systems and species that are currently being used to study tissue and organ regeneration. Each article provides background information about the phylogenetic position of the species, its life-cycle and habitat, the different organs and tissues that regenerate, and the experimental tools and techniques that are available for studying these organisms in a regenerative context. Importantly, these articles also give examples of how the study of these models has increased our understanding of regenerative mechanisms more broadly, and how some of the open questions in the field of regeneration may be answered using these organisms. To see the full collection as it grows, please visit: https://dev.biologists.org/collection/regeneration_models.

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Fig. 1. Phylogeny and phenotype of *Acomys*. Phylogenetic position of the genus *Acomys* among the 15 sub-families of the family Muridae. There are four genera including *Acomys* in the sub-family Deomyinae, the closest relatives of which are the gerbils. Adapted from Steppan and Schenk, 2017.

and mice (*Mus*), which are placed in the subfamily Muridae (Steppan and Schenk, 2017). Spiny mice of the genus *Acomys* are placed in one of the other subfamilies called Deomyinae (previously called Acomyinae) along with Rudd's mouse (*Uranomys*), the Congo forest mouse or link rat (*Deomys*), and the brush-furred mouse (*Lophuromys*) (Steppan and Schenk, 2017) (Fig. 1). The closest relatives to Deomyinae are the gerbils of the subfamily Gerbillinae (Chevret et al., 1993; Steppan and Schenk, 2017) from which they separated 17.6-20 million years ago.

There are at least five major groups in the genus *Acomys*: subspinosus, spinosissimus, russatus, wilsoni and cahirinus (Aghová et al., 2019). All species share morphological characteristics of dorsal spine-like hairs, which are golden brown, grey or rusty brown depending on the species. Ventral hairs are white. Spiny mice are distributed throughout Africa, the eastern Mediterranean, some Mediterranean islands, the Arabian Peninsula, Iran and Pakistan (Jeremy and Bates, 1994). Only three species have been tested for regenerative ability: *Acomys percivali* (from the wilsoni group), *Acomys kempi* (from the cahirinus group) and

Acomys cahirinus (Gawriluk et al., 2016; Seifert et al., 2012). All three have been shown to regenerate tissue of the ear following a biopsy punch, although only the regeneration of *A. cahirinus* has been studied extensively. Thus, we might assume that regeneration is a property of the genus.

The colonies that are currently maintained in Europe, USA and Australia are *A. cahirinus*, although one colony of *Acomys dimidiatus* is maintained in Geneva (Montandon et al., 2014). Because of their relatively long history of use as laboratory animals, there are several papers published on the care, maintenance and management of colonies, from early days (Strasser, 1968; Young, 1976) up until the present day (Dickinson and Walker, 2007; Haughton et al., 2016; Pinheiro et al., 2018). From these latter works it appears that their lifespan is usually 3-4 years, but can extend to 6 years.

Spiny mice have a complex social organization and are often housed in groups of up to 20 to permit communal breeding, although keeping three to five females and one male together in a cage allows the parentage of the pups to be determined (Haughton et al., 2016). Gestation lasts 39 days, considerably longer than in other rodents, and one to four pups (usually two or three) are produced. The pups are precocial and born with grey fur, open eyes and unfurled ears, and they are soon mobile and eating solid food. After 6 weeks, the grey coloured pups (Fig. 2) start producing the thick spiny hairs – from posterior to anterior, on the dorsum – and as a result change their coat colour to that of the adult (Fig. 2). They become sexually mature at 3-4 months of age (Dieterlen, 1961). Females have an 11-day oestrus cycle after which they undergo menstruation (Bellofiore and Evans, 2019). Mating does not result in the formation of a vaginal plug, making identification of the day of fertilization impossible, although later stages of gestation have been identified by ultrasound (Dickinson and Walker, 2007).

Although spiny mice have recently come to prominence because of their striking ability to regenerate several organs and tissues, as described below, they have been used as research animals and kept in breeding colonies since at least 1911 (Bonhote, 1911). Indeed, it is surprising that their regenerative abilities had not been observed before 2012 (Seifert et al., 2012). Previous research using spiny mice

Fig. 2. Hair colour and development in *Acomys*. Images of the hair colour at three stages of development of *Acomys cahirinus* viewed from the side (top row) and from above (bottom row). Pups (left column) are born with grey hairs; the golden spiny hairs first begin to appear on the caudal dorsum as sexual maturity approaches (middle column), spreading completely over the dorsum by adulthood (right column). See Jiang et al. (2019) for details on different hair types and their development.

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has included studies into diabetes, because these mice show spontaneous degeneration of the islets of Langerhans and are prone to hyperglycaemia and diabetes with obesity but without insulin resistance (Creutzfeldt et al., 1970; Pictet et al., 1967; Shafrir et al., 2006). They have also been used to study renal physiology because they have among the highest recorded urine urea concentration (of 4.7-4.8 M), likely linked to their desert dwelling (Shkolnik and Borut, 1969). Recent research has also described them as the first known menstruating rodent (Bellofiore and Evans, 2019; Bellofiore et al., 2017, 2018), despite early studies on their reproductive physiology (Dewsbury and Hodges, 1987; Peitz, 1981; Peitz et al., 1979).

