

Original paper

Ear wound regeneration in the Arabian spiny mouse (*Acomys dimidiatus*)

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Abstract: Skin shedding, skin wound regeneration, and extensive ear punch closure have been reported in three *Acomys* rodent species (*A. cahirinus*, *A. kempi*, and *A. percivali*) commonly known as African spiny mice. In this study, we examined the regenerative ability of *A. dimidiatus*, the Arabian spiny mouse. A 2 mm diameter punch hole was created in the proximal and distal parts of the pinna and observed for up to 35 days. That in the proximal region almost completely closed, and extensive elastic cartilage, adipose tissue, dermis, epidermis, and hair follicles appeared in the repaired area. More rapid skin regeneration was observed closer to the trunk. The punch hole in the distal part did not reach complete closure during the observation period. However, regeneration was observed in the repaired area as well as in the proximal part. These results corroborate the presence of regenerative capabilities of *A. dimidiatus* and indicate that *Acomys* species may be useful model animals for regeneration studies.

I. Introduction

Regeneration, the capacity to structurally and functionally restore damaged tissues and organs, has been observed in many taxonomic groups, including amphibians, crustaceans, and fish (Alvarado 2000, Brockes & Kumar 2005, Slack et al. 2008). However, there are few reports of regeneration in mammals, which show a greater tendency toward tissue repair with fibrous scarring than regeneration (Clark 1996). Regeneration has been confirmed in mammalian fetuses and the MRL/MpJ mouse strain, but these abilities are typically temporary or non-innate (Beare et al. 2006, Larson et al. 2010, Gawriluk et al. 2016). Notably, high regeneration ability was recently demonstrated in African spiny mice (*Acomys*), including *A. cahirinus* (Cairo spiny mice), *A. kempi* (Kemps spiny mice), and *A. percivali* (Percival's spiny mice) (Seifert et al. 2012, Matias Santos et al. 2016). These species were found to regenerate wound-defective skin tissue without undergoing the usual scarring or loss of function after fibrosis. Thus, these animals may be useful models for analyzing the cellular and molecular signals that control regeneration (Gaire et al. 2021).

Acomys cahirinus is the major spiny mouse species currently maintained and used as an experimental animal (Haughton et al. 2016). *Acomys dimidiatus* (the Arabian spiny mouse or eastern spiny mouse), which exists from southwestern Asia to northeastern Africa, is also maintained in laboratory colonies in Japan and Switzerland (Montandon et al. 2014, Mochida & Mekada 2017, Mekada et al. 2021). Because *A. dimidiatus* is a sister species to *A. cahirinus* (Kunze et al. 1999, Volobouev et al. 2007, Frynta et al. 2010, Mochida & Mekada 2017), it is expected to have the same regenerative ability as *A. cahirinus*. However, no detailed characterization of *A. dimidiatus* has been conducted to date. Therefore, we investigated the skin regenerative ability of *A. dimidiatus* by gross and histological observations of the temporal changes in the wound site of pinna tissue.

II. Materials and Methods

This study involved male and female (non-pregnant) of *A. dimidiatus* (Aco strain) (Mochida & Mekada 2017) and *Mus musculus* (BLAB/cCrSlc mice strain, purchased from Japan SLC, Inc., Hamamatsu, Japan) from the Department of Zoology, Faculty of Science, Okayama University of Science. The animals were housed at a room temperature of 24ºC and light/dark period of 12/12 h. They were fed commercial pellets (Labo MR breeder; Nihon Nosan Kogyo. Co., Yokohama, Japan), and both food and water were provided *ad libitum*. All animal experiments were conducted in accordance with the Regulations for Animal Experiments of the Okayama University of Science. All experimental protocols, including those involving animals (Exp2020-004), were approved by Animal Experiments Committee of the University.

