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# Complex tissue regeneration in *Lophuromys* reveals a phylogenetic signal for enhanced regenerative ability in deomyine rodents

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**Identifying why complex tissue regeneration is present or absent in specific vertebrate lineages has remained elusive. One also wonders whether the isolated examples where regeneration is observed represent cases of convergent evolution or are instead the product of phylogenetic inertia from a common ancestral program. Testing alternative hypotheses to identify genetic regulation, cell states, and tissue physiology that explain how regenerative healing emerges in some species requires sampling multiple species among which there is variation in regenerative ability across a phylogenetic framework. Here, we interrogate tissue healing across eleven rodents and show that brush-furred mice (*Lophuromys zena*) are capable of musculoskeletal regeneration where new tissue faithfully maintains axial polarity and tissue identity as previously observed in spiny mice (*Acomys spp.*). In contrast, we find that all nondeomyine rodents heal identical ear pinna injuries via fibrotic repair with scar tissue. Together, these data reveal a phylogenetic signal for enhanced regenerative ability in Deomyinae which is key to testing evolutionary hypotheses about the emergence of regenerative ability in mammals.**

regeneration | evolution | rodent | mammal | ear pinna

## Significance

Complex tissue regeneration is a phenomenon rarely observed among mammals. Exceptions include the distal most digit tip, rabbit ear punches, and deer antler velvet. Beyond these highly restricted examples, spiny mice (*Acomys spp.*) exhibit broader regenerative powers such that they regrow skin and musculoskeletal tissue, and functionally repair damaged spinal cords. Until now, their impressive regenerative ability was thought unique among rodents. Sampling across a nested group of eleven murid and nonmurid rodents, we identified musculoskeletal regeneration in another deomyine lineage—*Lophuromys*. These results provide a phylogenetic signal for the emergence of complex tissue regeneration in an enigmatic rodent subfamily and suggest that a unique set of genetic and cellular features evolved in this group to support regeneration.

Mammals have long been accused of having inferior powers of organ regeneration, particularly compared to a host of poikilothermic (cold-blooded) vertebrates that can rebuild damaged limbs, hearts, tails, and spinal cords. Still, mammals can regenerate compartmentalized tissues with dedicated stem cell populations quite well (e.g., epidermis, intestinal epithelium, endometrium, skeletal muscle, etc.) and regrow distal digit tips after amputation. Rabbits regenerate excised tissue in the ear pinna (1, 2), many cervids regrow their antlers on an annual basis and deer antler velvet regenerates after full-thickness wounding (3). Our previous discovery that three species of adult spiny mice (*Acomys dimidiatus, Acomys kempi, and Acomys percivali*) could regenerate full-thickness skin and musculoskeletal tissue reminded us that surprises abound in nature and revealed a new model for studying complex tissue regeneration in immunocompetent, adult mammals (1, 4).

Studies seeking to pinpoint genomic changes, molecules, or cell states that permit or inhibit complex tissue regeneration require within clade comparisons where the regenerative ability of a target tissue varies across species. While annelids and planarian flatworms have been studied in this fashion (5, 6), very few vertebrate regeneration studies utilize closely related species where one explicitly lacks regenerative ability. Rodents present an interesting test case. They are the most speciose group of mammals with subgroups that have rapidly diversified within the last 25 My (7). The presence of enhanced regenerative ability in spiny mice and the lack thereof in laboratory mice and rats established an evolutionary framework for more widespread sampling (1).

Studies over the last decade have reinforced the robust nature of the ear pinna regeneration phenotype in *Acomys* (1, 8, 9) supporting this tissue as an appropriate means for screening regenerative ability across mammalian species. Here we targeted nine murid rodents and two outgroup species. Among murids, we sampled *Lophuromys zena* (Deomyinae) the sister taxon to spiny mice (*Acomys*—Deomyinae). Using our 4 mm ear punch assay, we assessed (1) ear hole closure rate and (2) regenerative ability via cellular analysis of newly generated tissue. In the context of our comparative studies, our results show that only *Acomys* and *Lophuromys* can rebuild complex tissue structures revealing a phylogenetic signal for the emergence of enhanced regenerative ability in deomyine rodents.