Studies on the development of spiny mice have been expansive, examining olfactory response during early post-natal life (Janus, 1988, 1993; Porter and Etscorn, 1976; Porter et al., 1978a,b, 1982, 1986, 1989); fetal, parental and social behaviour (Makin and Porter, 1984; Nováková et al., 2008; Porter, 1976; Porter et al., 1977, 1980, 1981, 1983; Robinson and Smotherman, 1992), and the development of the kidney (Dickinson et al., 2005), lung (Oosterhuis et al., 1984), brain (Brunjes, 1989; Brunjes et al., 1989), endocrine system (Lamers et al., 1986; Quinn et al., 2013) and spiny hairs (Montandon et al., 2014). Recent research has also used spiny mice as a model of birth asphyxia (Hutton et al., 2009a,b). Finally, the precocial spiny mouse has been proposed as a better model for human pregnancy and birth compared with the altricial mice and rats normally used because the development of the kidney (Dickinson et al., 2005), liver (Lamers et al., 1986), lung (Oosterhuis et al., 1984) and various brain regions (Brunjes, 1989) is essentially completed by the time of birth, which is more similar to humans than the continued development and maturation during the neonatal period of other commonly used rodents (Dickinson and Walker, 2007).

Tools and techniques for studying *Acomys*

As the spiny mouse is a very new model organism to enter the field of regeneration, there has been little time to develop tools and techniques that would enable a molecular analysis of its regenerative ability. Furthermore, there are some characteristics that make it a difficult organism for developing such techniques.

As mentioned above, females do not plug following copulation, making the determination of the day of fertilization difficult. An alternative approach to obtaining embryos of precise stages is to coordinate breeding immediately following birth of a litter when the female is fertile in postpartum oestrus, thus providing the day of conception for the subsequent litter (Dickinson and Walker, 2007). This method has been used to obtain two-cell, four-cell and eight-cell embryos for studying gene transcription (Mamrot et al., 2018 preprint). In addition, ultrasound techniques have been developed for pregnant *Acomys* making it possible to detect fetuses from day 12 of gestation (Dickinson and Walker, 2007). However, female *Acomys* only produce two to three pups per pregnancy, making the large-scale production of embryos rather difficult, although *A. dimidiatus* tends to have a larger litter size (Frynta et al., 2011). Superovulation techniques are available though (Pasco et al., 2012), so the development of *in vitro* systems for *Acomys* embryo culture should be possible.

Cell culture of *Acomys* tissues is commonly used, with media composition based on that used to culture mouse tissues (Simkin et al., 2017; Stewart et al., 2018). In theory, the generation of immortalized cell lines, transfected cells for lineage studies and even induced pluripotent stem cells (iPSCs) should therefore now be possible. Likewise, using CRISPR to alter cells is feasible because genetic information is available from three published transcriptomes: one derived from ear regeneration (Gawriluk et al., 2016), one from early embryos (Mamrot et al., 2018 preprint) and one from skin regeneration (Brant et al., 2019). Annotated genomes will soon be available from several groups.

Immunocytochemistry is routinely performed with a range of commercially available antibodies that have been used to label a large variety of proteins in *Acomys* tissues, ranging from extracellular matrix (ECM) proteins, immune cell markers, cytoplasmic proteins to nuclear transcription factors (Table 1). There are at least 88 such antibodies reported and ELISAs have also been used to detect cytokines, thus there appears to be little problem of cross-reactivity with these antibodies, and where protein homology has been compared between *Acomys*, *Mus* and human

Table 1. Summary of the *Acomys* proteins that have successfully been detected using antibodies via immunocytochemistry, ELISA and flow cytometry analyses

Category	Proteins
ECM/adhesion proteins	Collagen I, collagen III, collagen IV, collagen VI, collagen VII, collagen XII, collagen XVII, fibronectin, tenascin C, laminin, laminin α2, vimentin
Cell surface/plasma membrane proteins	E-cadherin, dystrophin, PDGFRα
Signalling components/transcription factors	pSMAD1/5/8, β-catenin, LEF1, Nkx-2.2, Nkx-6.1, MafA, MafB
Cytokeratins/epidermis markers	Pan cytokeratin, KRT17, K14, K10, K17, AE13, AE15, K15, TRP63, MitfD5
Proliferation markers/cell cycle proteins	Ki67, PCNA, pHH3, p21, p27, p16, p19, p53, p63, pRB, gH2AX
Stem cell markers	SOX2, Pax7, CD34, CD200
Cytoplasmic proteins	α-Smooth muscle actin, MHCIIA, MHCIIB, MyoD, eMHC, perilipin, Nf-κB, Lamp1, fractin
Enzymes	P450c17, cytochrome b5, 3bhsd, tyrosine hydroxylase, caspase 3, Arg1
Neural proteins	β-Tubulin, neurofilament H, synaptophysin, GFAP, Neu-N, peripherin, CNPase
Hormones and receptors	Prolactin, Era, ERb, PR, AR, vasopressin, glucagon, insulin, somatostatin, pancreatic polypeptide, peptide YY
Immune cell proteins	F4/80, MOMA-2, CD206, IBA1, CD86, MPO, CD3, CD11b
Factors detected by ELISA	IL1a, IL1B, IL1ra, IL2, IL4, IL5, IL6, IL12, IL16, IL17, CXCL2, CSF2, CCL2, CCL3, CCL5, CXCL1, CXCL13, TNFα, IFNγ, C5
Factors successfully detected via flow analyses	F4/80, CD3, CD196, CD11b, CD206, CD49b
Factors unsuccessfully detected via flow analyses	CD45, TCRab, TCRgd, CD4, CD8, CD25, CD69, CD196, CD49b, CD19, CD23, CD40, CD44

