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Developmental genetics in emerging rodent models: case studies and perspectives

Ricardo Mallarino[1], Hopi E Hoekstra[1] and Marie Manceau[2]

For decades, mammalian developmental genetic studies have focused almost entirely on two laboratory models: *Mus* and *Rattus*, species that breed readily in the laboratory and for which a wealth of molecular and genetic resources exist. These species alone, however, do not capture the remarkable diversity of morphological, behavioural and physiological traits seen across rodents, a group that represents >40% of all mammal species. Due to new advances in molecular tools and genomic technologies, studying the developmental events underlying natural variation in a wide range of species for a wide range of traits has become increasingly feasible. Here we review several recent studies and discuss how they not only provided technical resources for newly emerging rodent models in developmental genetics but also are instrumental in further encouraging scientists, from a wide range of research fields, to capitalize on the great diversity in development that has evolved among rodents.

Addresses
[1] Department of Organismic & Evolutionary Biology, Department of Molecular & Cellular Biology, Museum of Comparative Zoology, Howard Hughes Medical Institute, Harvard University, Cambridge, MA, USA
[2] Center for Interdisciplinary Research in Biology, Collège de France, Paris, France

Corresponding author: Manceau, Marie (marie.manceau@college-de-france.fr)

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Introduction

Understanding how morphological traits form and evolve is a fundamental challenge in biology. Because of their small body size, short generation time, and high fecundity in captivity, rodents traditionally have served as major laboratory models to study mammalian genetics and development, in addition to a variety of other research fields, including, behaviour, physiology, and biomedical studies relevant in human disease. Two murine species have been the primary workhorses: the house mouse (*Mus musculus*) and the laboratory rat (*Rattus norvegicus*). Their key role in uncovering fundamental principles in developmental genetics has been predicated on the wealth of molecular and genomic resources that have been generated for these species — the genomes of *Mus* and *Rattus* were among the first mammalian genomes sequenced and annotated [1,2]; numerous laboratory strains have been produced and are maintained; and molecular and cellular tools for tissue staining, imaging and functional testing abound.

The availability of such resources has made rodent models, particularly *Mus*, a prominent system in which to infer the molecular basis of trait formation and evolution in other species for which direct experimentation is difficult. For example, functional studies relying on transgenic mice have allowed researchers to test hypotheses related to diverse morphological traits, including limb formation in bats [3], limb loss and penis formation in snakes [4••], sensory vibrissae and penile spine loss in humans [5], human hair pigmentation [6••], as well as hair thickness and eccrine sweat glands in specific human populations [7]. On the other hand, computational and molecular approaches have been used in laboratory mice to construct predictive developmental models. For example, experimental data from *in vitro* reconstruction of tooth formation in *Mus* allowed for the prediction of dentition patterns among rodents with various diets [8]. More recently, it was shown that modulation of signalling factors can recreate the dental transformations that occurred during rodent evolution [9••]. These studies, however, are limited to traits present in laboratory rodents, and identifying causal genetic variants and developmental processes is often difficult due to the evolutionary distance between the species compared and the extent of character variation.

Despite the unquestionable importance of *Mus* and *Rattus* in biological research, both species are members of a single family {Muridae} and thus, their morphologies, behaviours and physiologies represent only a subset of the range of phenotypic diversity that has evolved among rodents. Here we highlight why rodents, a large group of species displaying a remarkable diversity of traits, should no longer be viewed only as a 'passive' tool for understanding biological processes occurring in other species. Instead rodents can be used both as a prominent study system in which to investigate the origin and evolution of natural variation in a range of phenotypes (evolutionary biology) as well as testable systems to explore the molecular bases of

tissue and organ development (developmental biology). Due to recent advances in genomic and molecular techniques, we can now turn to the use of new rodent models that display ecologically and developmentally relevant traits to explore the molecular basis of character formation and evolution in unprecedented detail.