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## Results

**Complete Ear Hole Closure Is Restricted to Deomyine Rodents.** We examined tissue healing across a range of species representing the three main murid subfamilies (Murinae, Gerbillinae, and Deomyinae) and two outgroup families (Nesomyidae and Gliridae) (Fig. 1 *A*–*F*). Given our previous discovery of complex tissue regeneration in *A. kempi* and *A. percivali,* two wild-caught spiny mouse species from Kenya, we trapped additional sympatric murids *Aethomys hindei* (Hinde's rock rat or bush rat), *Gerbilliscus vicinus* (fringe-tailed gerbil), and *Myomyscus brockmani* (Brockman's rock mouse) in the same savanna habitat. We also captured *Saccostomus mearnsi* (Mearn's pouched mouse—Nesomyidae) and *Graphiurus murinus* (forest African dormouse—Gliridae) as outgroup species from the same habitat. To broaden sampling within Deomyinae, we located and trapped *L. zena* in two forest locations (Fig. 1*B*). After allowing animals to acclimate in captivity for several weeks, we assessed ear hole closure and regenerative capacity in healthy adult animals (Fig. 1 *C*–*F*). We also collected the same data from three captive bred species: *A. dimidiatus* (Arabian spiny mouse–previously referred to as *Acomys cahirinus*; see Methods), *Meriones unguiculatus* (Mongolian gerbil), and outbred *Mus musculus.* Both sexes were analyzed together based on previous work showing that sex does affect regenerative ability (1).

Analyzing hole closure over 85 d we observed that every ear punch from *L. zena* (26/26) completely closed mirroring results from all three *Acomys* species: *A. kempi* (21/21), *A. percivali* (33/33), *A. dimidiatus* (82/82) (Figs. 1*D* and 2*A*). In stark contrast to these four deomyines, and with the exception of two ear holes from *A. hindei* specimens (2/41), we did not observe appreciable ear hole closure in any of the other murids: *A. hindei* (2/41), *G. vicinus* (0/22), *M. brockmani* (0/12), *M. unguiculatus* (0/8), *M. musculus* (0/30), or outgroup species, *G. murinus* (0/9) and *S. mearnsi* (0/5) (Figs. 1 *E* and *F* and 2*A*). Across all sampled species, animals either did or did not completely close 4 mm ear holes (Fig. 1 *C*–*F*). A notable observation was that *Lophuromys* exhibited a pattern of asymmetric tissue production similar to *Acomys* (Fig. 1 *G* and *H*). Importantly, *A. hindei* was representative of the other