The last row shows those antibodies that are commonly used for *Mus*, but have failed to identify immune cell types in *Acomys* using flow cytometry. Data from: Bellofiore et al., 2017; Brant et al., 2015, 2016, 2019; Castel and Hockman, 1978; Gawriluk et al., 2016, 2019; Gustavsen et al., 2009; Hułas-Stasiak and Gawron, 2007, 2010, 2011; Hutton et al., 2009a,b; Jiang et al., 2019; Maden 2018; Maden et al., 2018; Matias Santos et al., 2016; Montandon et al., 2014; Okamura et al., 2018 preprint; Pennello et al., 2006; Quinn et al., 2013; Saxena et al., 2019; Seifert et al., 2012; Simkin et al., 2017; Streeter et al., 2019.

it is expectedly high (Gawriluk et al., 2019 preprint). In contradiction, however, very few antibodies used for *Mus* T cell analysis by flow cytometry cross-react with *Acomys* (Gawriluk et al., 2019 preprint; Pennello et al., 2006), suggesting that an investment in antibody production in this area of research would be highly beneficial.

Thus, the typical reagents and techniques used in regeneration research are mostly available for use with *Acomys*. Once our understanding of the embryology and reproductive physiology of *Acomys* has advanced, it should be possible to generate transgenic animals, although the small litter size will presumably continue to be a significant drawback to rapid progress.

Tissue regeneration in *Acomys*

Despite more than a century of keeping colonies, the regenerative abilities of *Acomys* were not reported until 2012 (Seifert et al., 2012). Then, it was reported that spiny mice captured in the wild with large areas of dorsal skin missing could regenerate their skin successfully; it was also noted that their skin was weak and could tear easily. Their weak skin was previously reported in the context of the care and general biology of spiny mice. These early reports advised to avoid tail-handling, as the spiny mice tail is weak and can deglove (Bate, 1903; Shargal et al., 1999), and suggested that frequent bite-wounds could be treated with antibiotic spray 'until the fur re-grows' (Dickinson and Walker, 2007). Since these early reports, research into the regeneration of spiny mouse tissues has expanded from skin and ear punches to include skeletal muscle, kidneys and the spinal cord (Fig. 3, Table 2).
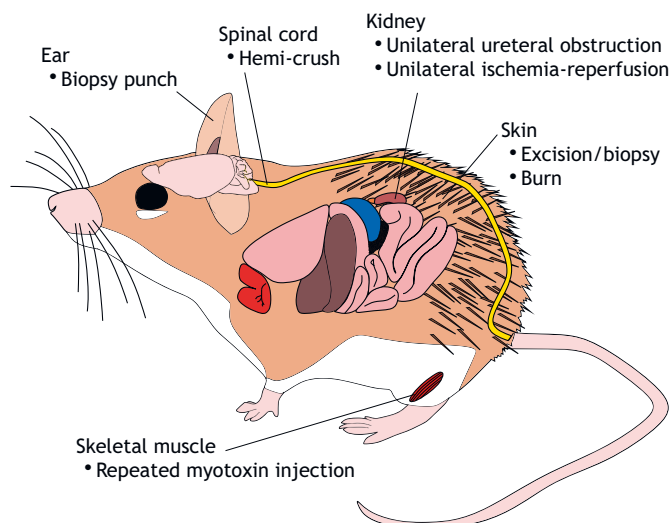
Skin

After full-thickness skin removal or full-thickness burn injury, all the components of the *Acomys* skin are eventually regenerated, with each component following their own time scale (Brant et al., 2019, 2016, 2015; Maden, 2018; Seifert et al., 2012). Scab formation and haemostasis is rapid, and more than 50% of the wound area can close within 24 h after injury (Seifert et al., 2012). The epidermis – the outermost layer of the skin – responds immediately by inducing

Fig. 3. Tissue and organ regeneration in *Acomys*. Schematic of *Acomys cahirinus* highlighting the tissue types that can regenerate as well as the methods that have been used to investigate them.