Trait diversity in rodents

Rodents represent the most taxonomically diverse order of mammals, comprising more than 2000 extant species that diverged between the Pleistocene and the middle Miocene, and radiated into multiple environments (reviewed in: [10]). Present on all continents but Antarctica, rodents have invaded all terrestrial habitats, adapting to desert heat (e.g., jerboas, kangaroo rats), extreme cold (e.g. lemmings, siberian hamsters), altitude (e.g., deer mice, guinea pigs), semi-aquatic life (e.g., beavers, nutria), and fossorial life (e.g., gophers, mole rats), among others. Because they occupy such a range of habitats, rodents have evolved a diversity of specialized morphologies. Skeletal architecture, for example, is a highly variable trait–individuals, populations and species have evolved differences in digit number, limb length, tail length, head and snout shape, and/or vertebrae number (reviewed in: [11,12]). Coat colouration, often offering background-matching camouflage in rodents, ranges from completely white to melanic, and may be homogeneous or distributed in a bicolored (i.e., light ventrum and dark dorsum) or more complex, periodic pattern (e.g., longitudinal stripes and dotted lines of chipmunks and pacas). Fur can be sparse (e.g., naked mole rats) or extremely dense (e.g., chinchillas), and hair can thicken to form spines (e.g., spiny mice, porcupines). Variation in behaviour has also evolved among species: rodents may be diurnal or nocturnal, solitary or gregarious, monogamous or promiscuous, herbivorous or insectivorous or even carnivorous. Finally, although less documented, physiological diversity exists: for example, the kidneys of desert-dwelling rodents concentrate urine [13]; wood rats and other mice have evolved resistance to toxic compounds found in plants [14]; and hibernating rodents, like thirteen-lined ground squirrels, have evolved thermogenic proteins that are activated during hibernation to support nervous tissue function at low temperatures [15]. Natural populations of rodents thus constitute a wide source of variation that is an opportunity to study directly, beyond the limits of comparative approaches with traditional laboratory models, the developmental pathways and cellular events (morphogenesis) governing the formation of mammalian traits and the mechanisms shaping their evolution.

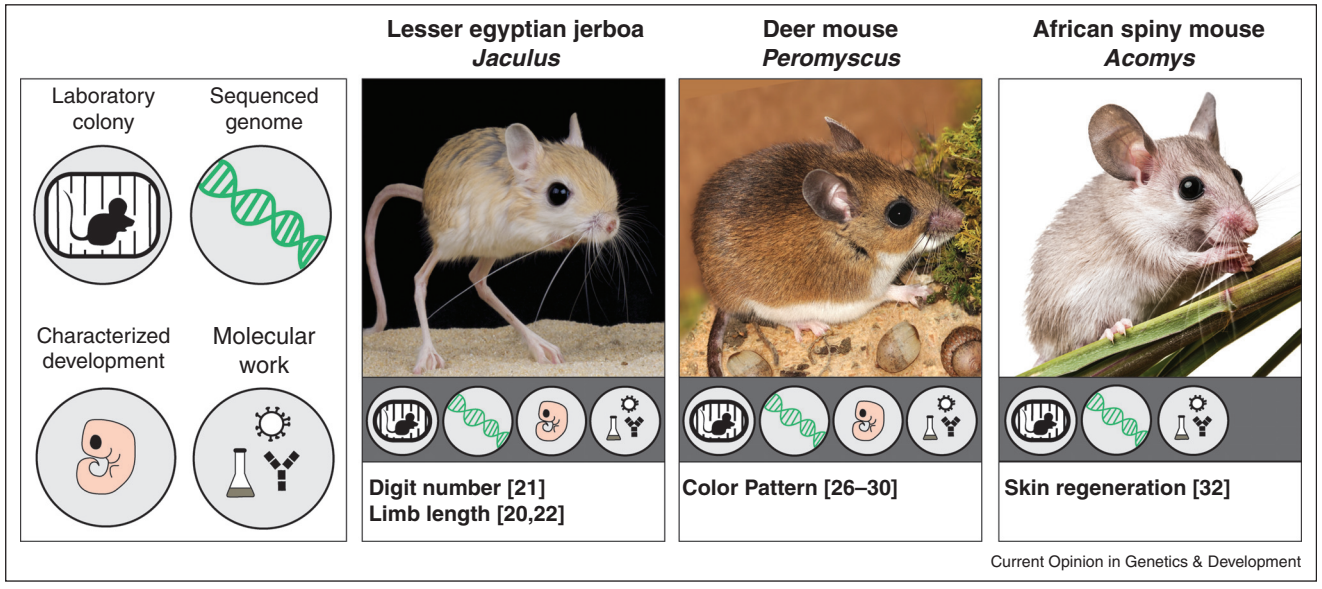
Beyond *Mus* and *Rattus*: developmental genetics in emerging rodent models