Under isoflurane inhalation anesthesia, a 2 mm diameter ear punch was used to create punch holes in the proximal part of the right pinna

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(closer to the trunk) and the distal part of the left pinna (farther from the trunk), avoiding major ear vessels and resulting in minimal bleeding. The mice were divided into two groups according to the starting age of treatment: 3 weeks (W3 group) and 9 weeks (W9 group). After treatment, the animals were euthanized every 7 days until day 35, at which time the pinna tissues were collected. In total, 40 mice were used (8 [4 females and 4 males] in each group at each collection time point). The collected pinna fragments were subjected to histopathological analysis after the status of the wound was observed under a stereomicroscope and digital images were acquired. The area of the wound site was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

The ear samples were fixed in 4% paraformaldehyde overnight, dehydrated in a graded ethanol series and embedded in paraffin wax. Transverse microtome sections with a thickness of 4 µm were deparaffinized in xylene and rehydrated in a graded ethanol series. A commercial Masson's trichrome staining kit (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) was used to stain the sections, which were then were rinsed, dehydrated, and mounted.

III. Results

The *A. dimidiatus* and *M. musculus* mice used in this study included both females and males, but no statistically significant differences in the area of the ear punch holes were found between the sexes at each sampling time point (Brunner-Munzel Test, $P > 0.05$). Because a previous study of *A. cahirinus* showed differences between the sexes (Matias Santos et al. 2016), we used a combined value in this study.

In the proximal pinna of *A. dimidiatus*, a rapid decrease in the wound area was observed between days 7 and 35 after punching treatment in both the W3 and W9 groups (proximal part in Figs. 1 and 2). The median wound area on day 7 was 3.1 and 3.2 mm^2 in the W3 and W9 groups, respectively, indicating the beginning of wound repair in both groups. On day 14 after treatment, the median wound area was 1.5 and 1.8 mm^2 in the W3 and W9 groups, respectively, and new skin formation was observed proximal to the wound in both groups. On day 21 after treatment, the median area was much smaller in the W3 group (0.0 mm^2), and complete closure of the wound was observed in five of the eight animals. In the W9 group, the median wound area was 0.1