proliferation around the wound margin, which thickens as a result and then migrates across the wound. The rate of epithelial migration is notably quicker than that observed in mouse or rat skin (Seifert et al., 2012), and *in vitro* wound healing experiments reveal that keratinocytes migrate twice as fast in *Acomys* compared with *Mus* (Stewart et al., 2018). By the end of the second week, new hair placodes are seen in the wound epithelium and regenerate through defined stages, exhibit high proliferation and reuse molecular pathways from embryonic hair follicle development. The hair follicles arise in response to Wnt signalling as determined by expression of LEF1, a nuclear transcription factor that is a readout of Wnt signalling (Brant et al., 2019; Seifert et al., 2012). *Wnt7a*, which is known to induce hair follicles in transgenic mice (Ito et al., 2007), is also expressed at this time (Brant et al., 2019), suggesting similar pathways are used in *Acomys* and *Mus* for hair follicle induction in the epidermis.

During hair development in *Mus*, the epidermal placode appears first and is induced by a signal from the dermal mesenchyme, the so-called 'first dermal signal', the nature of which is unknown but may be another Wnt signal (Millar, 2002). Fibroblast growth factors (FGFs) and *Shh* are also involved as mesenchymal signals in hair development and, based on studies of the wound-induced hair follicle neogenesis (WIHN) mouse model, it is known that *Fgf9* overexpression increases the number of hairs induced by signalling a feedback loop in dermal fibroblasts involving *Wnt2a* (Gay et al., 2013). Similarly, ectopic expression of *Shh* in the WIHN mouse model induces extra hair follicles (Lim et al., 2018). Moving forward, it will be interesting to see whether the same events occur during follicle regeneration in *Acomys*.

Following hair follicle induction, hair placodes in the *Acomys* wound epithelium grow and deepen into the newly formed tissue of the wound bed to generate a hair with a dermal papilla. The three types of hair normally present in *Acomys* skin (guard, awl and zigzag) are regenerated in the same proportions (Jiang et al., 2019). In the fourth week, sebaceous glands develop, along with erector pili muscles that elevate the new hairs, making them fully functional (Brant et al., 2016).

Fibroblasts within the dermis – the layer beneath the epidermis – migrate into the wound bed to replace the missing tissue. These cells generate a new matrix that is lower in cell density than that in the corresponding wound site in *Mus*. As might be expected, there is a different collagenous organization and composition between scarring *Mus* wounds and regenerating *Acomys* wounds, with the wound bed of *Acomys* being arranged more loosely and being lower in density than that of *Mus* (Brant et al., 2016). Concerning the composition, *Acomys* have low levels of collagens compared with *Mus*, particularly collagen 12a1 (Brant et al., 2015, 2016), which is known to stabilize the ECM and is present in dense connective tissues. The most obvious matrix-associated differences are in the matrix remodelling proteins (MMPs), which are highly induced in *Acomys*, particularly MMP2 and MMP9 (Brant et al., 2015). By contrast, tissue inhibitors of MMPs (TIMPs), are highly induced in *Mus*. Considering MMPs and TIMPs in *Acomys* and *Mus*, it is possible that ECM degradation is more active in *Acomys* wound healing. Another noteworthy and highly upregulated matrix-associated gene in *Acomys* wounds is the collagen triple helix repeat-containing gene, *Cthrc1* (Brant et al., 2019). Interestingly, the function of this secreted protein has primarily been investigated in cancer metastasis, which Cthrc1 promotes by enhancing migration, reducing collagen I expression and production (Pyagay et al., 2005) and upregulating Wnt signalling and MMP9 expression (Guo et al., 2017), all highly relevant to skin regeneration. This

Table 2. Summary of the tissues that have been used in *Acomys* regeneration studies

Organ/tissue	Injury method	Reference(s)	General conclusions/comparison with *Mus*
Skin	4 mm excision	Seifert et al. 2012	*Acomys* rapidly re-epithelializes, subsequently regenerating hair follicles, hair with dermal papilla, sebaceous glands, erector pili muscles and skeletal muscle of the panniculus carnosus. There are significant differences in cytokines, immune cells and matrix composition between *Mus* and *Acomys*.
	1.5 cm excision	Jiang et al. 2019; Seifert et al. 2012	
	8 mm biopsy punch	Brant et al. 2015, 2016, 2019	
	1.5 cm burn (100°C)	Maden 2018	
Ear	4 mm biopsy punch	Gawriluk et al. 2016; Matias Santos et al. 2016; Seifert et al. 2012	*Acomys* re-epithelializes then generates a thickened epithelium with underlying mesenchyme resembling a blastema. Blastema grows across the biopsy in a proximal-distal direction. Hair, skin and cartilage regenerate. Histological analysis 3 months after wound closure indicates variable presence of regenerated muscle.
	8 mm biopsy punch	Gawriluk et al. 2016	
Skeletal muscle	Repeated myotoxin injection	Maden et al. 2018	*Acomys* and *Mus* regenerate muscle after single injection, yet *Acomys* does so quicker. After five successive injections 3 weeks apart, *Mus* fails to regenerate and replaces damaged muscle with fat cells, whereas *Acomys* continues to regenerate.
Kidney	Unilateral ureteral obstruction	Okamura et al. 2018	*Acomys* shows full restoration of kidney function after injury without fibrosis, contrasting with renal failure and fibrosis observed in *Mus*.
	Unilateral ischaemia-reperfusion injury	Okamura et al. 2018	
Spinal cord	Hemi-crush of cervical region	Streeter et al. 2019	*Acomys* has reduced spinal inflammation and fibrosis, with reduced collagen deposition. Bladder clearing suggests restoration of function within 48 h, contrasting with 21 days in *Mus*.

