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Research Article

Potential use of brush-tailed mice for evolutionary developmental biology studies: *Calomyscus elburzensis* an appropriate rodent species

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Abstract
Rodents (mainly mice and rats) are the most commonly used animal models for studies of cancer biology, immunology, genetics, developmental biology and also embryo transfer techniques in domestic and endangered animal species, and even in humans. Herein, we aimed to evaluate Goodwin's brush-tailed mouse (*Calomyscus elburzensis*) as a suggested model species for evolutionary developmental biology (Evo-Devo) studies. Trapping procedure was performed in Khaje-Morad rocky region, south center of Mashhad, Razavi Khorasan province, Iran between April and September 2014. Breeding procedure for 15 adult females and 12 adult males was conducted in captivity between 2014 and 2017. Brother × sister mating in each generation was used as laboratory rearing method for breeding three generations (F1-F3). The average of pregnancy period and the differences in the sex of newborns in different parturition were measured using descriptive statistical analyses (independent-sample t-test). We observed that Goodwin's brush-tailed mice were relatively social rodents, and due to their high level of tolerance in harsh conditions, no hibernation, low cost, small size, the potential to be bred all round the year, nearly short pregnancy period (about 4.5 weeks) and long lifespan in captivity (generally between 4 to 5 years) have the potential to be bred and used in different fields of evolutionary studies. However, low reproductive output (known up to five till now) and late sexual maturity (at about four months) as compared with *Mus* and *Rattus*, are some drawbacks associated with *Calomyscus*, which might be improved by gradual genetic changes and artificial selection.

Keywords: Laboratory models, Rodentia, Goodwin's brush-tailed mouse, Breeding strain, Iran.

Introduction
Animal models are mostly used as vital tools in medical, immunological, physiological, pathological, developmental, genetic, cancer and neuroscience studies. For more than one hundred years, every progress in different fields of biological studies has been virtually affected by using animal models (Willis-Owen and Flint 2006). These models still have important roles in our understanding of the function of genes, the mechanisms of various diseases, the effectiveness and toxicity of different chemicals and also many other fields (Patten and Hall-Porter 2009, Makvand-Hosseini *et al.* 2014). Moreover, study of animal models is required to preserve endangered and nearly extinct species such as the tamarins of Brazil and California by applying new reproductive techniques. All in all, use of animal models in related studies is an essential factor to develop new techniques which are suitable for characterizing biological phenomena both in

humans and animals (Willis-Owen and Flint 2006, Patten and Hall-Porter 2009, Makvand-Hosseini *et al.* 2014).

The most commonly used model animals are murine species such as house mice (*Mus musculus*) and brown rats (*Rattus norvegicus*), which have made the basis for our current knowledge in many fields including cancer, aging, immunology, and genetics as well as many other aspects of science. They are also used to develop and complete the embryo transfer techniques in domestic and endangered animal species, and even in humans (Suckow *et al.* 2005; Hedrich 2012). Moreover, the other rodent species such as Mongolian gerbils (*Meriones unguiculatus*), Syrian or golden hamsters (*Mesocricetus auratus*) and Guinea pigs (*Cavia porcellus*) are also used as animal models (Mills *et al.* 1996). Mongolian gerbil has been used in different fields of physiology and medicine (e.g. evaluating the efficacy of therapeutic agents, and the study of epilepsy or defining the disease pattern of encephalitic form of Rift Valley fever (RVF)) (Somova *et al.* 2000). However, use of this species as animal model has decreased in recent years. Some hamster species (*e.g. Mesocricetus auratus, Phodopus sungorus, Cricetulus griseus*) thrive in captivity and are easily bred in this condition, so they are used in laboratories for behavioral and physiological studies (Kuhnen 2002, Fox 2006, Mitchell and Tully 2008). Guinea pigs are also used for medical researches mainly in the fields of immunology, pathology and physiology (Dominioni *et al.* 1984; Fernandez *et al.* 2006). Experimental models as well as genetically modified and negative models of the house mice species *Mus musculus* are commonly used in evolutionary studies. For example, many studies have performed on the activities of different genes during dental development (e.g. Nadiri *et al.* 2004; Hamidi *et al.* 2017b). A proper animal model for a research should be selected on the basis of the background knowledge of biological properties, cost and availability, ease and adaptability to experimental manipulation, and ecological consequences. The animal model should be chosen based on its adequacy to the planned procedures (Johnson and Besselsen 2002).