While largely unexploited due to the technical challenges associated with studying novel species, studying the mechanisms underlying the remarkable diversity highlighted above is now a real possibility. First, a wealth of natural history is documented on the majority of species representing the major rodent groups, and phylogenetic relationships of the main evolutionary lineages have been well resolved [10,16,17]. Second, a growing number of species already have established breeding colonies: wild-caught house mice, deer mice, grasshopper mice, jumping mice, jerboas, gerbils, voles, thirteen-lined ground squirrels, hamsters (e.g. Chinese, Syrian, dwarf), guinea pigs, mole rats and chinchillas, to name a few. The feasibility of breeding additional new rodent species in modern laboratories has been demonstrated by the fact that some of these colonies are maintained (after a decontamination process) in pathogen-free animal facilities. Third, with the large number of soon-to-be available rodent genomes (e.g., Genome 10k Project; Broad Institute of MIT and Harvard Project; [18]) and the advent of techniques amenable to virtually any species [19,20], such as innovations in high throughput DNA and RNA sequencing analytical technologies (e.g., double digest restriction associated DNA sequencing [21], *de novo* transcriptome assembly), new molecular tools (e.g., retroviral and lentiviral constructs, gene editing through the use of CRISPR/Cas9), and imaging techniques–scientists from various disciplines are starting to integrate fields to take advantage of the endless phenotypic variation of wild animals. Here we review work from three groups that have done so in emerging rodent models displaying variation in limb structure, coat colouration and skin regeneration (see Figure 1).

Jerboas and limb modifications

Because limb formation is arguably one of the primary models for developmental patterning and tissue differentiation, bipedal rodents have attracted much interest. Several desert rodents commonly display modifications in the number of digits or length of the limb, likely as an adaptation for long-distance bipedal locomotion, often observed in species that occupy open habitats and cover great distances to find food and shelter. Cooper and colleagues [22] compared chondrogenesis of hind limb bones in lesser Egyptian jerboas (*Jaculus jaculus*; see Figure 1), which have fused and drastically elongated metatarsals in the hind limb, compared with laboratory mice. They showed that chondrocytes undergo several phases of growth during embryonic development, providing insights into the mechanisms of skeletal size regulation. This study established the utility of jerboas as a new rodent system in which to study the processes governing limb evolution. A second study focused on the three-toed jerboa (*Dipus sagitta*), a species in which the anterior-most and posterior-most digits and associated metatarsals are absent. Using histological and expression analyses for marker genes known for their role in digit formation in mice (i.e., *Shh*, *Gli1*, *HoxD13*, and *Ptch1*), they showed that during early development the three-toed jerboa undergoes patterning of five digits, similar to what is seen in

Figure 1



Examples of emerging rodent models in developmental genetics. Rodent species discussed in this review, which have contributed new insights into developmental genetic processes and their evolution. Icons indicate that for all four species, breeding colonies have been established and genome sequences are available (or soon available). Developmental (i.e., description of embryonic stages and experimentation on embryos) and molecular (i.e., studies of protein and transcript expression) work has been performed in deer mice and jerboas.

laboratory mice, five-toed Jerboas (*Allactaga elater*), and ungulates (e.g., camels and horses). Later in development however, the tissue undergoes extensive cell death and two of the digits are lost, a process likely controlled by *Msx2*. Interestingly, a different mechanism acts in pigs in which early patterning of missing digits is impaired, correlating with a loss of *Ptch1* expression, thereby showing there is flexibility in the developmental events causing digit loss in mammals [23••]. Interestingly, in jerboas, digit loss is developmentally independent of limb elongation or metatarsal fusion [24], providing an excellent system in which to study the modularity of developmental processes shaping limb evolution. Further experimentation in jerboas for which stable breeding colonies have been established [11] may allow for the uncovering of the molecular and morphogenetic underpinnings of limb formation in these mammals.

Deer mice and pigmentation

Coat colouration is an iconic example of adaptive variation that repeatedly evolved to provide camouflage — the dorsal coat of many rodents closely matches their local soil colour. Moreover, pigment mutations in laboratory mice have helped uncover some of the fundamental principles of heredity and as a consequence, skin histology and pigment production processes have been widely characterized [25]. Thus, pigmentation has provided a nice framework for uncovering the developmental pathways and morphogenetic events that govern colour variation downstream of known genetic changes.