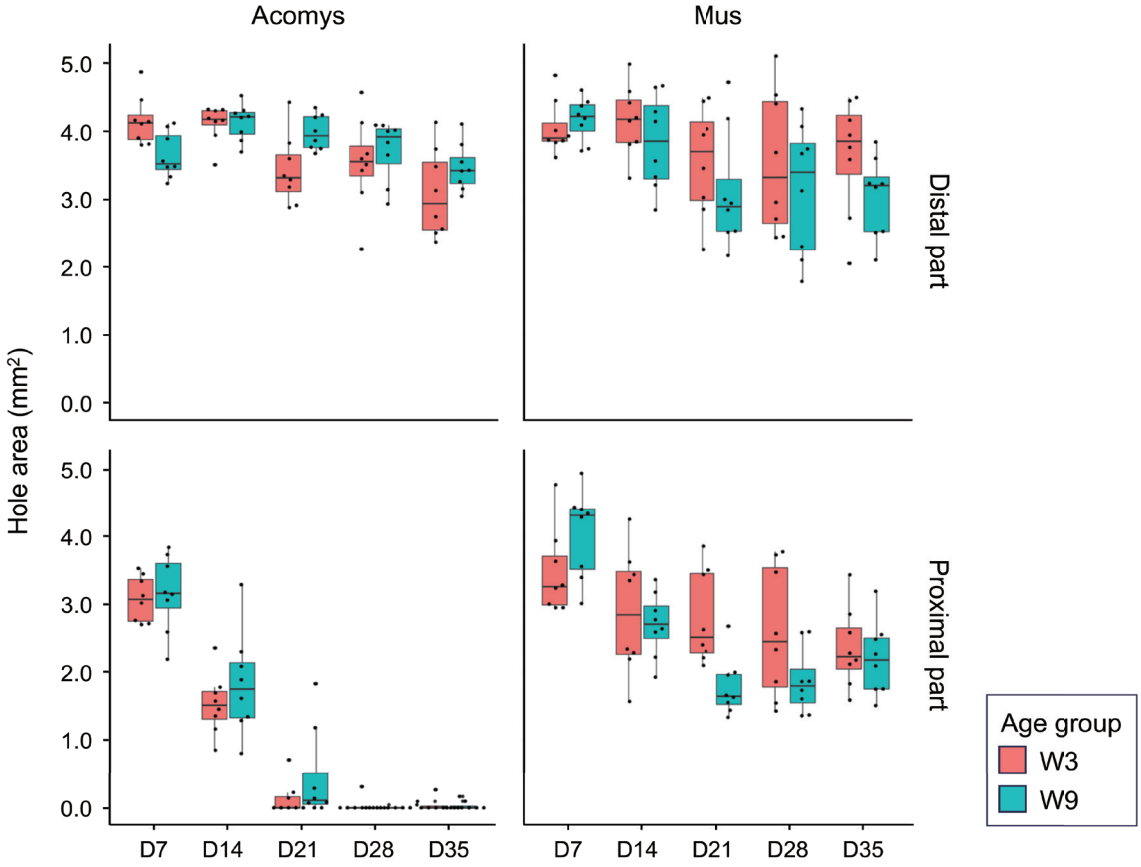

Fig. 1. Scatter boxplots of ear punch hole area (by proximal and distal parts) from day 7 (D7) to day 35 (D35) in *Acomys dimidiatus* and *Mus musculus* (BALB/c) of the W3 group versus W9 group.