matrix protein, along with tenascin C, tenascin N, fibronectin, laminin α1, fibrillin 2 and aggrecan, produces a loose 'regenerative matrix' in the *Acomys* wound bed (Brant et al., 2019; Seifert et al., 2012).

There is also a layer of skeletal muscle at the base of the skin, the panniculus carnosus, which is particularly well developed in rodents. Normally, holes punched through skeletal muscle in mice cannot regenerate because the connective tissue component of the muscle driving regeneration has been removed (Ciciliot and Schiaffino, 2010). As such, muscle fails to regenerate in a *Mus* skin wound and the formed scar is thinner than the original skin. However, the muscle in *Acomys* can fully regenerate (see Skeletal muscle section). In the second and third weeks after skin wounding, the levels of embryonic myosin (Myh3) rise 450-fold compared with baseline levels, and strong staining of this protein can be seen across the wound bed. After the completion of skin regeneration, at ~7-8 weeks, mature muscle myosin can be seen in the regenerated muscle and new neuromuscular junctions demonstrate that this regenerated muscle of the panniculus carnosus is functional (Brant et al., 2019, 2016).

Ear punches

Observing the effect of a through-and-through ear punch has been a quick and easy method for assessing the regenerative ability of mammals for many years (cf. Goss, 1987). The ear consists of a plate of cartilage covered by skeletal muscle (with more on the dorsal side), adipocytes, connective tissue, dermis, and epithelium containing hairs with their associated sebaceous glands and erector pili muscles. Thus, full regeneration of the ear punch is clearly a complex phenomenon, requiring the replacement of a considerable number of tissues, and it greatly resembles full-thickness skin regeneration apart from the mode of epithelial healing and the absence of cartilage in skin.

Holes, typically with a diameter of 4 mm (ranging from 2-8 mm), are regenerated in all species of *Acomys* that have been examined (Gawriluk et al., 2016; Matias Santos et al., 2016; Seifert et al., 2012). Following damage, the epidermis migrates over the wound to cover the internal tissues, which begin to lose their differentiated characteristics as if they were dedifferentiating, as occurs during

salamander limb regeneration. Unlike the skin, epithelial closure of the wound occurs more rapidly in *Mus* than in *Acomys* (Gawriluk et al., 2016) or at a similar rate (Matias Santos et al., 2016). The epidermis becomes thickened, another characteristic of the amphibian limb, and by the end of the second week a structure resembling a 'blastema' (the regenerating tissue mass in salamanders) forms (Gawriluk et al., 2016; Seifert et al., 2012). Proliferation of blastemal cells elongates the structure and, by the end of the third week, re-differentiation of a variety of tissues occurs. New hair follicles differentiate in a proximal to distal spread, whereas new cartilage differentiates abutting the cut end of the old cartilage. By 8 weeks, the 4 mm wound has completely filled in, with new hairs and skin covering the regenerated plate of cartilage and new muscle (Matias Santos et al., 2016).

Interestingly, the hole does not regenerate equally around the circle created by the wound; instead, regeneration occurs from the proximal part (closer to the head), whereas the distal part hardly regenerates at all, making the final closure of the hole displaced from the centre (Matias Santos et al., 2016). This is most likely because, again like the salamander limb, regenerating ear punches depend on a functional nerve supply (Buckley et al., 2011) and the nerve fibres are differentially distributed across the wound (Gawriluk et al., 2016). The proximal semicircle of the punch wound has a plentiful supply of axons from the auricular nerve, but the axons in the distal semicircle of the wound are severed, making the distal part effectively denervated. The role of nerves in axolotl limb regeneration is well established (Stocum, 2019) and acts via the axonal and probably Schwann cell-based synthesis of the growth factor Neuregulin 1, which is released into the blastemal milieu to stimulate blastemal cell proliferation (Farkas et al., 2016). It would be of great interest to determine whether Neuregulin 1 is present in the *Acomys* auricular nerve and whether its inhibition prevents ear hole regeneration. This would re-ignite the debate of whether the absence of regeneration in various systems is related to an insufficiency of nerves and/or neuregulin growth factors.