Deer mice (genus *Peromyscus*; see Figure 1) have long been the subjects of study by natural historians; hence variation in coat colour has been well documented both within and between species. One *Peromyscus* species, in particular, has emerged as a promising system for studying the developmental changes associated with pigmentation and pattern. Populations of the oldfield mouse (*Peromyscus polionotus*) display intraspecific variation in colour and pattern resulting from adaptation to visual predation in their respective habitats [26]. Specifically, *P. p. subgriseus*, inhabiting dark loamy fields, are characterized by a dark brown dorsum and light grey ventrum, a pattern typical of many vertebrates, while *P. p. leucocephalus*, which recently invaded white sand beaches of Florida, have a comparably lighter dorsum, and their ventral area extends dorsally and is devoid of pigment. A quantitative trait loci (QTL) analysis performed on a genetic intercross between these two subspecies combined with in vitro and in vivo functional testing in embryonic and adult specimens showed that three genes act together to produce colour changes [27,28]. In these beach mice, the melanocortin receptor *Mc1r* contains a coding mutation that lowers its activity at the surface of pigment producing cells and results in overall lighter colouration [29]. Its antagonist, the signalling molecule *Agouti*, contains a *cis*-regulatory modification that changes its level and spatial expression during development. To understand the effects of alterations in *Agouti* expression, Manceau and colleagues adapted a technique pioneered in *Mus* — ultrasound-assisted retrovirus injections — that

allows modification of the expression of a given gene in targeted regions of the developing embryos [30]. Using this tool, they showed that ectopic expression of *Agouti* in the developing skin of *Peromyscus* embryos impairs pigment cell terminal differentiation [28]. In beach mice, changes at the *Agouti* locus increase and dorsally extend its expression, causing a complete inhibition of pigment cell differentiation leading to the observed ventral colour and pattern changes [28]. The possibility of combining forward genetic screens with powerful molecular tools for testing gene function, such as ultrasound-assisted viral injections, will facilitate investigations of other identified causal loci and allow reconstructions of the evolutionary history of developmental modifications. Doing so for other species displaying similar variation (e.g., mutations at the *Agouti* locus are associated with lighter colouration in deer mice, *Peromyscus maniculatus*; [31,32]) will provide insights into the developmental bases of convergent evolution.

Spiny mice and skin regeneration

The previous two examples have highlighted traits that form during embryonic development. We now shift the focus to discuss an example of a developmental process that occurs in the adult: tissue regeneration. The skin, the largest vertebrate organ, is the first line of defense against external injuries and as such, it frequently undergoes trauma. When this occurs, most mammals, including humans, are not able to functionally replace the injured tissue (regeneration) and instead undergo a process of scarring (fibrosis), which involves the excessive production of connective tissue without the appearance of organs such as hair follicles and sweat glands. Although a few mammalian species are capable of regenerating different components of the skin tissue [33], a recent study identified African spiny mice (*Acomys spp.*; see Figure 1) as a promising system in which to understand the mechanisms that promote skin regeneration over scarring [34]. The skin of African spiny mice is extremely weak, compared to laboratory mice, and can easily tear when tension is applied, a process thought to aid in escape from predators. Seifert and colleagues inflicted wounds in the dorsal skin of two species of African spiny mice, *A. kempi* and *A. percivali*, and, using histological and molecular markers, found that a few days after wound infliction, the dermal and epidermal architecture had been re-established and closely resembled that of intact skin [34]. Specifically, they found that, relative to Swiss Webster laboratory mice, *Acomys* had a faster rate of reepithelialisation, a slower deposition of collagen type I, a protein that is produced at high levels during the formation of fibrous scars, and detectable expression of *Lef1*, a marker of hair follicle placodes [34]. As they display extensive variation in skin morphology, including porous extracellular matrix (allowing tearing) or thickened hair, ongoing developmental studies in spiny mice will provide important insights as to why this species is capable of undergoing skin regeneration whereas other mammalian species cannot.

Future directions

By integrating histological analyses with expression assays, in the case of jerboas, forward genetics and functional experimentation in deer mice, and histological/molecular markers with measurements of physical parameters in spiny mice, the studies discussed above highlight ways in which linking phenotypic change to specific developmental processes in diverse rodents is possible, and new developmental insights are starting to emerge. In general, we envision that emerging rodent models may prove instrumental, at least initially, for three developmental fields. First, the study of tissue patterning will benefit from diversity in limb and skin patterns (i.e., limb and digit form and number, integumentary appendage and pigment distribution across the body, and skin regeneration) in diverse rodent species, such as jerboas, deer mice, spiny mice and other species. Second, tissue homeostasis has been investigated in the context of bone formation in jerboas, but more generally, variation in the size of other tissues such as internal organs and teeth, which can be highly variable in natural populations of rodents, can serve as a model in which to study tissue growth. Finally, specific, derived characters such as spines of porcupines or extended skin membranes of flying squirrels may provide insights into the mechanisms of tissue differentiation.

