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Newly breeding an inbred strain of ischemia-prone Mongolian gerbils and its reproduction and genetic characteristics

Xiaoyan DU[1,2], Dongping WANG[3], Ying LI[3,4], Xueyun HUO[1], Changlong LI[1], Jing LU[1], Ying WANG[1,2], Meng GUO[1,2], and Zhenwen CHEN[1]

[1]*School of Basic Medical Sciences, Capital Medical University, No.10 Xitoutiao, Youanmen, Fengtai District, Beijing 100069, P.R. China*
[2]*Department of Laboratory Animal, Capital Medical University, No.10 Xitoutiao, Youanmen, Fengtai District, Beijing 100069, P.R. China*
[3]*Institute of Jingfeng Medical Laboratory Animals, No. 20 Dongdajie, Fengtai District, Beijing 100071, P.R. China*
[4]*Animal Science and Technology College, Jilin Agricultural University, No. 2888 Xin Cheng Da Jie, Changchun 130118, P.R. China*

Abstract: The Mongolian gerbil has been a useful laboratory animal in many research fields, especially in ischemia studies. However, due to the variation of the circle of Willis (COW), the ischemic model is unstable and various. To solve this problem, we newly established an inbred strain of gerbils, restricting breeding and keeping to F_{23}. The data on the breeding and growth of the animals are described in the present study. The genetic characteristics of F_4 to F_{20} detected by microsatellite DNA and biochemical markers are also shown here. The results demonstrated that the frequency of ischemic model by unilateral carotid occlusion and the frequency of incomplete COW increased, increasing from 50% and 75% in F_1 to 88.89% and 100% in F_{20}, respectively. The ratios of consistent patterns of COW in parents were positively related with the number of inbred generations. A reproductive performance analysis indicated that the average size of litters in the inbred gerbils was less than that of outbred gerbils and that adult body weight was also lower in inbred gerbils; also, the pups in the 2nd litter were the best ones chosen to reproduce. The genetic detection results indicated that 26 out of 28 microsatellite loci and all 26 biochemical markers were homozygous in F_{20}, showing comparably identical genetic composition in inbred gerbils. All the data demonstrated that an inbred strain of ischemia-prone gerbil has been established successfully. This strain can be used in stroke research and can largely reduce the number of animals needed in experiments.
Key words: biochemical marker, inbred strain, ischemic model, microsatellite DNA, Mongolian gerbil

Introduction

The Mongolian gerbil (*Meriones unguiculatus*) is a laboratory animal that has been popularly used in many research fields. It has been captured in the basin of the Amur river in China and domesticated as a laboratory animal for about 80 years history [21]. The gerbil has been called multiple-function laboratory animal because

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Address corresponding: Z. Chen, School of Basic Medical Sciences, Capital Medical University, No.10 Xitoutiao, Youanmen, Fengtai District, Beijing 100069, P.R. China
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it has been able to benefit a great deal of research, such as research on gastritis and gastric cancer [14], the study of parasites [4], viral infections [23], epilepsy[8, 11], behavioral aging [12, 21], and hearing [16, 17].

One of the most useful human diseased models of the gerbil has been that used in the study of strokes since the pioneering work of Levin and Payan [13]. Because of the lack of communicating arteries between the posterior vertebral arteries (through the basilar artery) and the internal carotid arteries anteriorly, there is no significant collateral flow from the vertebral blood supply to the forebrain; thus, transient bilateral common carotid artery (CCA) occlusion can induce consistent ischemic injury [7, 10]. The relative simplicity of carotid occlusion makes this model very useful for the study of brain ischemic mechanisms [3, 9]. However, because of the variation of circle of Willis (COW) patterns, gerbil exhibit a lower frequency (30–40%) of the ischemic model [7]. Therefore, to resolve this problem, we established an ischemia-prone population of gerbils and increased the model percent from 40% to 70% in F_1 to F_5 by selectively breeding. Unfortunately, when the outbred colony was bred by randomly mating, the successful model ratio decreased. Thus, we hypothesized that selectively breeding an inbred strain would be a more sufficiently method to fix this biological character. In the present study, we report the results of selectively breeding an inbred gerbil with a higher frequency of ischemic model that was inbred for 8 years from 2008 after an ischemia-prone outbred gerbil was established [7]. An evaluation of the genetic quality and reproductive performance of this inbred gerbil was also performed.