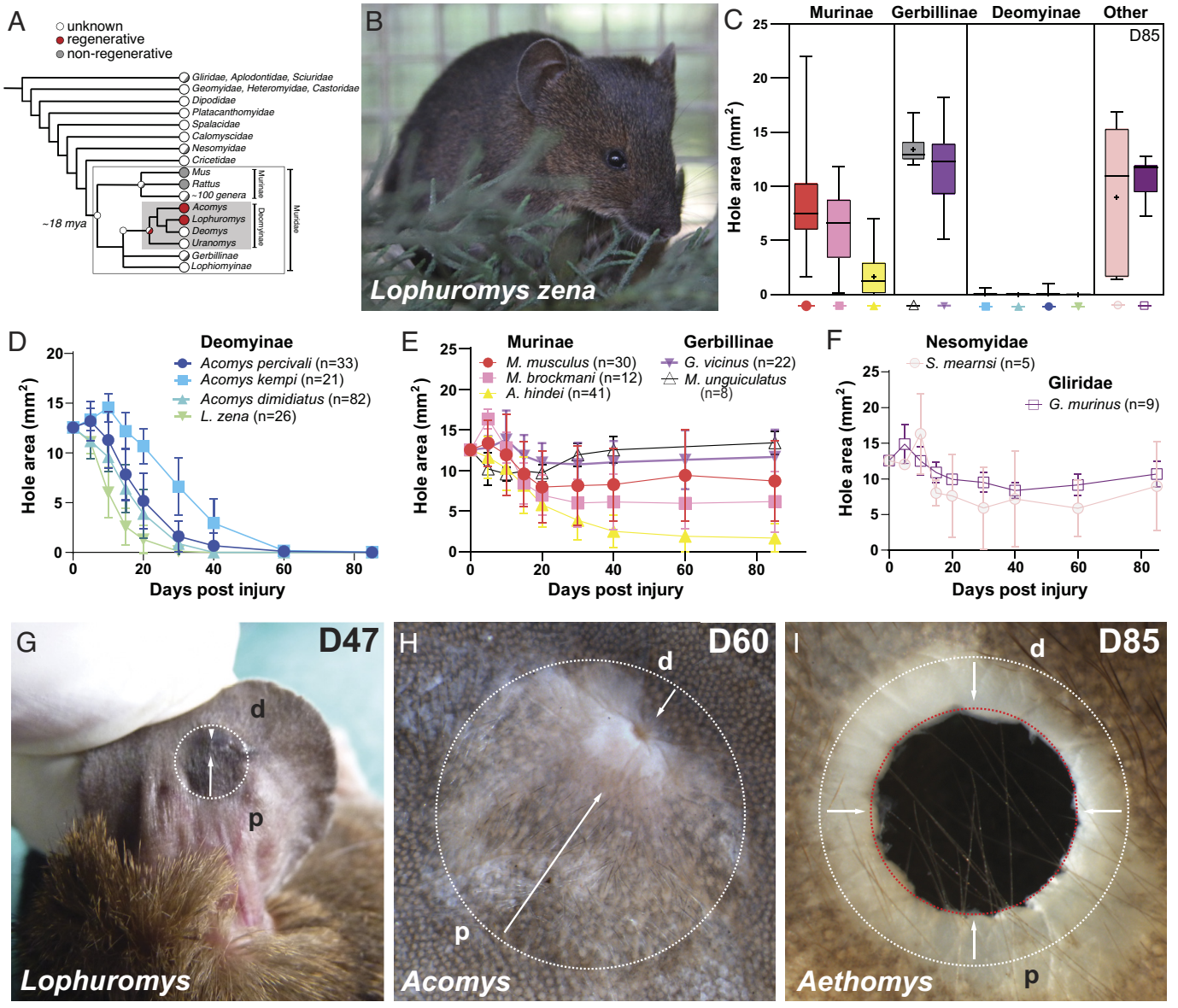


**Fig. 1.** Complete ear hole closure is restricted to deomyine rodents. (*A*) Simplified phylogeny showing genera used in this study with gray and red shading showing subfamilies sampled. (*B*) *L. zena*. (*C*) 4 mm ear hole area at D85 post injury. (*D*–*F*) Ear hole area measured over time across eleven rodent species. Number of ears per species indicated after each species. (*G*–*I*) Whole mount photographs showing proximally biased new tissue production and pigmented hairs in *L. zena* (*G*) and *A. dimidiatus* (*H*) and concentric scar tissue production in *A. hindei* (*I*). Distal and proximal relative to the skull are denoted d and p, respectively.