Skeletal muscle

In mammals, skeletal muscle normally regenerates repeatedly throughout life, owing to the presence of Pax7-positive stem cells,

called satellite cells (Chargé and Rudnicki, 2004; Musarò, 2014). This regeneration occurs following physical or toxic insults to skeletal muscle, such as injection of cardiotoxin, physical injury from freezing or crushing, or chemical injury. In general, these insults induce the breakdown of cell membranes, the invasion of immune cells, destruction of actin/myosin filaments, activation of satellite cells, the re-expression of embryonic and developmental myosins and myogenic regulatory factors, and subsequent regeneration of muscle fibres. In mice, a common muscle for investigation is the tibialis anterior, which is found at the front of the lower leg. After myotoxin injection, the regeneration of this muscle is unexpectedly fast and is essentially complete by the third week (14-16 days) after damage (Chargé and Rudnicki, 2004).

Not surprisingly, *Acomys* can also regenerate the muscle fibres of the tibialis anterior following similar insults, but does so faster – within 10 days – when compared with *Mus* (Maden et al., 2018). Within 6 days, newly regenerated myofibres are present and the regenerating muscle expresses embryonic myosin and higher levels of dystrophin. Following a consistent theme, there are lower levels of inflammation (see below) and fibrosis as measured by Nf-κB levels, as well as lower levels of collagen I, collagen III and collagen XII, and less necrosis. The damaged muscle becomes hugely infiltrated with M2 pro-regenerative macrophages, which also occurs in *Mus*, but strikingly lacks MI pro-inflammatory macrophages and has higher levels of the anti-inflammatory chemokine Cxcl12.

A more dramatic difference between *Mus* and *Acomys* muscle emerges when the tibialis anterior muscle is subjected to repeated rounds of regeneration (Maden et al., 2018). After five sequential myotoxin injections, spaced 3 weeks apart, the *Acomys* tibialis anterior continues to regenerate perfectly, as it did after only one round of regeneration. By contrast, the *Mus* tibialis anterior fails to regenerate and becomes largely composed of fat cells, which replace the muscle fibres and differentiate in the interstitium between the fascicles, a result typical of repeated muscle injury and showing striking resemblance to muscle in advanced cases of Duchenne muscular dystrophy (Uezumi et al., 2010, 2011).

As described above, a hole punched through the panniculus carnosus of *Acomys*, which is a skeletal muscle layer present at the base of the dermis, regenerates completely in ~8 weeks and forms new neuromuscular junctions, suggesting it is functional (Brant et al., 2019). This is a striking result because the same damage in *Mus* does not trigger regeneration (and instead leads to volumetric muscle loss) as there is no connective tissue component remaining to guide regeneration. The fact that *Acomys* can regenerate this guidance tissue/cue suggests that a special property resides in the connective tissue fibroblasts.

Kidney
Typical fibrosis-inducing models in the kidney involve unilateral ureteral obstruction, whereby one ureter is ligated, and ischaemia reperfusion injury, whereby vascular supply to the kidney is clamped for a period of time and the contralateral kidney removed. After ureter obstruction in *Mus*, the kidney shows clear hydronephrosis (kidney swelling) after 14 days with a shrinking of the parenchyma and weight loss, which is accompanied by an increase in collagen content and extensive fibrosis (Okamura et al., 2018 preprint). In contrast, the *Acomys* obstructed kidney preserves its structure and does not lose its weight, increase its collagen content or show fibrosis. In addition, there are reduced numbers of myofibroblasts and F4/80 macrophages in the *Acomys* obstructed kidney, and tubular integrity is preserved.

Thus, the *Acomys* kidney does not respond to damage by inducing fibrosis and tissue loss.

In the context of the ischaemia reperfusion model, in which ischaemia is performed for 40 min and the contralateral kidney is removed after 24 h, both *Mus* and *Acomys* show equivalent levels of elevated blood urea nitrogen and equivalent levels of tubular injury and tissue damage (Okamura et al., 2018 preprint). This indicates that the *Acomys* kidney suffers significant tissue damage after ischaemia. However, if the contralateral kidney is not removed at the time of ischaemia (thereby preventing death from kidney failure), there is an almost complete absence of fibrosis after 14 days and preservation of renal mass in *Acomys* compared with the severe fibrosis and 40% loss of renal mass observed in *Mus*. Thus, after ischaemia, *Acomys* can almost completely restore its kidney function compared with the progressive renal failure that *Mus* normally undergoes.

Spinal cord
Damage to the *Mus* spinal cord typically results in the appearance of a fibrotic glial scar, which is thought to be inhibitory to axonal regrowth across the site of damage and thus prevents any restoration of function. As a lack of fibrosis is characteristic of the response of *Acomys* to the various damages described above, it is also possible that the same may occur after spinal cord injury. To test this, *Mus* and *Acomys* were subjected to a hemi-crush of the spinal cord in the cervical region (Streeter et al., 2019). This study revealed that, in *Mus* at 3 days post-injury, several pro-inflammatory genes such as *Il6*, *Cxcl3*, *Ccl12*, *Ccl7*, *Il1b* and fibrosis genes such as *Tgfb1*, *Serpine1* and *Timp1* are induced. In contrast, the majority of upregulated genes in *Acomys* encode growth factors such as brain-derived neurotrophic factor and glial cell-derived neurotrophic factor, or components of the Wnt pathway, or are genes associated with neural stem cells such as *Sox2*, *Notch1* and *Ascl1*, or axonal guidance such as *Robo1*, *Efnb1* and *Ntn1*. Thus, there appears to be a completely different spectrum of genes induced in the two species in response to the same injury. At later sampling times, *Acomys* shows reduced immunoreactivity for collagen IV and GFAP, which are associated with spinal scarring and fibrosis, and reduced immunoreactivity for IBA1 (also known as AIF1), again suggesting a reduced immune response. These initial studies need to be extended to determine whether the reduced immune and fibrotic response in *Acomys* results in improved axonal regrowth across the damage site and improved outcomes, as it does in the regenerative situations described above.