Material and Methods

Ethics

All of the experimental procedures were conducted in accordance with the Guidelines of the Capital Medical University Animal Experiments and the Experimental Animals Management Committee (No. AEEI-2017–032).

Animals and housing

A total of 969 Mongolian gerbils (newborn to 18 months old) were involved. They were housed 2 or 3 animals per cage at a temperature of 22 ± 4°C and a humidity of 40–65% and were maintained under an artificially illuminated light and dark cycle (12-h light/dark cycle) with ad libitum access to standard laboratory diet (a commercial compound diet for mice, Production License: SCXK-2012–0003) and tap water.

Selective inbreeding of gerbils

The original 4 pairs of gerbils, whose parents had incomplete COW patterns and were from an outbred ischemia-prone population we established previously [7], were chosen as the original gerbils to be inbred. After weaning of the first litter in the second generations, we mated them with sisters and brothers. When the third generations were produced, the grandparents underwent unilateral carotid occlusion (UCO). Then the UCO animals were observed under a dissecting microscope (Leica EZ4) to confirm their patterns of COW in situ. These processes were repeated until the parents with consistent COW patterns appeared. From then on, we maintained and mated the litters from parents with consistent COW patterns as much possible to ensure that sufficient enough pairs of gerbils in each generation remained and continued this strategy sequentially until F_{20}.

UCO ischemic model of gerbil

When the parents produced two generations, we processed them for the UCO ischemic model. In brief, each animal was anesthetized with isoflurane (induced by 3%, maintained with 2%, 30% O_2/70% N_2O) in preparation for a ventral midline cervical incision to expose the right CCA. We tightly wrapped the CCA with a 4/0 silk thread, confirmed the blockage of blood flow, and placed each animal in an individual cage after closing the incision with glue. Using a heated blanket, we maintained the cage temperature at approximately 36.5°C until the animals were awake. To evaluate the effects of surgical stress on the brain, a sham-operated group (a total of 10 animals) underwent the same procedures and received the same treatment as the experimental group except for ligation of the right CCA. After confirming that the operation had no influence on the animals' neurological symptoms, we did not use the sham-operated group for subsequent evaluations.

We observed the post-UCO gerbils for 2 h after surgery and rated them according to a scale used in a previous reports [7]. This scale ranges from 0 to 5, with 0 representing normal behavior and 5 representing death. After scoring their behavior, all of the live animals were euthanized with an overdose of pentobarbital (150 mg/kg, intraperitoneal injection). The brains of all animals (including dead animal before euthanization) were removed and the patterns of COW were evaluated using a dissecting microscope (Leica EZ4).

The characteristics of reproduction in the inbred gerbil

When the F_{20} litters were produced, we mated them and their progeny by the inbreeding method and marked their information on white and green cards. In the case of the third generation after F_{20}, we mated their litter by randomly breeding and marked their information on yellow cards. Birth body weight, litter birth body weight, litter size, body weight daily for the first 7 days after birth, body weight weekly for the first 8 weeks after birth, and body weight at 8 weeks old and 3 to 4 months old were recorded or calculated.

Genetic detection in the growing F_3–F_{20} generations with 28 microsatellite loci

Genetic detection was performed with 28 microsatellite loci we selected in a previous study [6]. For this, the genomic DNA of 2–5 animals in F_3–F_{20} was extracted from frozen liver or kidney specimen by a standard phenol-chloroform method [24]. The DNA quality was analyzed by a micro-volume spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA, USA). The primers of the 28 microsatellite markers and PCR conditions are shown in Supplementary Table S1. The PCR reactions were performed with a total volume of 20 μl that contained 50 ng of genomic DNA, 0.5 μM forward primer and reverse primer, 2 μl of 10 × buffer with $MgCl_2$, 200 μM of deoxynucleoside triphosphates, and 1.0 U of Taq DNA polymerase. The PCR cycles were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at a primer-specific gradient temperature for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. The PCR products were assessed on 1.5% agarose gels stained with 10 μg/μl of ethidium bromide and visualized using a UV transilluminator (VilBer LouRMAT Inc.) to genotype each locus according to marker.