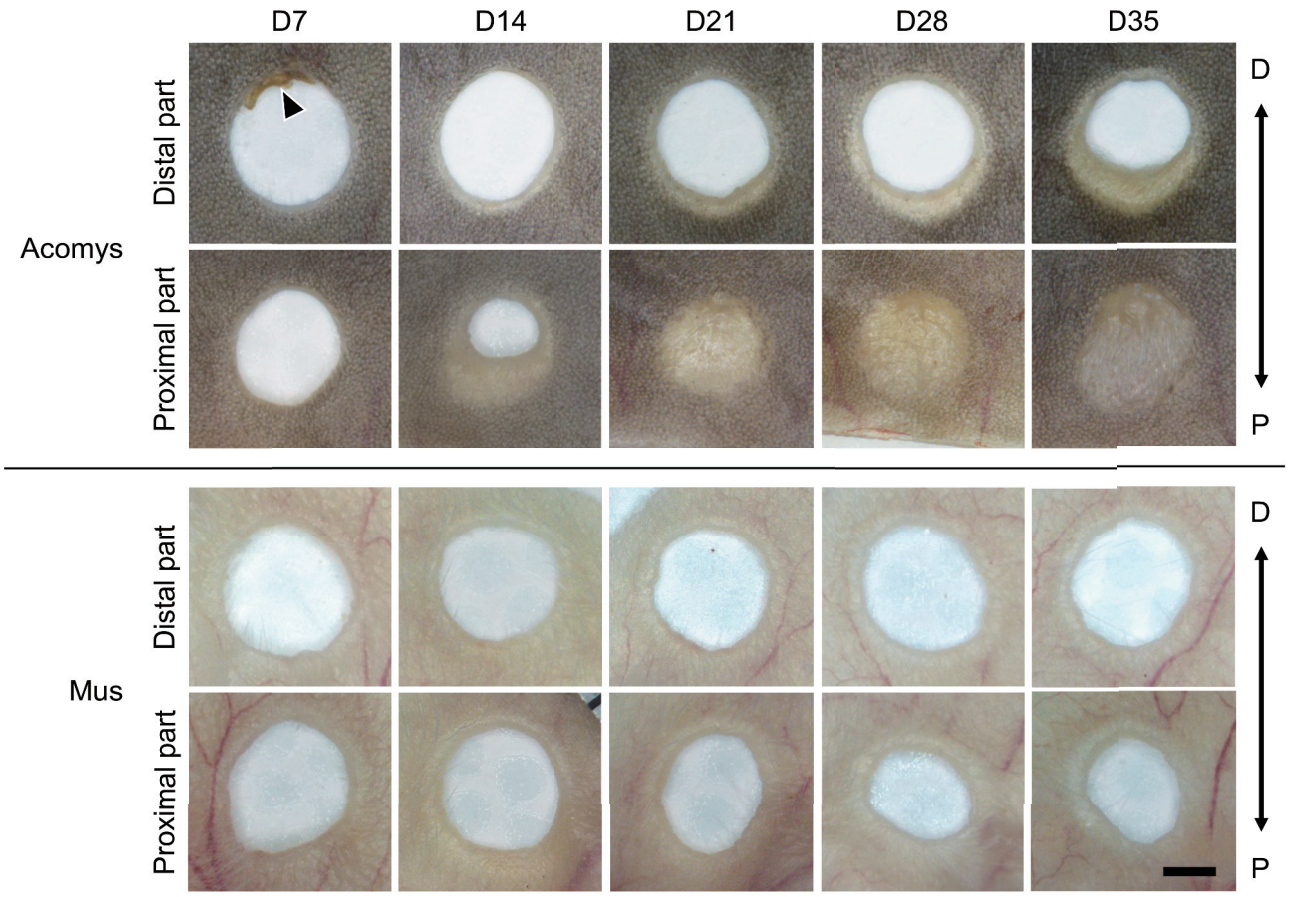

Fig. 2. Representative images of ear punch closure along timeline from day 7 (D7) to day 35 (D35) in *Acomys dimidiatus* versus *Mus musclus* (BALB/c) of W3 group. The distal–proximal axis is shown vertically with distal (D) at the top and proximal (P) at the bottom for all panels. The arrowhead indicates necrotic skin tissue. Scale bar = 1 mm.