Insights into the mechanisms of regeneration
Stem cells
Many regenerative processes are associated with the presence of stem cells, for example the satellite cells of skeletal muscle or the stem cells of the liver, so it is possible that *Acomys* has more stem cells than non-regenerating mammals or that a stem cell population is present in an *Acomys* tissue where none exists in other species. In the one tissue in which this has been examined, the skeletal muscle of the tibialis anterior, neither of these situations pertains (Maden et al., 2018). There were more absolute numbers of satellite cells (Pax7$^+$) in the *Acomys* muscle fibres, but when corrected for an increased size of fibres and quantitated relative to myonuclei, the same value of satellite cells relative to myonuclei was obtained in both species.

Extrapolating from this very limited data, if there is no difference in the number of stem cells present in *Acomys* it is possible that the special feature that this organism possesses is the ability to

regenerate its stem cell niches and repopulate them. This may be why *Acomys* can regenerate skeletal muscle after the connective tissue has been removed as well as the fibres themselves. The ability to regenerate and repopulate stem cell niches is clearly seen in the case of regenerating hairs, which contain several stem cell populations in the hair bulge, in the dermal papilla and in the sebaceous glands. As functional hairs regenerate in the *Acomys* skin, this implies these stem cell populations are regenerated from the basal stem cells that re-epithelialize the wound. Thus, the *Acomys* epithelium may exhibit unique properties or it may receive unique signals compared with the epidermis from other mammals. Some of these signals are beginning to be identified. For example, the overexpression of *Wnt7a* in *Mus* epidermis induces hair follicle regeneration after wounding (Ito et al., 2007) and we see high levels of *Wnt7a* early during *Acomys* regeneration (Brant et al., 2019). The induction of *Shh* in *Mus* wounds also induces hair follicle regeneration (Lim et al., 2018) and we see induction of *Shh* during *Acomys* regeneration (Brant et al., 2019). Uncovering these signals and finding ones that are unique to *Acomys* will be a fruitful avenue for investigation into why *Acomys* can regenerate but *Mus* and humans cannot.

Immune-based regulation

The immune system has long been thought to play a role in regeneration, with an immature system correlating with the regenerative ability of lower vertebrates and the skin regenerative abilities of mammalian fetuses (Mescher and Neff, 2005; Seifert and Maden, 2014). In both larval frogs and mammalian fetal skin, the ability to regenerate is lost during development and this loss correlates with the ability of the immune system to mount an inflammatory response in the damaged tissues. We might therefore expect *Acomys* to generate an immature or embryonic-like immune response to wounding.

In each of the regenerating systems discussed above, there is undoubtedly a blunted cytokine and macrophage response. In skin wounds, several cytokine genes such as *Cxcl3*, *Cxcl5*, *IL1b*, *Cxcl1* are massively upregulated in *Mus* (at least at the gene level) compared with undamaged levels, but this does not happen in *Acomys*, which expresses far fewer pro-inflammatory cytokines (Brant et al., 2015, 2019). Furthermore, the anti-inflammatory molecule *IL10* is strongly upregulated in *Acomys* wounds, over 100-fold compared with *Mus* wounds (Brant et al., 2019). The macrophage response is also different. In *Mus*, the early wound is infiltrated with large numbers of both M1 and M2 macrophages, whereas the regenerating *Acomys* dermis displays highly reduced numbers of macrophages (Brant et al., 2015). There are plenty of macrophages present in the underlying fascia and at wound margins but, even then, there is a dearth of F4/80 macrophages and reduced numbers of IBA1 macrophages, both of which are pro-inflammatory.

A cytokine analysis in the regenerating ear similarly identified IL6, CCL2 and CXCL1 expressed at higher levels in *Mus* fibrotic wounds, whereas regenerating *Acomys* ears exhibit higher levels of IL12 and IL17 (Gawriluk et al., 2019 preprint). There is also a different macrophage profile in the regenerating ear blastema of *Acomys* compared with *Mus* (Simkin et al., 2017). It is nearly devoid of classically activated (M1) macrophages, as marked by CD86 staining, but shows plenty of M2 macrophages, as marked by CD206 staining. There is still an inflammatory phase in *Acomys* (IL12 and IL17), which is also marked by a more robust production of reactive oxygen species from macrophages and there is a strong influx of CD3$^+$ T cells showing the characteristics of activated cytotoxic and regulatory T cells (Gawriluk et al., 2019 preprint).