Genetic detection in 4 generations with 26 biochemical markers

The gerbils in F_{14}, F_{17}, F_{19}, and F_{20} were involved in genetic detection with 26 biochemical markers (detailed information of these markers including name, locus, sample, and staining, is shown in Supplementary Table S2). After anticoagulant and coagulant blood collection, the animals were euthanized, and the liver, kidneys, testis, and pancreas were collected. The blood was centrifuged to extract the plasma and serum, and then water were added to the remained red cell to prepare hemolysin. The tissues were homogenized by adding solution or buffer and then evaluated by electrophoresis. After electrophoresis, the proteins or enzymes were stained by different methods referred to previous reports [19, 22].

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). Comparisons of production performance data in the 1st, 2nd, and 3rd litters were analyzed by ANVOA. A P value<0.05 was considered statistically significant.

Results

The ischemic model and COW patterns in F_1–F_{20}

We detected the frequency of ischemic model and ratio of gerbils with incomplete COW patterns in each generation from F_1 to F_{20}. The results showed that the frequency of UCO ischemic model increased from 50% in F_1 to 88.89% in F_{20} (Fig. 1A, Table 1). The ratio of gerbils with incomplete COW patterns increased from 75% in F_1 to 100% in F_{20} (Fig. 1A, Table 1). The most noteworthy result was that when the first pair with consistent patterns of COW appeared in F_4, the ratio of consistent patterns of COW in pairs (parents) (the number of pairs with the consistent patterns of COW / total numbers of pairs detected) increased from 15% in F_4 to 50% in F_{20} (Fig. 1A, Table 1). This trend encourages us to believe that it may be possible to continue to inbreed this inbred gerbil strain to obtain a 100% of consistent patterns.

The characteristics of reproduction in F_{21}–F_{23}

The reproductive performance of 30 breeding pairs of gerbils in F_{21}, F_{22}, and F_{23} was recorded (Table 2). Birth body weight, birth weight per litter, size of litter, and individual weaning body weights in the first litter (1st), second litter (2nd), and third litter (3rd) were recorded separately. The results showed that although the average birth body weight exhibited no significant difference between 3 litters (P=0.101,>0.05), values for the 2nd and the 3rd litter was higher than that of the 1st. Litter size also displayed no significant difference among 3