Because the use of diverse rodent species currently is still rare in developmental genetics, the isolated case studies presented in this review, though providing insights into adaptive trait formation, do not yet allow us to draw of a general picture of the mechanisms underlying the developmental bases of trait evolution in natural populations. Although two of the examples described above pointed towards late acting tissue modifications (i.e., secondary cell death in jerboas limbs or terminal differentiation of pigment cells in deer mouse skin), it is unknown whether they represent a common theme in developmental processes across rodents. Most likely, they exemplify only a subset of the many ways in which phenotypic variation can originate. Thus, we advocate for an increased use of diverse rodents in developmental biology as more techniques become available. Such effort will open new avenues in the search for principles governing the evolution of form and will provide further experimental insights into the role that key processes in evolutionary developmental biology - such as modularity, convergent evolution, heterochrony, and co-option - play in driving biological diversity.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Mouse Genome Sequencing Consortium, Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M *et al.*: **Initial sequencing and comparative analysis of the mouse genome**. *Nature* 2002, **420**:520-562.

2. Genome Therapeutics, Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, Scott G, Steffen D, Worley KC *et al.*: **Genome sequence of the Brown Norway rat yields insights into mammalian evolution**. *Nature* 2004, **428**:493-521.

3. Cretekos CJ, Wang Y, Green ED, Martin JF, Rasweiler JJ, Behringer RR: **Regulatory divergence modifies limb length between mammals**. *Genes Develop* 2008, **22**:141-151.

4. •• Infante CR, Mihala AG, Park S, Wang JS, Johnson KK, Lauderdale JD, Menke DB: **Shared enhancer activity in the limbs and phallus and functional divergence of a limb-genital cis-regulatory element in snakes**. *Develop Cell* 2015, **35**:107-119.

This study uses a combination of enhancer analyses and genetics in snakes, lizards and mice to show that limbs and genitalia formation involves common patterns of enhancer activity during development, that have likely been partially lost in snakes.

5. McLean CY, Reno PL, Pollen AA, Bassan AI, Capellini TD, Guenther C, Indjeian VB, Lim X, Menke DB, Schaar BT *et al.*: **Human-specific loss of regulatory DNA and the evolution of human-specific traits**. *Nature* 2011, **471**:216-219.

6. •• Guenther CA, Tasic B, Luo L, Bedell MA, Kingsley DM: **A molecular basis for classic blond hair color in Europeans**. *Nat Genet* 2014, **46**:748-752.

By characterizing and functionally testing the regulatory region of the Kit ligand gene linked to blond hair color in humans, authors of this study identified a SNP in an enhancer driving Kit's expression in hair follicles that causes a reduction of the responsiveness of the crucial transcription factor LEF1.

7. Kamberov YG, Wang S, Tan J, Gerbault P, Wark A, Tan L, Yang Y, Li S, Tang K, Chen H *et al.*: **Modeling recent human evolution in mice by expression of a selected EDAR variant**. *Cell* 2013, **152**:691-702.

8. Kavanagh KD, Evans AR, Jernvall J: **Predicting evolutionary patterns of mammalian teeth from development**. *Nature* 2007, **449**:427-432.

9. •• Harjunmaa E, Seidel K, Hakkinen T, Renvoise E, Corfe IJ, Kallonen A, Zhang Z-Q, Evans AR, Mikkola ML, Salazar-Ciudad I *et al.*: **Replaying evolutionary transitions from the dental fossil record**. *Nature* 2014, **512**:44-49.

Using a combination of empirical and computational approaches, this study shows that varying concentrations of ectodysplasin A and inhibition of Sonic hedgehog signaling reproduce predicted phenotypic variation in rodent dentition, providing an innovative analytical approach to study the developmental bases of character evolution.

10. Kay EH, Hoekstra HE: **Rodents**. *Curr Biol* 2008, **18**:R406-R410.

11. Cooper KL: **The lesser Egyptian jerboa, *Jaculus jaculus*: a unique rodent model for evolution and development**. *Cold Spring Harb Protoc* 2011, **2011**:1451-1456.

12. Vaughan TA, Ryan JM, Czaplewski NJ: **Mammalogy**. *Jones and Bartlett Learning* 2013.

13. Feldhamer GA, Drickamer LC, Vessey SH, Merritt JF, Krajewski C: *Mammalogy*. JHU Press; 2015.

14. Malenke JR, Skopec MM, Dearing MD: **Evidence for functional convergence in genes upregulated by herbivores ingesting plant secondary compounds**. *BMC Ecol* 2014, **14**:1-16.