Goodwin's brush-tailed mouse as a new rodent model

The newly introduced animal model with novel biological characters in comparison with the previously introduced animal models increase the diversity of our available research models and supports expanding our fields of study and scientific approaches. Herein, we intended to consider the possibility of using Goodwin's brush-tailed mice (*Calomyscus elburzensis* Goodwin, 1938) as a new animal model.

Brush-tailed mice are a group of small rodents belonging to family Calomyscidae Vorontsov and Potapova, 1979. They are distributed around Near East and Middle Asia and interestingly, are limited to the Iranian plateau and its neighborhoods; they can be usually found in semi mountainous areas in desert regions of Iran, Afghanistan, Pakistan, Turkmenistan, Azerbaijan and Syria (Nowak 1999, Musser and Carleton 2005). They represent an early split from the rest of the mouse-like rodents (such as mice and rats), and also are the sole member of a clade basal to the radiations of other Muroidea (Jansa and Weksler 2004). Brush-tailed mice have maximum lifespan among muroid rodents. They regularly live over four years in captivity; nonetheless there are several records indicating that they can live as long as nine years in captivity. Hence, it may suggest that these rodents are very similar in life history traits to much larger rodents such as sciurids and hystricognaths, which both of them can live over ten years in captivity (Carleton and Musser 1984, Meyer and Malikov 1996).

Calomyscidae contains eight valid species belonging to a single genus, *Calomyscus* Thomas, 1905. Goodwin's brush-tailed mouse has been mainly reported from mountains of north and northeast of Iran (Lebedev *et al.* 1998, Musser and Carleton 2005). In Europe, a species of *Calomyscus* is popularly kept as a pet; they are labeled *C. bailwardi*, and probably represent either *C. mystax* or *C. elburzensis*. However, they can be rarely found in European pet stores but commonly presented in dedicated breeders (Volf and Volf 2003). In Russia, they have been used in laboratories for different sorts of researches and also sold as pets in many pet shops. In United Kingdom, they have been imported for exhibits in zoos (Acron web 2000). Herein, assuming the hypothesis of using Goodwin's brush-tailed mouse as a new animal model, we aimed to evaluate several features of this species, as one of the representative members of Calomyscidae, based on the author's long term previous and recent field and lab studies, and also discuss about the possibilities and limitations which may occur in the way of breeding and maintaining the colonies of this exotic old evolutionary rodent for using as a novel laboratory animal model.

Materials and methods

Trapping was performed in Khaje-Morad heights (36°08′-37°03′N and 59°13′-59°42′E at an altitude of 1146 m a.s.l.), south center of Mashhad, Razavi Khorasan province, Iran between April and September 2014 (Hamidi *et al.* 2015). This well drained, barren rocky region is located in cold and dry climatic condition with generally flat topography and some mounds. The typical vegetation of this area is mainly composed of Mount Atlas pistache (Anacardiaceae; *Pistacia atlantica* Desf.), shrubby horsetail (Ephedraceae; *Ephedra* sp.) and sun spurge (Euphorbiaceae; *Euphorbia helioscopia* L.) as well as some lands under cultivation and several industrial and residential constructions around it (Hamidi *et al.* 2015, 2017a).

Fifteen adult females and twelve adult males of Goodwin's brush-tailed mice were captured using custom-made mesh live traps. Captured rodents were transferred to the animal house and kept in captivity for breeding programs between 2014 and 2017 (Hamidi *et al.* 2017b; also author's unpublished data). A single male and a single female were paired in a separate cage. Mating cage was an oblong shaped box with following dimensions: $40 \times 60 \times 50$ cm^3. The frame of the cage was made of chewing-resistant transparent polyvinyl chloride (PVC), with galvanized wire fencing in some sides of the cage. The laboratory rearing method was organized on brother × sister (full-sib) mating in each generation. Wild animals were kept and raised for up to three generations (Filial 1-3) in captivity.