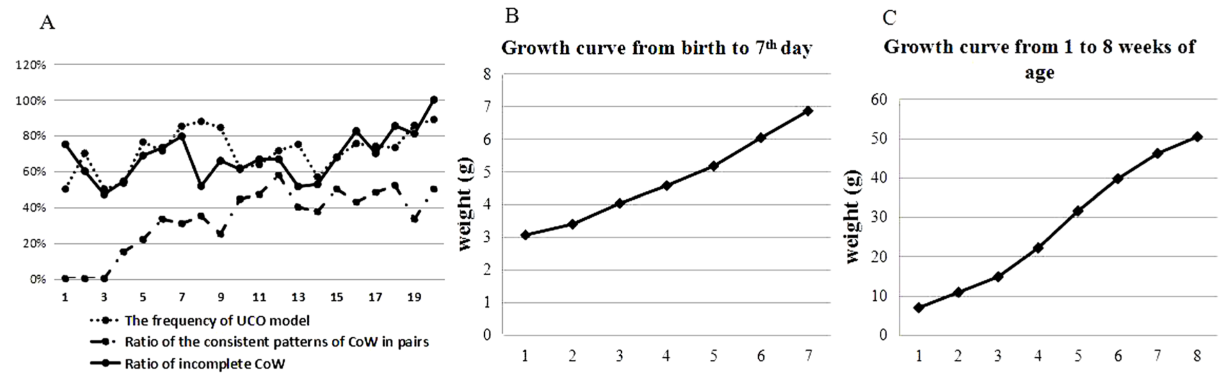

Fig. 1. Detection of the ischemic model in F_1 to F_{20} and the growing characteristics of the inbred gerbil. The frequency of UCO ischemic model, the ratio of gerbils with incomplete COW patterns, and the ratio of the consistent patterns of COW in pairs (parents) were detected and calculated in F_1 to F_{20} (A). All increased with the number of generations in the inbreeding process. The growth curves for the inbred gerbils were calculated from birth to the 7th day (B) and weekly from 1 week to 8 weeks of age (C).

Table 1. Frequency of UCO ischemic model and ratio of gerbils with incomplete COW patterns in each generation of breeding gerbils

Genertions	Number detected	Number of gerbils with incomplete COW	Ratio of erbils with incomplete COW (%)	Number of ischemic model gerbils	Frequency of UCO ischemic model (%)	Ratio of the consistent patterns of COW in pairs
F_1	4	3	75%	2	50%	–
F_2	10	6	6%	7	70%	–
F_3	34	16	47.06%	17	50%	–
F_4	92	50	54.35%	49	53.26%	15%
F_5	131	90	68.70%	100	76.34%	21.87%
F_6	108	79	73.15%	77	71.30%	33.33%
F_7	39	31	79.49%	46	85.19%	30.76%
F_8	60	31	51.67%	36	87.80%	35%
F_9	41	27	65.85%	38	84.44%	25%
F_{10}	36	22	61.11%	21	61.76%	44.44%
F_{11}	36	24	66.67%	21	63.64%	47.05%
F_{12}	39	26	66.67%	25	71.43%	57.89%
F_{13}	35	18	51.43%	24	75.00%	40%
F_{14}	57	30	52.63%	29	56.86%	37.5%
F_{15}	59	40	67.80%	40	67.80%	50%
F_{16}	57	47	82.46%	43	75.44%	42.85%
F_{17}	50	35	70.00%	37	74.00%	48.15%
F_{18}	41	35	85.36%	30	73.17%	52%
F_{19}	21	17	80.95%	18	85.71%	33.33%
F_{20}	9	9	100%	8	88.89%	50%

Notes: UCO, unilateral carotid occlusion; COW, circle of Willis.

litters (P=0.191, >0.05), but the 2nd litter was the largest. There was no difference in birth weight per litter or individual weaning body weight among the 3 litters, and again, the 2nd litter has the largest values. We also calculated the average body weights at 8 weeks and 3–4 months of age, respectively. The data demonstrated no statistically significant difference (50.3 vs. 55.61, P=0.119, >0.05), indicating that we could regard the 8 weeks as an adult age in the inbred gerbils. Furthermore, we weighed the baby gerbils daily for the first 7 days after birth and weekly for the first 8 weeks after birth, and then created the birth curve as shown in Fig. 1B and C. The curves told us that the gerbils grew pretty quickly during the first week after birth and over the course of the 8 weeks after birth. After that, body weight became stable.

Table 2. Characteristics of reproduction were examined in inbred gerbils from F_{21} to F_{23} including birth body weight (BW), birth weight per litter, size of litter, and average individual weaning BWs in the first litter (1st), second litter (2nd), and third litter (3rd); and BWs at 8 (8W) and 12 to 16 weeks of age (12–16W)