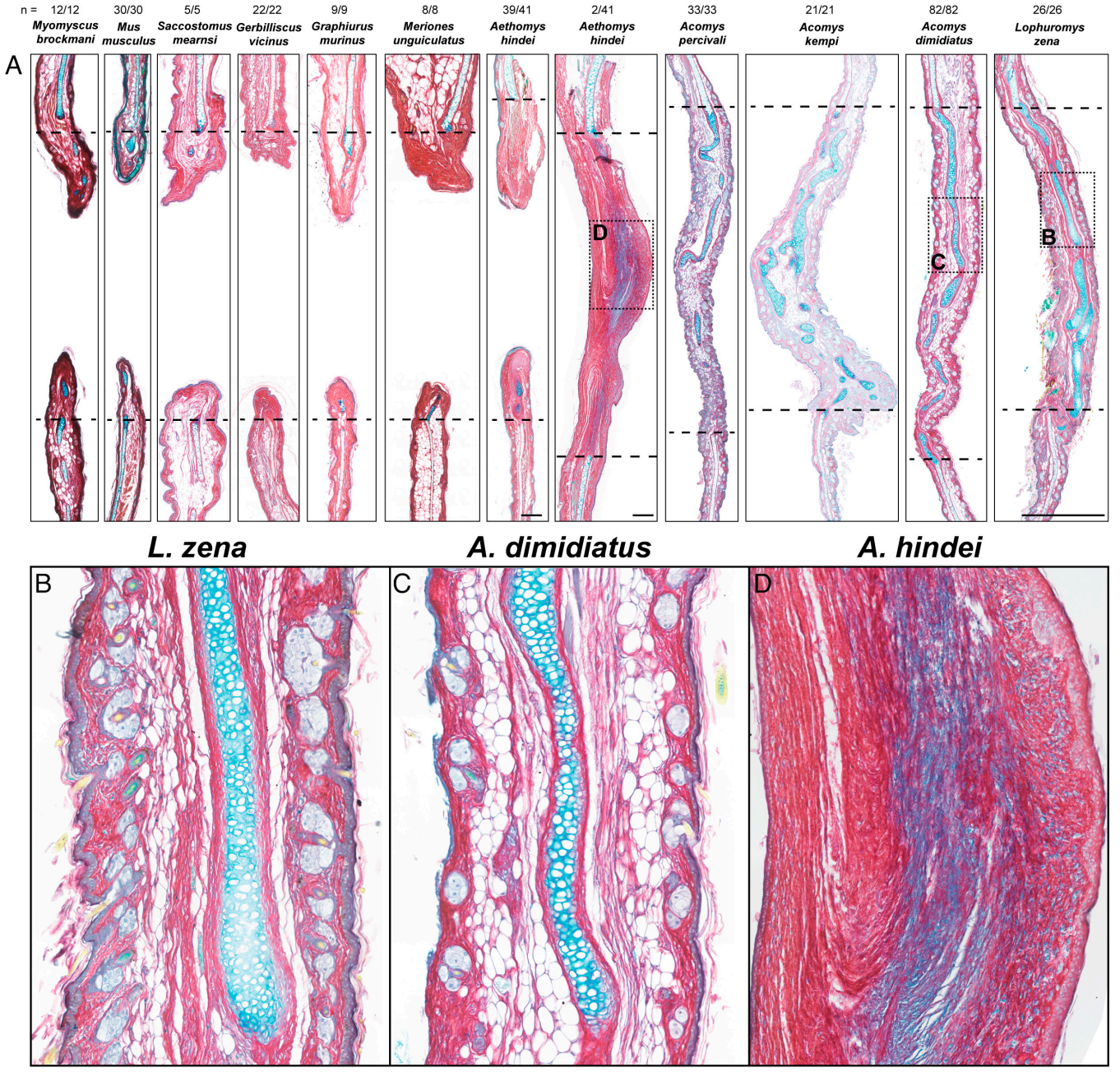


**Fig. 2.** *Lophuromys* exhibit patterned tissue regeneration during ear hole closure. (*A*) RGB Trichrome (RGBT) staining of D85 ear tissue from all eleven species used in this study. Number of ear holes per species relative to the resultant histology result indicated above species name. (*B–D*) *Insets* from *L. zena* (*B*), *A. dimidiatus* (*C*), and one of the two *A. hindei* ears that closed (*D*). New tissue in *Acomys* and *Lophuromys* exhibit patterned tissue regeneration including replacement of elastic cartilage, new hair follicles, sebaceous glands, adipose tissue, and dermis. *A. hindei* generated scar tissue. Scale bar in *Lophuromys* (*A*) representative for all species: 1 mm, except for *A. hindei*: 500 μm.

species and exhibited an even distribution of scar tissue production around the entire hole supporting that the pattern of new tissue production is tied to the type of healing observed (Fig. 1*I*).

***Lophuromys* Exhibit Patterned Tissue Regeneration during Ear Hole Closure.** The capacity to generate new tissue and close open ear punches does not necessarily indicate tissue regeneration. Proper restoration of individual tissue compartments and structures needs to occur. In *A. dimidiatus*, evidence for complex tissue regeneration includes restoration of patterned elastic cartilage, adipose tissue, skeletal muscle, and full thickness skin with associated hair follicles and sebaceous glands (Fig. 2 *A* and *B*). Similarly, *L. zena* restored all of these structures indicating musculoskeletal regeneration in this species (Fig. 2 *A* and *C*). We did observe two outlier *Aethomys* (2/41) that completely closed ear holes with new tissue (Fig. 2 *A* and *D*). However, when this new tissue was analyzed with Alcian Blue (to visualize glycosaminoglycans) we observed a scar-like matrix with no mature cell types or complex structures in the dermal compartment (Fig. 2*D*). Moreover, we observed a dense band of scar tissue instead of new elastic cartilage through the central portion of the pinna and we did not observe new hair follicles, adipose tissue, or muscle (Fig. 2*D*). The other *Aethomys* that did not close ear holes and that we examined (39/41) showed concentric scar-like deposition and the occasional cartilage nodule but no evidence of regeneration (Figs. 1*I* and 2*A*).