Statistical analyses (descriptive analyses) for developmental studies including the duration of pregnancy and the differences in the sex of newborns in different parturition have been performed using the SPSS 16.0 (SPSS Inc. 2007). Animal care was performed in compliance with the "Guideline for the care and use of laboratory and experimental animals, Rodentology Research Group, Ferdowsi University of Mashhad" (Darvish 2015).

Results

During present study focused on behavioral and biological features of *Calomyscus elburzensis*, we found that this species can be potentially considered as a relatively social rodent; Goodwin's brush-tailed mice were successfully kept in small colonies and bred in captivity for more than three years. Our observations are summarized below:

Feeding

They were generally herbivorous. Their natural diet in north and northeastern Iran was mainly *Pistacia atlantica* seeds (also see Hamidi *et al.* 2015, 2017a). During trapping process, they were mainly attracted to the baits resembling their preferred diet such as sunflower seeds and gourd seeds. So these seeds as well as hard small fruits such as walnuts, hazelnuts, chestnuts or almonds were used as their nutrient requirements in captivity. However, when they fed on a high fat diet, they tended to store the fat and became obese, therefore restricted feeding was necessary. In females, the fat accumulation was associated with some reproductive difficulties. Our observation showed that they had weak willing to use commercial feeds (rodent dry pellets). Adults were daily fed with about 10 g of seeds. Water was not critically necessary to provide directly for the mouse during rearing period because it could be supplied by providing apple slices and lettuce leaves.

Keeping in captivity

Lighting was a very important factor in the breeding of Goodwin's brush-tailed mice and a 12-12 h light-dark cycle was generally used. The mice avoided the areas with high level of light and tended to be hidden in their nests; thus using nest boxes instead of simple cages is suggested. Activity was higher during hours of darkness. They were reasonably cold-tolerant animals. Hence, the temperature at 19-23 ˚C was suitable for rearing them. The adequate humidity range for keeping this species was 25-50%.

Stress was often occurred when individual animals were put together later in life or a new one was introduced into another one's cage. For evading this tragedy, selecting a paired mating scheme and also not regrouping Goodwin's brush-tailed mice during breeding project was often applied. For breeding, two adults with different sexes were housed together at the age of about four months. The female may rarely become a bit aggressive toward the male if kept together for too long after mating. In some very rare cases, the males were killed after being attacked by the female (intersexual aggression). We could breed these rodents for up to three generations (F1, F2 and F3) in captivity. In addition, based on our observation, they could survive for at least five years in captivity.

Behavioral features

Goodwin's mice were nocturnal especially during the spring and summer, however in winter, they were found active in the afternoons too. Individuals of *C. elburzensis* did not generally have hibernation in captivity. They were relatively social rodents and rarely showed pugnacious tendencies in encountering with their congeneric and hence, could get together during a short time after housing in captivity. They tended to form colonial relationships and group housing was possible, although special care was essential in separation of semi-aggressive animals. At puberty stage, they often tended to exhibit light fighting or other weak aggressive behaviors. In contrary, young animals had very exploratory actions and engaged in social play for a long time; the males were more inquisitive than the females. They were clever animals and extensively used their sense of smell and acoustic abilities to evaluate their surroundings (their ears are very rounded in shape, devoided of hair and elongated 17 to 20 mm). Their hearing system is highly developed and severely sensitized to noise. They were very calm animals. However, sudden and extremely loud noises would cause them to escape or produce a special voiced sound.

Goodwin's brush-tailed mice were able to jump high and therefore, cages with minimum 50 cm heights were used. The minimum facilities included refuges (e.g. nest boxes) and nesting materials such as napkins, wadding or pelletized wood dust. They rarely bite unless mishandled and became tame when handled gently and frequently. When they were suddenly excited, they jumped and dart to escape from the stimulus agent.