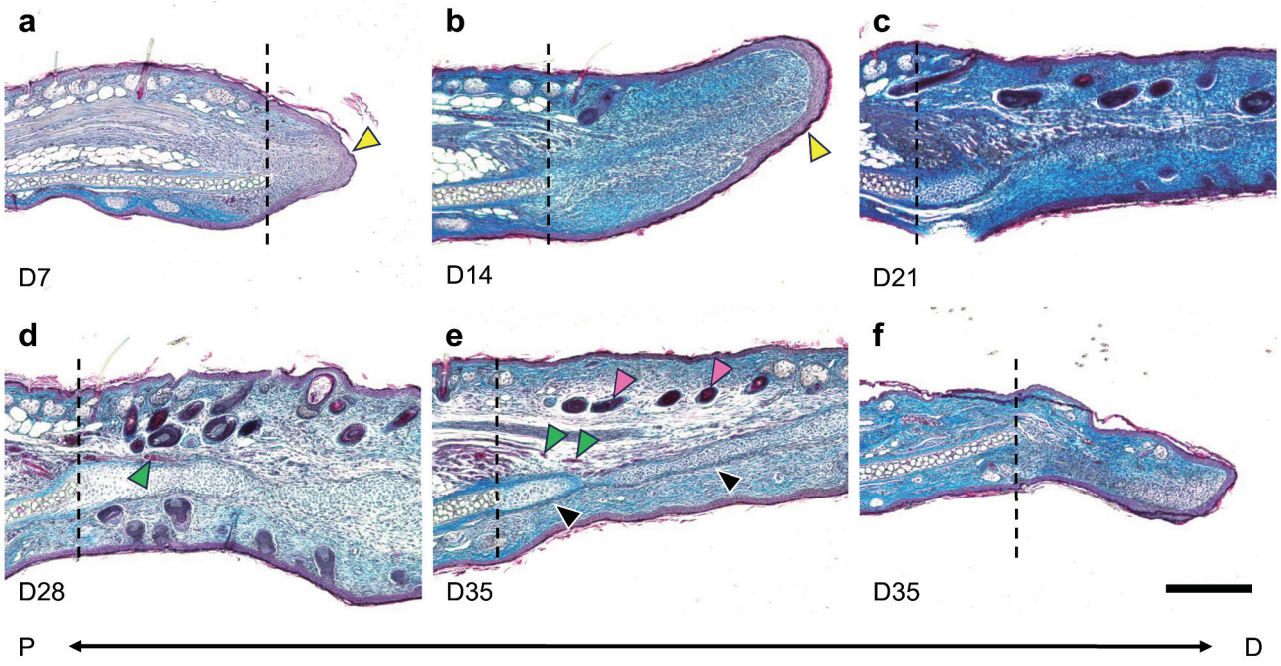

Fig. 3. Representative histological sections of *Acomys dimidiatus* and *Mus musculus* (BALB/c) of W3 group, stained with Masson's trichrome stain. *Acomys dimidiatus* on (a) at day 7 (D7), (b) day 14 (D14), (c) day 12 (D12), (d) day 28 (D28), and (e) day 35 (D35). (f) *M. musculus* on day 35 (D35). Arrowheads indicate thickened epidermis (yellow), hair follicles (pink), muscle (green), and cartilage (black). Vertical dotted lines indicate the original plane of wounding (identified by the presence of mature cartilage). The distal–proximal axis is shown horizontally with distal (D) to the right and proximal (P) to the left for all panels. Scale bar = 0.3 mm.

mm^2, and complete closure of the wound was observed in two of the eight animals. On day 35 after treatment, the median wound area was 0.0 mm^2 in both the W3 and W9 groups, resulting in a nearly closed wound area in both groups.

In the distal pinna of *A. dimidiatus*, the wound area tended to gradually decrease between days 7 and 35 after treatment in both groups (distal part in Figs. 1 and 2). The median wound area on day 7 after treatment was 4.1 and 3.5 mm^2 in the W3 and W9 groups, respectively, and necrotic skin tissue was observed at the edges of the wounds in all mice of the W9 group. The median wound area on day 14 after treatment was 4.2 and 4.2 mm^2 in the W3 and W9 groups, respectively, and the beginning of wound repair was observed in the W3 group. On day 21 after treatment, the median wound area was 3.3 and 3.9 mm^2 in the W3 and W9 groups, respectively, and new skin formation was observed on the proximal side of the wound in the W3 group. At day 35 after treatment, the median wound area was 2.9 and 3.4 mm^2 in the W3 and W9 groups, respectively, and closure of the wound from the proximal to distal side was observed in all *A. dimidiatus* animals. In *M. musculus* animals, both the proximal and distal pinnae showed a decrease in the wound area in the W3 and W9 groups, but complete closure of the area was not observed during the study.

In the W3 group, the wound in the proximal pinna of *A. dimidiatus*, showed re-epithelialization by day 7 after ear punch treatment, below which a mass of loosely packed tissue was observed (Fig. 3a). By day 14 post-treatment, the distance between the original wound surface and the wound boundary had increased (Fig. 3b). On day 21, the formation of elastic cartilage extending from the pinna cartilage beyond the wound surface and the formation of hair follicles were observed (Fig. 3c). On days 28 and 35, more hair follicles as well as muscle formation were observed. Extensive growth of elastic cartilage was present in the regenerated area (Fig. 3d, e). The recovery of the wound area of the distal pinna of *A. dimidiatus* in the W3 group and the proximal and distal pinnae in the W9 group exhibited changes similar to these (data not shown). Although some epidermal regeneration was also observed at the wound sites of *M. musculus* by day 35 (data not shown), no complicated structural change such as those seen in *A. dimidiatus* were observed on day 35 (Fig. 3f).