## Discussion

When epimorphic regeneration was originally discovered in two wild-caught *Acomys* species (4) it raised the question of whether enhanced regenerative abilities might be more prevalent among mammals than previously appreciated or instead uniquely evolved in a group of murids that exhibit a suite of distinctly nonmurid phenotypes (e.g., weak skin, precocial development, menstruation, tail osteoderms, etc.). Although we observed that *L. zena* skin exhibited a propensity to tear, captive females gave birth to altricial young supporting a mix of traits shared with *Acomys* (Fig. 3). By challenging eleven rodent species to generate new ear pinna tissue, we found that the brush-furred mouse (*L. zena*), like *Acomys spp*., exhibited the ability to regenerate musculoskeletal tissue while no other species assayed did so. Placed in an evolutionary context, our results reveal a phylogenetic signal for the emergence of complex tissue regeneration in the Deomyinae clade. Future studies with the monotypic *Deomys* and *Uranomys* will provide additional phylogenetic resolution. The four deomyine genera were phylogenetically united by DNA sequence only until a recent museum study using single specimens from each genus uncovered the presence of osteoderms in the tail (10). Beyond osteoderms, no other morphological or physiological characters were known to separate deomyines from the other murid subfamilies (11). Now the presence of regenerative ability in *L. zena* provides a second character uniting at least two of the deomyine genera.

We also observed that new tissue generation among *Lophuromys* and *Acomys* was proximally biased. Previous work in *Acomys* demonstrated an asymmetric growth pattern consistent with gene expression (9), cell proliferation, and extracellular matrix composition (1, 12) unique to the proximal area of the ear hole along the proximal–distal (PD) axis. This proximal bias in new tissue production was not observed in the other nonregenerating rodents where concentric growth of fibrotic scar tissue was deposited evenly around the open hole. Even though two ear holes in *A. hindei* achieved hole closure, the holes were closed with scar tissue that was produced concentrically. The basis for these divergent types of new tissue growth during regeneration and fibrotic repair awaits further investigation.

Contrary to opinions that tissue repair occurs along a continuous spectrum, we found that healing type (i.e., regeneration versus scar formation) appears to occur as a binary phenotype. It remains unclear with current species coverage the degree to which specific tissue-type regeneration exists in other species. For example, *M. brockmani* appear to regenerate some hair follicles despite failing to regenerate patterned tissue or close ear holes. Similar investigations of skin regeneration in laboratory mice and deer antler velvet supports the loss of a tissue-specific niche and regenerative cell types with aging (3). Our published data on *Acomys* regeneration support that, beyond a cell type deficiency, other innate cellular features are required for the expression of regenerative ability in adult mammals.

As we have yet to explore the genetic and cellular basis for regeneration in *Lophuromys*, future studies are necessary to ascertain whether these species also exhibit specific cellular features associated with regeneration in *Acomys*. These include stromal cells that exhibit a baseline redox state streamlined for low reactive oxygen species production via highly efficient mitochondria, resilience to oxidative stress via strong intrinsic antioxidant activity (13), maintaining elevated ERK activation after inflammatory resolution (12), the capacity for continued cell cycle progression among stromal cells (1, 9) and a unique tissue-resident macrophage phenotype that reduces inflammatory magnitude and cellular stress (14).