In the regenerating *Acomys* skeletal muscle, kidney and spinal cord the story is the same: a reduced inflammatory cytokine response, reduced numbers of pro-inflammatory M1 macrophages and many pro-regenerative M2 macrophages are observed. Importantly, when macrophages are depleted from the regenerating ear, regeneration is blocked, showing that they have a crucial, positive role in regeneration, specifically in histolysis and re-epithelialization during the early phases of regeneration (Simkin et al., 2017). Therefore, it is likely that the macrophage phenotype plays a role in *Acomys* regeneration, guiding regeneration rather than fibrosis following tissue damage.

The reduced cytokine response and the reduced macrophage response may be responsible for the regeneration versus scarring seen in *Acomys* versus *Mus*. If this is the case, then suppression of the inflammatory response in *Mus*, or the deletion of cytokines, could be used as strategies to generate an improved regenerative response, and there are indeed some good examples of this (Ferreira et al., 2006).

Biomechanics

Another aspect of cell biology that, surprisingly, interacts with the cytokine response concerns the ECM. It was originally observed that *Acomys* skin is weak and tears easily (Seifert et al., 2012) and we now know that this is a property not only of the skin but of the internal tissues as well (Maden et al., 2018). In addition, the *Acomys* skin wound ECM has a different composition to that of *Mus*, supporting the idea that there is a pro-regenerative matrix, a concept which is a guiding principle of attempts to use artificial matrices to induce regeneration: a pro-regenerative matrix ideally generates a pro-regenerative microenvironment that promotes tissue regeneration (He et al., 2018). It is often assumed that a pro-regenerative matrix contains growth factors that can promote regeneration, but it is also possible that the biomechanical properties of the matrix itself are responsible for a reduced inflammatory response and consequent regeneration. There are several examples of the relationship between biomechanical forces and stem cell differentiation, but there is also a relationship between biomechanical forces on a wound and cytokine induction. For example, it has long been known that decreasing the mechanical forces on wounds decreases scar formation and, conversely, that increasing mechanical forces increases scarring (Wong et al., 2012). Mechanical forces from the matrix are transmitted to the cell via cell-surface integrins and relayed to the actin cytoskeleton via a molecule called focal adhesion kinase (FAK; PTK2). Increasing mechanical forces on a wound increases FAK activation. Remarkably, FAK also modulates cytokine and immune signalling; FAK-knockout wounds have reduced levels of macrophage chemotactic protein (MCP-1; Mcpt1) and Ccr2 (the cell surface receptor for MCP-1), and reduced levels of F4/80 macrophages (Wong et al., 2012), precisely the phenotype of *Acomys* skin wounds.

Thus, it is possible that the evolution of a weak skin phenotype, which is thought to help *Acomys* escape from predators, may have had unexpected consequences on the regeneration of tissues. Moving forward, we should aim to learn from evolution and apply this knowledge to understand how to induce regeneration in regeneration-incompetent mammals.

Conclusions

A. cahirinus is a mammalian model organism that has been used in research for more than 60 years, but it has only recently been

discovered that it shows striking powers of regeneration (Fig. 3, Table 2). The reason for this regenerative potential may be because *Acomys* does not fibrose in response to damage, as most mammals do. Uncovering the molecular and cellular basis of this lack of fibrosis and learning how to prevent it in other mammals such as humans may lead to the discovery of therapies for the induction of regeneration. But before this can happen, the full power of modern molecular and genetic techniques needs to be applied. Although the *Acomys* genome has been sequenced and transcriptomes published, techniques for manipulating the genome need to be developed to generate transgenic animals, which have been valuable in unravelling gene function in the laboratory mouse. Moreover, although this new species has the advantages associated with small rodents as laboratory models, it should be noted that the small number of embryos produced per litter and the lengthy gestation time remain as obstacles to rapid progress in understanding regeneration.

Moving forward, it would be fascinating to survey the regenerative ability of the close relatives of *Acomys*, namely Rudd's mouse (*Uranomys*), the Congo forest mouse or link rat (*Deomys*), and the brush-furred mouse (*Lophuromys*), which are members of the same subfamily of Deomyinae (Fig. 1), to determine whether the regenerative properties described above have only evolved in the genus *Acomys* or are present throughout the subfamily. In the same regard, the closest subfamily, the Gerbillinae, which includes gerbils, have been used extensively in research, especially in studying the central nervous system, but there is no evidence from the literature that there is any enhanced regeneration potential compared with the typical mammal; indeed there is an anecdotal report that gerbils do not regenerate a hole punched through the ear (Goss, 1980). At present, it thus appears that only the genus *Acomys* has the ability to regenerate tissues. However, this comparative approach is certainly a valuable one towards understanding the evolution of regenerative ability in mammals and, as emphasized earlier, it may be that there are other previously unrecognized regenerative species out there waiting to be discovered.

Competing interests
The authors declare no competing or financial interests.

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