Item	Average ± SD			95% confidence interval		
Litter	1st	2nd	3rd	1st	2nd	3rd
Average individual birth BW (g) (N)	2.89 ± 0.36 (48)	3.08 ± 0.33 (74)	3.17 ± 0.24 (48)	2.65–3.13	2.91–3.25	3.02–3.30
Average birth weight per litter (g) (N)	11.78 ± 5.81 (11)	14.93 ± 5.56 (17)	11.28 ± 6.28 (13)	7.88–15.68	12.07–17.78	7.48–15.07
Size of litter (N)	4.00 ± 1.84 (11)	4.94 ± 2.04 (17)	3.62 ± 2.10 (13)	3.78–5.02	3.77–5.71	2.73–4.46
Average individual weaning BW (g) (N)	31.04 ± 6.14 (56)	32.23 ± 7.38 (51)	30.16 ± 1.89 (19)	30.20–32.82	3.77–5.71	2.73–4.46
BW (8W) (g) (N)	50.35 ± 5.86 (90)			49.18–51.52		
BW (12–16W) (g) (N)	55.61 ± 5.06 (48)			54.05–57.17		

Note: N, number of samples (individuals or litters).

Table 3. Genetic dynamic characteristics during the gerbil inbreeding process from F_3 to F_{20} detected with 28 microsatellite loci

Generations / locus	F_3 ♂	F_3 ♀	F_4 ♂	F_4 ♀	F_5 ♂	F_5 ♀	F_6 ♂♀	F_7 ♂♀	F_8 ♂♀	F_9 ♂♀	F_{10} ♂♀	F_{11} ♂♀	F_{12} ♂♀	F_{13} ♂♀	F_{14} ♂♀	F_{15} ♂♀	F_{16} ♂♀	F_{17} ♂♀	F_{18} ♂♀	F_{19} ♂♀	F_{20} ♂♀
AF200942	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
AF200943	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
AF200944	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
AF200946	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
AF200945	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
AF200941	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
AF200947#	+/−	+/−	+/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
D16Mit7	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D16Mit26	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D1Mit362	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D8Mit184	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D7Mit33	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D6Mit37	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D5Mit31	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D12Mit201	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D2Mit22	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−
D15Mit124	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D11Mit36	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D7Mit71	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D2Mit76	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D3Mit130	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D19Mit1	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D11Mit35	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D17Mit38	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−
DXMit17	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D8Mit56	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D10Mit66	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
D13Mit1	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+

Notes: #, the locus AF200947, which was heterozygous in F_3 and became homozygous after F_5. Symbols: +/+, homozygous; +/−, heterozygous; −/−,locus was heterozygous from the beginning and became homozygous after several inbreeding generations.

The genetic features of F_3–F_{20} detected with 28 microsatellite loci

We detected dynamic genetic characteristics during the gerbil inbreeding process from F_3 to F_{20} with 28 microsatellite loci. The microsatellite loci were referenced from our previous report [6]. The results showed that 26 of the loci (92.85%, 26/28) became genetically homozygous in F_{20} (Table 3). At the beginning of F_3, the locus *AF200947* was heterozygous, with 2 different alleles. In F_4, the male gerbil was heterozygous at this locus, whereas the female gerbil showed homozygous. From F_5, this locus became homozygous and remained this way consistently to F_{20} (Fig. 2A, Table 3). There were 2 loci (*D2Mit22* and *D17Mit38*) that were heterozygous in all the generations from F_3 to F_{20} and in F_{22} (data not shown). These results showed that the number