Reproduction and developmental features

From what we observed in nature in northeastern parts of Iran *Calomyscus elburzensis* were breeding from mid-February to mid-June (generally with two times pregnancy in this period). Hence, they exhibited some appreciable seasonality acts in respect to reproduction and breeding events in nature. It is worth to mention that we were able to breed them successfully while in captivity all year round which restricted to 39 newborns (first filial: F1) descended from 11 pregnant females (out of 15 captured females). The duration of pregnancy was 31.5± 2.1 days on average, and females beard up to 5 offspring. Total of 15 pups were produced by a female belonging to the second filial (F2; raised in captivity), which happened during her six parturitions within about nine months. The breeding intervals for F2 females in captivity were 53.4± 0.6 days on average. In total, there was no significant difference in the sex of newborns of F1, F2 and F3 (independent-sample t-test; $p < 0.05$).

The young mice were unable to feed or care for themselves independently for a long period of time after birth (altricial). The litters were hairless and blind. Their body weight was 2 g, and their body length was about 53 mm on average. They were helpless for the first 15 days of their life when their eyes opened for the first time, both male and female contributed almost equally to breed their newborns. The litters were weaned no earlier than four weeks (mostly up to the postnatal day 35). The mice usually began to explore outside the nest at the age of five weeks (Fig. 1). The newborn males could be distinguished from the newborn females by their greater anogenital distance and larger genital papilla. The female pups had generally larger size than males. Nipples in the female pups could be observed about ten days after birth and testes in the males could be observed in the scrotum at fourth week if the pup was held with its head up. The first pregnancy in the captive females was occurred at the age of about four months, and the duration of postnatal growth and development was about six months. Significant events happening during the life history of Goodwin's brush-tailed mouse newborns are listed in Table 1.

Table 1. The most significant events in the life history of Calomyscus elburzensis newborns

Postnatal day (PN)	Significant event(s)
PN0	Dark body without hair; closed eyes; ears separated from the head and with visible blood vessels; enlarged moist bulge of olfactory organ around the nose covered with long whiskers
PN5	Emergence of the hair (body fur)
PN10	Appearance of six nipple glands in female pups
PN15	Eyes opening; enable to walk; grasp objects
PN20	Changing in the grayish color of dorsal fur near the head to the brownish (grey hairs change to brown initially from the proximal part of the back of the body to the distal part); emergence of the tail tuft as some dark color assembled hairs
PN35	Weaning from milk and start feeding on seeds; beginning to explore outside the nest
PN40	Separation of pups from mum; each was kept in a separate cage

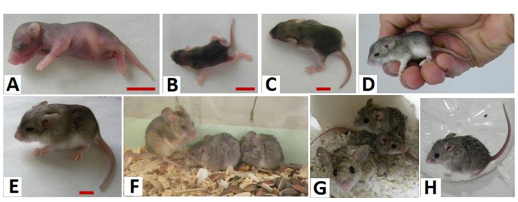

Figure 1. Life history of *Calomyscus elburzensis* newborn pups: A, B, C, D, E, F, G and H represent postnatal days 0, 5, 10, 15, 20, 30, 35 and 40, respectively. Mum is seen near her pups in F and G.

Discussion

As new laboratory techniques develop and available data related to biology, life style and reproduction characters of many organisms increase, new animal models will be introduced and old ones may be ignored. Some laboratory rodents e.g. the house mouse, have been very well characterized genetically and have undergone genetic manipulations and artificial selections to produce animals with uniformly heritable phenotypes (e.g. Hedrich 2012, Kuramoto 2012).

Different breeding systems, selective breeding, and genetic-engineering techniques have been used to produce strains and stocks of rodents for particular experimental purposes: inbred strains, transgenic strains, recombinant inbred strains, hybrid strains, and outbred stocks. A strain is a group in which all members are closely identical from the point of genetic traits. In laboratory mice and rats, this is accomplished through inbreeding. Full-sib inbreeding for 20 generations will result in more than 98% genetic homogeneity, at which point the members of the stock are isogenic and the stock is considered an inbred strain (Hedrich and Bullock 2004; Hedrich 2012). Many inbred strains of mice are widely used in different types of research. A few inbred strains of rats, Guinea pigs, Syrian hamsters, and gerbils have also been developed. By having this kind of isogenic populations, it is possible to conduct researches on the roles of genes, or carry out experiments that do not require genetic variations. By contrast, outbred populations are used when genetic variations among members are required or identical genotypes are unnecessary. These populations are usually referred to as stocks rather than strains and are used primarily when genetic heterogeneity is desired (National Research Council and Institute of Medicine 1988, Mills *et al.* 1996; Krinke 2000).