IV. Discussion

In previous studies of ear punch regeneration in *A. cahirinus* and *A. kempi* treated with ear punches twice the size of those of the present study (4 mm in diameter), the punch holes completely closed within 2 months, and histologic examination showed extensive formation of elastic cartilage, adipose tissue, dermis, epidermis, and hair follicles at the site of repair (Gawriluk et al. 2016, Matias Santos et al. 2016). In the present study, complete closure of the proximal pinna wound was observed in most *A. dimidiatus* mice (Figs. 1 and 2). The regenerated auricular injury site exhibited epidermis, hair follicles, elastic cartilage, and muscle, and the tissue structure resembled that of the normal skin (Fig. 3). Hence, *A. dimidiatus*, like previously reported *Acomys* species, was found to have the ability to reconstruct skin wounds structurally and functionally.

Although regeneration occurred in the wounded area of the distal pinna of *A. dimidiatus*, complete closure of the wound was not observed during the study period (35 days). The regenerative process progressed from the proximal to distal side of the wound hole, suggesting a greater regenerative capacity closer to the trunk. In experiments involving *A. cahirinus*, in which punch procedures were performed in the middle of the auricle without distal–proximal distinction, skin regeneration progressed from the proximal to the distal side of the wound (Matias Santos et al. 2016). Although no studies to date have performed quantitative measurement of the expression profiles of genes noted during regeneration of *Acomys* species, studies of laboratory mice have shown that calpain in neovascular vessels promotes wound healing and tissue formation (Miyazaki et al. 2018). Because blood vessels are more abundant closer to the trunk, the presence of blood vessels may have influenced the large difference in regenerative capacity between the proximal and distal locations in the present study, with wounds in more vascularized locations having greater regenerative capacity. The wound area reduction tended to be faster when the animals were injured at 3 weeks of age (W3 group) than at 9 weeks (W9 group), but the difference was slight. We would not expect a significant difference in regenerative capacity over this age range.

This analysis revealed that *A. dimidiatus* has the ability to structurally and functionally reconstruct skin wounds. *Acomys cahirinus* and the present *A. dimidiatus* colonies were raised as experimental animal strains, facilitating a comparative biological research approach using both species. We expected that *Acomys* species will become an even more useful model animal in the field of regeneration research.

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References

Alvarado, A. S. (2000) Regeneration in the metazoans: why does it happen? Bioessays 22(6): 578-590.

Beare, A. H. M., Metcalfe, A. D. & Ferguson, M. W. (2006) Location of injury influences the mechanisms of both regeneration and repair within the MRL/MpJ mouse. Journal of Anatomy 209(4): 547-559.

Brockes, J. P. & Kumar, A. (2005) Appendage regeneration in adult vertebrates and implications for regenerative medicine. Science 310(5756): 1919-1923.

Clark, R. A. F. (1996) Overview and general considerations of wound repair. *In*: Clark, R. A. F. & Henson, P. M. (eds.) The Molecular and Cellular Biology of Wound Repair, pp. 3-33, Plenum Press, New York.

Frynta, D., Palupcikova, K., Bellinvia, E., Benda, P., Skarlantova, H., Schwarzova, L. & Modry, D. (2010) Phylogenetic relationships within the *cahirinus–dimidiatus* group of the genus *Acomys* (Rodentia: Muridae): new mitochondrial lineages from Sahara, Iran and the Arabian Peninsula. Zootaxa 2660: 46-56.

Gaire, J., Varholick, J. A., Rana, S., Sunshine, M. D., Doré, S., Barbazuk, W. B., Fuller, D. D., Maden, M. & Simmons, C. S. (2021) Spiny mouse (*Acomys*): an emerging research organism for regenerative medicine with applications beyond the skin. npj Regenerative Medicine 6(1): 1.

Gawriluk, T. R., Simkin, J., Thompson, K. L., Biswas, S. K., Clare-Salzler, Z., Kimani, J. M., Kiama, S. G., Smith, J. J., Ezenwa, V. O. & Seifert, A. W. (2016) Comparative analysis of ear-hole closure identifies epimorphic regeneration as a discrete trait in mammals. Nature Communications 7: 11164.