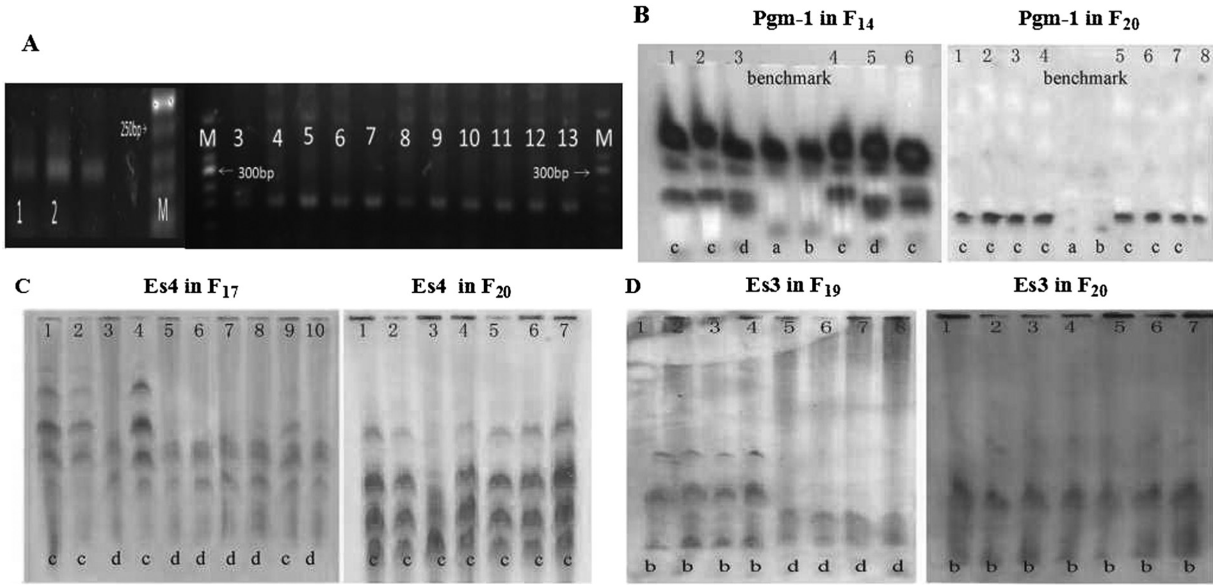

Fig. 2. Results of genetic detection with 28 microsatellite loci and 26 biochemical markers. The allele of locus *AF200947* analyzed by agarose gels electrophoresis in F_3 (lane 1–2), F_4 (lane 3–4), F_5 (lane 5–6), F_6 (lane 7–8), F_7 (lane 9–10), and F_8 (lane 11–13). M represents a marker of 50bp (A). Illustrations of typical zymogram patterns for 3 biochemical markers, Pgm-1 (B), Es4 (C), and Es3 (D), that exhibited monomorphism in F_{20} after exhibiting polymorphism in F_{14}, F_{17}, and F_{19}, respectively. The numbers and the letters (B–D) indicate individual gerbils detected in F_{14}, or F_{17}, or F_{19} and the zymogram patterns of these biochemical markers, respectively. The benchmark was the standard marker.

of homozygous loci increased as the number of the generations increasing.

The genetic features of 4 generations examined with 26 biochemical markers

We explored the genetic features of F_{14}, F_{17}, F_{19}, and F_{20} with 26 biochemical markers we selected previously that could be used to detect gerbil genetic quality [22]. The results are shown in Table 4 and Figs. 2B–D. The results were partly different from those of the microsatellite loci. A total of 23 markers were homozygous in all generations examined. There were 3 markers that became homozygous after being from heterozygous during the breeding process: the marker Es-3 showed 2 genotypes in F_{14}, F_{17}, and F_{19}; the marker Pgm-1 in F_{14}, and the marker Es-4 showed 2 genotypes in F_{14}, F_{17}, and all exhibited one allele in F_{20}. These data indicated that we successfully bred an inbred gerbil strain in which all 26 biochemical markers were homozygous.

Discussion

The gerbil is one of the widely used laboratory animals. However, the available strains of gerbils are very

Table 4. Summary of the zymogram patterns of the 26 biochemical markers

Locus	F_{14}	F_{17}	F_{19}	F_{20}
Gpd-1	c	c	c	c
Es-3	b, c	b, c	b, c	b
Gdc-1	c	c	c	c
Gus-1	c	c	c	c
Es-2	d	d	d	d
Car-2	c	c	c	c
Akp-1	a	a	a	a
Ldr-1	b	b	b	b
Idh-1	c	c	c	c
Mod-1	a	a	a	a
Ce-2	a	a	a	a
Pgm-1	c, d	c	c	c
Pep-3	d	d	d	d
Gpi-1	a	a	a	a
Hbb	e	e	e	e
Sep	c	c	c	c
Trf	c	c	c	c
Es-1	c	c	c	c
Amy-1	b	b	b	b
Es-6	a	a	a	a
Es-8	c	c	c	c
Es-9	c	c	c	c
Es-4	c, d	c, d	c	c
Cs-1	b	b	b	b
Es-10	c	c	c	c
Es-12	a	a	a	a