15. Laursen WJ, Mastrotto M, Pesta D, Funk OH, Goodman JB, Merriman DK, Ingolia N, Shulman GI, Bagriantsev SN, Gracheva EO: **Neuronal UCP1 expression suggests a mechanism for local thermogenesis during hibernation**. *Proc Natl Acad Sci USA* 2015, **112**:1607-1612.

16. Steppan SJ, Adkins RM, Anderson J: **Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes**. *Syst Biol* 2004, **53**:533-553.

17. Steppan SJ, Adkins RM, Spinks PQ, Hale C: **Multigene phylogeny of the Old World mice, Murinae, reveals distinct geographic lineages and the declining utility of mitochondrial genes compared to nuclear genes**. *Mol Phylogenet Evol* 2005, **37**:370-388.

18. Koepfli K-P, Paten B: **Genome 10K community of scientists, O'Brien SJ: The Genome 10 K Project: a way forward**. *Annu Rev Anim Biosci* 2015, **3**:57-111.

19. Milinkovitch MC, Tzika A: **Escaping the mouse trap: the selection of new evo-devo model species**. *J Exp Zool B Mol Dev Evol* 2007, **308B**:337-346.

20. Abzhanov A, Extavour CG, Groover A, Hodges SA, Hoekstra HE, Kramer EM, Monteiro A: **Are we there yet? Tracking the development of new model systems**. *Trends Genet* 2008, **24**:353-360.

21. Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE: **Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species**. *PLoS ONE* 2012, **7**:e37135.

22. Cooper KL, Oh S, Sung Y, Dasari RR, Kirschner MW, Tabin CJ: **Multiple phases of chondrocyte enlargement underlie differences in skeletal proportions**. *Nature* 2013, **495**:375-378.

23. •• Cooper KL, Sears KE, Uygur A, Maier J, Baczkowski K-S, Brosnahan M, Antczak D, Skidmore JA, Tabin CJ: **Patterning and post-patterning modes of evolutionary digit loss in mammals**. *Nature* 2014, **511**:41-45.

This study provides an embryological explanation for digit loss in mammals, showing the crucial role of localized cell death and variation in expression of genes involved in digit formation. By performing empirical experiments in several key species, authors show that there is developmental flexibility in the mechanisms that control convergent digit loss in mammals.

24. Moore TY, Organ CL, Edwards SV, Biewener AA, Tabin CJ, Jenkins FA, Cooper KL: **Multiple phylogenetically distinct events shaped the evolution of limb skeletal morphologies associated with bipedalism in the jerboas**. *Curr Biol* 2015, **25**:2785-2794.

25. Silvers WK: *The Coat Colors of Mice*. Springer Science & Business Media; 2012.

26. Vignieri SN, Larson JG, Hoekstra HE: **The selective advantage of crypsis in mice**. *Evolution* 2010, **64**:2153-2158.

27. Steiner CC, Weber JN, Hoekstra HE: **Adaptive variation in beach mice produced by two interacting pigmentation genes**. *PLoS Biol* 2007, **5**:e219.

28. Manceau M, Domingues VS, Mallarino R, Hoekstra HE: **The developmental role of Agouti in color pattern evolution**. *Science* 2011, **331**:1062-1065.

29. Hoekstra HE, Hirschmann RJ, Bundey RA, Insel PA, Crossland JP: **A single amino acid mutation contributes to adaptive beach mouse color pattern**. *Science* 2006, **313**:101-104.

30. Punzo C, Cepko CL: **Ultrasound-guided in utero injections allow studies of the development and function of the eye**. *Dev Dyn* 2008, **237**:1034-1042.

31. Linnen CR, Kingsley EP, Jensen JD, Hoekstra HE: **On the origin and spread of an adaptive allele in deer mice**. *Science* 2009, **325**:1095-1098.

32. Linnen CR, Poh Y-P, Peterson BK, Barrett RDH, Larson JG, Jensen JD, Hoekstra HE: **Adaptive evolution of multiple traits through multiple mutations at a single gene**. *Science* 2013, **339**:1312-1316.

33. Seifert AW, Maden M: **New insights into vertebrate skin regeneration**. *Int Rev Cell Mol Biol* 2014, **310**:129-169.

34. Seifert AW, Kiama SG, Seifert MG, Goheen JR, Palmer TM, Maden M: **Skin shedding and tissue regeneration in African spiny mice (Acomys)**. *Nature* 2012, **489**:561-565.