Haughton, C. L., Gawriluk, T. R. & Seifert, A. W. (2016) The biology and husbandry of the African spiny mouse (*Acomys cahirinus*) and the research uses of a laboratory colony. Journal of American Association for Laboratory Animal Science 55(1): 9-17.

Kunze, B., Dieterlen, F., Traut, W. & Winking, H. (1999) Karyotype relationship among four species of Spiny mice (*Acomys*, Rodentia). Zeitschrift für Säugetierkunde 64: 220-229.

Larson, B. J., Longaker, M. T. & Lorenz, H. P. (2010) Scarless fetal wound healing: a basic science review. Plastic Reconstructive Surgery 126(4): 1172-1180.

Matias Santos, D., Rita, A. M., Casanellas, I., Brito Ova, A., Araújo, I. M., Power, D. & Tiscornia, G. (2016) Ear wound regeneration in the African spiny mouse *Acomys cahirinus*. Regeneration (Oxf) 3(1): 52-61.

Mekada, K., Oyake, A., Miyake, S., Yamaguchi, H., Hiraki, E., Yoshida, S., Tokumatsu, E. & Mochida, H. (2021) Basic reproductive features of captive Arabian spiny mice (*Acomys dimidiatus*). The Bulletin of Okayama University of Science 57A: 7-11.

Miyazaki, T., Haraguchi, S., Kim-Kameyama, J.R. & Miyazaki, A. (2019) Endothelial calpain systems orchestrate myofibroblast differentiation during wound healing. FASEB Journal 33(2): 2037-2046.

Mochida, H. & Mekada, K. (2017) Taxonomic status of Aco strain derived from spiny mice. Proceeding of Okayama Association for Laboratory Animal Science 33: 22-25. [in Japanese]

Montandon, S. A., Tzika, A. C., Martins, A. F., Chopard, B. & Milinkovitch, M. C. (2014) Two waves of anisotropic growth generate enlarged follicles in the spiny mouse. EvoDevo 5: 33.

Seifert, A. W., Kiama, S. G., Seifert, M. G., Goheen, J. R., Palmer, T. M. & Maden, M. (2012) Skin shedding and tissue regeneration in African spiny mice (*Acomys*). Nature 489(7417): 561-565.

Slack, J. M., Lin, G. & Chen, Y. (2008) The *Xenopus tadpole*: a new model for regeneration research. Cell and Molecular Life Sciences 65(1): 54-63.

Volobouev, V., Auffray, J. C., Debat, V., Denys, C., Gautun, J. C. & Tranier, M. (2007) Species delimitation in the *Acomys cahirinus–dimidiatus* complex (Rodentia, Muridae) inferred from chromosomal and morphological analyses. Biological Journal of Linnean Society 91(2): 203-214.

公受暁子・目加田和之：アラビアトゲマウス（*Acomys dimidiatus*）の耳介損傷の再生

要約

アフリカトゲマウスとして一般的に知られる*Acomys* に属する*A. cahirinus*, *A. kempi* および*A. percivali* の３種の齧歯類において，皮膚の脱落，皮膚の再生，広範な耳介閉鎖が報告されている．本研究では，第４の種である*A. dimidiatus* の再生能力を検証した．耳介の近位部ないし遠位部に直径２mm のパンチ穴を開けたトゲマウスを35日間まで経時的に観察をしたところ，近位部のパンチ穴はほぼ完全に塞がり，修復部位には弾性軟骨，脂肪組織，真皮，表皮，毛包が広範囲に形成された．遠位部に関しては，本研究での観察期間では，完全な閉鎖までは至らなかったが，同様に皮膚の再生が観察された．これらの結果は，*A. dimidiatus* の再生能力を裏付けるものであり，*Acomys* 属が再生研究のモデル動物としてさらに注目されるべきものであることを示した．

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