Brush-tailed mice are considered as the only existing members of the subfamily Myocricetodontinae Lavocat, 1961 which occurred since early Miocene (about 13.7-18 Ma) (Wessels 1996), and they are also called living fossils. Other common rodent models have been evolved later. For example, *Mesocricetus* species were evolved during the late Miocene about 7-12 Ma ago (McKenna and Bell 1997; Kowalski 2001) and the evolutionary time of species thought to be on the line leading to *Mus*, is considered as 10.4 Ma ago (Jacobs and Flynn 2005). Comparative evolutionary developmental biology (Evo-Devo) studies focusing on the similarity in dental morphology of *Calomyscus* species (with considering Calomyscidae as a basal group) with many other muroid rodents (such as members of Murinae, Acomyinae, Dendromurinae and Cricetomyinae) (Jansa and Weksler 2004, Wessels 2009) can be very helpful to understand the way of convergent (or maybe parallel) evolution of these rodents. In other words, studies on changes in the gene expression during pre- and postnatal development of *Calomyscus* species which are bred in the lab would add excellent data in this regard.

Furthermore, because of the long life history of brush-tailed mice and different climatic impacts on their habitat and distribution range, they can remarkably suffer harsh conditions. Goodwin's mice may be considered as an ideal selection for specific laboratory experiments especially for evolutionary studies. Some advantages of these animals include no hibernation, low cost, small size, easier handling, nearly short pregnancy period (about 4.5 weeks), long lifespan in captivity (generally between 4 to 5 years) and karyological variations (Meyer and Malikov 1996, Nowak 1999, Graphodatsky *et al.* 2000, Grzimek 2004, Musser and Carleton 2005, Hamidi *et al.* 2015). They can be bred and used in different fields of scientific studies such as basic biological research, educational and applied research, veterinary medicine, breeding programmes etc. Moreover, their relatively slow growth rate makes them suitable for chronic studies (e.g. their usefulness in prenatal protocols which last between 4 to 5 weeks vs. about 3 weeks in mice). Numerous experiments can be designed on Calomyscidae members based on the karyological variations observed in this family (e.g. Graphodatsky *et al.* 2000). It would also be possible to breed different strains of Goodwin's brush-tailed mice in captivity which result in producing enough numbers of genetically identical samples for genetic studies.

On the other hand, Goodwin's brush-tailed mice have lower reproductive outputs (known up to five till now), a relatively longer period of growth and development (between five to seven months) and later sexual maturity (at about four months) (Meyer and Malikov 1996, author's unpublished data) than the members of family Muridae (e.g. mice and rats). These characters may hamper the studies which require several generations of offspring during a reasonable period of time. These limitations can be improved by gradual genetic changes resulted through genetic manipulation, genetic improvement of valuable traits, and artificial selection. Therefore, Goodwin's brush-tailed mice can be introduced as an alternative ideal rodent model for Evo-Devo studies.

Conclusion

The long history of Goodwin's brush-tailed mice, and the basal situation of Calomyscidae among those radiations representing Muroidea in different molecular trees can provide a great opportunity for scientists to have an animal model with the potential of more suitability for comparative phylogenetic studies and especially in the field of Evo-Devo biology. Chance of finding genetic markers which are closely linked to major genes affecting some important quantitative traits will be increased by completing our knowledge about the genome of this species. However, it is necessary to continue comprehensive research on biology, ecology and behavior of this exotic animal. In conclusion, beside some limitations which may be improved by genetic manipulations and artificial selection, brush-tailed mice have a bright future perspective among other animal models for using in Evo-Devo studies.

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