Rigorously testing evolutionary hypotheses requires phylogenetically informed sampling to create multispecies systems for experimental investigation (6, 15, 16). The multispecies assemblage reported here adds a remarkable system in which to test important, unresolved questions in vertebrate regeneration. For example, it remains unknown whether regeneration as observed in specific lineages represents cases of convergent evolution or is the result of phylogenetic inertia from a common, ancestral gene program. Testing these alternative hypotheses is now possible in vertebrates. Additionally, research with spiny mice provides some support for the possibility that regeneration (from injury through functional tissue replacement) may be the natural outcome of how particular cell states arise in response to injury and behave against the background of permissive tissue physiology. As such, we hypothesize that a specific set of cellular phenotypes as outlined above may have facilitated tissue regeneration to emerge in this group. Although regenerative ability is commonly promoted as a complex trait under direct selection, multispecies systems like that described here will allow us and other biologists to consider an alternative hypothesis; that regeneration as a collection of interrelated processes is an emergent phenomenon. This in fact may help explain why regenerative ability is observed as an all or none phenotype across different lineages.


**Fig. 3.** Newborn *L. zena* pups are altricial. Captive female *L. zena* give birth to altricial newborn pups (i.e., blind, hairless, etc.). In contrast, newborn *Acomys* pups (*A. dimidiatus*) are precocial (i.e., eyes open, haired, etc.).

## Materials and Methods

**Animals, Husbandry, and Ethics.** *A. kempi*, *A. percivali*, *M. brockmani*, *S. mearnsi*, *A. hindei*, and *G. vicinus* were live-captured in Laikipia, Kenya at Mpala Research Centre and surrounding ranches. *L. zena* were captured in the Mau Forest in the Rift Valley and at Loita Hills. Animals were transported to the University of Nairobi where they were held in a dedicated animal house for study. Each rodent species was separated by sex and housed in metal wire cages (Quality Cage Company), fed mouse pencils (Argrocide Inc., Nairobi, Kenya) 1× per day and exposed to natural light through windows (roughly equivalent to a 12:12 light:dark cycle in Nairobi). *Acomys dimidiatus*, outbred Swiss Webster *M. musculus* (Envigo–ND4 strain), and *M. unguiculatus* were housed in our breeding colony at the University of Kentucky in Lexington, Kentucky. Captive *Acomys* colonies whose provenance traces back to animals captured in Israel (which are not *A. russatus*) have erroneously been referred to as *A. cahirinus* but are instead *A. dimidiatus*. A forthcoming genome paper will address this issue with genomic and transcriptomic data. The genome for our captive animals is available through ENSEMBL–GCA_907164435.1 (mAcoDim1_REL_1905). In anticipation of this study, the *Acomys* research community, in consultation with the genome paper authors, has elected to correct the species information in all publications moving forward.

All animal work in Kenya was approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC) under protocol 2016-2490, Kenyan Wildlife Service, and the University of Nairobi Faculty of Veterinary Medicine Animal Care and Use Committee. Research in Kenya was approved by the Kenyan National Council for Science and Technology (NACOSTI). All wild species trapped were species of least concern. All work in this study performed at the University of Kentucky was approved under IACUC protocol 2019-3254.

**Ear Hole Closure and Regeneration Assay.** Animals were anesthetized under 2% isoflurane and a 4 mm ear punch was made through both ear pinna using a sterile biopsy punch as previously reported (1). Measurements of ear-hole area were subsequently taken on days 5, 10, 15, 20, 30, 60, and 85 post injury (D5 to D85) by measuring along the PD and medial-lateral axes. For specific time points, healing tissue was collected using an 8 mm biopsy punch which was fixed overnight in 10% neutral-buffered formalin, washed 3× in phosphate-buffered saline, washed 3× in 70% EtOH, and stored at 4 °C in 70% EtOH prior to paraffin embedding

**Tissue Histology.** Paraffin-embedded tissue blocks were cut at 5 μm and sections stained with a modified RGBT stain as recently described (17). Our modification for RGBT staining used 0.4% Fast Green FCF to attain sufficient contrast.

**Microscopy and Data Visualization.** Brightfield images were captured on an Olympus IX81 inverted, epifluorescent scope and exported through Olympus Cellsens (ver. #4.2.1). Polarized images were captured with a Zeiss Axio Scan Z.1 slide scanner and exported through Zeiss ZEN (ver. #3.1). Plots were produced in GraphPad Prism 10 and annotated in Adobe Illustrator.

**Data, Materials, and Software Availability.** All study data are included in the main text.

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