limited. Loskota *et al.* reported that a seizure-sensitive (WJL/UC) and seizure-resistant (STR/UC) gerbil strains were bred with "closed colony" technique [15]. We merely found two inbred strains of gerbil including low seizure susceptible and seizure-prone gerbils that had been previously reported [8]. In the current study, we reported an inbred strain of ischemia-prone gerbils. We found that the ratio of gerbils with an incomplete COW pattern was obviously increased in F_{20} (100%) compared with that of the beginning generation (F_1, 75%). The frequency of UCO ischemic model was also higher in F_{20} than in F_1 (88.89% vs. 50%). This value was also higher than that for the outbred ischemia-prone population we established in a previous report [7]. Thus, we exceeded our primary target, as increasing the UCO ischemic model ratio has the potential to reduce the number of animals used in experiments. The more interesting finding is that the ratio of consistent COW patterns (the ratio of the number of pairs of parents with consistent COW patterns to all pairs detected) also increased as the number of generations increased. Thus, it encouraged us to continue breeding and to obtain a strain with a completely uniform pattern of COW.

It has been thought that this species would be easy to inbreed because of its less genetic variation [2]. However, it was difficult for us to establish an inbred gerbil because the outbred population we used originated from a CMU colony that was established and cultivated from approximately 400 pairs of wild gerbils captured in 1986 from the district of Hohehot Municipality, China. This gerbil population exhibited far higher genetic variation than that of gerbils sold by another international company [5]. After 20 generations of inbreeding by sister-and-brother mating method, we found that our inbred strain was indeed established successfully and confirmed by genetic detection using microsatellite loci and biochemical markers. The 2 microsatellite loci that remained heterozygous in F_{20} were unexpected. We suspect that these 2 microsatellite loci may be linked important homozygous genes causing lethal or severe disease genes. When they are heterozygous, animals survived and continue to live, whereas when they become homozygous, the animals die or develop lethal diseases. Another possibility may be that these 2 microsatellite loci are not quite the right ones to detect inbred gerbil genetic quality even though they have been successfully used for population genetic structure analysis in outbred gerbils [6]. Thus, this result also reminds us that we should select more effective microsatellite loci to detect the genetic quality of this inbred gerbil strain and show that it is genetically homogenous. On the other hand, it is gratifying that the results for the 26 biochemical markers also demonstrated that this new inbred strain of gerbil was genetically homogenous.

We explored the reproduction characteristics by calculating birth body weight, birth weight per litter, litter size, and individual weaning body weights in the first litter (1st), second litter (2nd), and third litter (3rd) in 3 generations, respectively. After considering these reproductive performance factors, we found that the second litter is the best choice to maintain as seed animals or for reproduction. The litter size for our inbred gerbils was about 4, which is a bit lower than that report earlier for gerbil breeding (mean of 5.1 pups per litter) [1], much lower than that in a recent report concerning outbred gerbil (about 6 for the 1st and 2nd litters) [20], and less than that (7 pups) in gerbils fed by different methods [18], indicating that the reproduction ability of the inbred gerbil was obviously decreased. The average body weights at 8 weeks and 3–4 months of age in the present inbred gerbil was also much lower than those of outbred gerbils in a previous report [19].

Although we spent 8 years to establish this new inbred strain, a lot of researches on this strain still needs to be performed. For instance, would the lifespan of these inbred gerbils be shorter? Is the nutritional requirement of these inbred gerbils the same as for outbred gerbils? Would the behavior characteristics, biochemical indice, and physiological indices of these inbred gerbils be different from outbred gerbils? We will investigate these questions in the future and reveal and enrich more knowledge about this inbred strain of gerbil.

Conflict of Interest

There are no conflicting interests to declare.

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