Analysis of Mandible of Newly Developing Strain of Japanese White Rabbit (Nlb:JWNS)

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Abstract: In the present paper, we applied analysis of the mandible to a new strain of Japanese White rabbit being developed (Nlb:JWNS), at 5th and 8th generations (F5 and F8), and evaluated the process of establishment, making comparison with the parent colonies (I, K and L), and other established JW colonies (A, E, O and R). The mandibles were measured at 12 sites and the data obtained were calculated by principal component analysis and discriminant analysis. As a result of principal component analysis, the parent colonies were found to be close to one another with intermediate size of the mandible and height of condyloid and angular processes, but in F5 and F8 the mandibles were slightly shorter in height and different from those in the parent colonies. On the other hand, results of discriminant analysis revealed that mandibles were discriminated correctly 100% in colonies A, O and R, and 90% in E and I. Colonies A, O, R, E and I were therefore regarded as established strains. In the developing colonies, the discriminant rate was 70% in F5 and 80% in F8. One mandible from F5 and two from F8 were wrongly classified to the parent colony L, which was the lowest discriminant rate among the colonies examined (61.5%). The results of both statistical analyses suggest that the JWNS are almost established at 8th generation as a new strain.

Key words: Discriminant analysis, Japanese White rabbit, mandible analysis, principal component analysis, selective breeding

Introduction

From the end of the 1860s into the 1870s many kinds of rabbits, such as Lop, Angora and Himalayan, were imported into Japan as pets. They were crossed with

one another. Later newly imported rabbits such as the Flemish Giant and New Zealand White were also involved in cross mating. The descendants of this cross breeding have been called the Japanese White breed (JW) [6, 7, 10, 13], but JW rabbits exhibited large varia-

(Received 5 February 1996 / Accepted 12 June 1996)

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tion in body size, weight and features, because they were bred from the complex origin mentioned above without a common principle to obtain improvement. At present there are about 20 breeding colonies producing JW throughout Japan, and because the animals have been bred in each colony independently, it is said that the responses of the JW rabbits in toxicity tests, pyrogen tests and bioassays vary depending on the producing colonies.

The Japanese Society of Laboratory Animals (JSLA) surveyed the morphological variation in JW rabbits from 17 breeding colonies by mandible analysis in 1988 [9]. As a result, the morphological variation was observed to be remarkable not only between colonies, but also within them. Of 17 breeding colonies, only 4 colonies were recognized as strains, because there was less variation within the colonies [9]. On the basis of the report of surveillance by JSLA, the National Livestock Center Nagano Station (Nlb) began breeding rabbits to develop a new strain of JW (Nlb:JWNS), which is representative of JW with medium body size and uniform genetic characteristics, for laboratory use. The present paper describes the results of mandible analysis of the newly developing strain of JW, compared with parent and other established colonies.

Materials and Methods

Animals: The breeding of JWNS was begun by cross breeding rabbits from three breeding colonies (I, K and L) in 1989. As shown in Fig. 1, rabbits in colony I were mated with those in L, then their F_1 progenies were mated with rabbits in colony K to produce F_2 . We regarded the offspring between F_1 and F_2 as a fundamental stock (generation 0, F_0), advanced their generation, and carried out selective breeding for the following traits: large erect ears with clear veins which are convenient for blood collection, good reproduction, large litter size, early sexual maturity, medium in body size about 4 kg in adult body weight, moderate in temper, and other characteristics useful for experiments. In each generation, about 30 males and 60 females were maintained. Mating pairs were selected at less than a 25% coefficient of relationship. One male was mated with 3 females or fewer.

At the present time, the breeding of JWNS is proceeding to the 8th generation (F_8). For mandible

analysis, female rabbits from the 5th and 8th generations (JWNS F_5 and JWNS F_8) were sacrificed. Rabbits from the following colonies were also sacrificed and analyzed to compare: I, K, and L as parent colonies, and A (Nlb:JWNL), E, O and R as established colonies.

All animals used for analysis were female from 10 to 24 months old. In each colony the animals were selected from different litters, and the numbers of animals were 10 to 20 as shown in Table 1. Pellets for food and breeding conditions such as room temperature, humidity and others were different in each colony. In the Nlb the F_5 , F_8 and A (Nlb:JWNL) rabbits had been raised in a room conditioned to a temperature of 18–23°C and a humidity of 55–60%. They were fed pelleted food (ORC-4, Oriental Yeast Co., Ltd., Tokyo) and tap water *ad libitum*.

Preparation of mandible: Rabbits from each breeding colony were sacrificed by ether anesthesia and decapitation. The heads were autoclaved and frozen at below -20°C , and then sent to the National Institute of Animal Health (NIAH) for preparation of the mandible, measurement and statistical analysis. In the NIAH the heads were thawed and skinned, and then the muscle and soft tissues were removed. Mandibles isolated from the head were soaked in 0.5% papain solution at 37°C

Fig. 1. Breeding schedule of a new strain of Japanese White rabbits (Nlb:JWNS). I, L and K are parent colonies of Japanese White. F_5 and F_8 are 5th and 8th generation from fundamental stock, which is counted as 0 generation.

Table 1. Strains means for mandible measurement variables (mm)

Colony	No. of rabbits	Variable											
		X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂
A	10	4.054	25.913	43.693	51.173	27.212	24.972	23.581	44.429	55.647	62.270	67.953	73.199
E	10	3.338	23.801	41.507	49.690	25.739	23.401	20.173	39.561	48.945	55.327	61.662	65.826
I	10	3.442	24.053	38.889	46.720	23.319	22.160	23.280	43.116	56.169	60.289	66.861	70.895
K	10	3.610	24.055	39.488	46.577	22.784	21.721	21.995	41.718	55.214	59.104	66.200	69.722
L	13	3.619	23.968	39.677	47.160	22.288	21.215	22.669	42.771	55.849	60.131	66.657	69.230
O	14	2.620	21.737	35.047	42.454	19.509	18.461	21.074	40.036	51.129	54.940	61.392	64.360
R	20	3.390	23.529	40.063	48.189	25.077	23.369	20.530	39.419	49.337	55.548	60.596	65.704
F5	10	3.379	22.975	39.515	46.729	22.780	21.791	23.379	43.221	56.626	61.194	68.468	70.555
F8	10	3.024	22.376	39.433	46.439	22.012	21.537	23.162	43.708	57.347	61.921	68.600	70.313

Fig. 2. Mandible measurements. Numericals 3, 4 and 5 show the height of the coronoid, condyloid and angular processes, respectively.

for 24 hr, thoroughly washed in running water, dried, and stored until the time of measurement.

Measurement of mandible and statistically analysis: The mandible, with incisor teeth removed, was measured at 12 sites on the right side, as shown in Fig. 2. Six sites from X₁ to X₆ were height from an X axis, and the other 6 sites from X₇ to X₁₂ were length from a Y axis. Measurements of the mandible were carried out with a profile projector (Nikon, V-20A) at a magnification of 5X (1/1000 mm minimum).

The data obtained were subjected to principal component analysis and discriminant analysis with software produced by Dr. Takayasu Takizawa for personal computers.

Results

1. Principal component analysis—Analysis of morphological characteristics of mandible and relationship between colonies—

Figure 3 shows photographs of mandibles selected randomly from each colony. Means of mandible measurements at 12 sites (variables X₁–X₁₂) in 9 colonies are shown in Table 1, but it is difficult to estimate the morphological characteristics of the mandibles in each colony based on the means of 12 variables and their standard deviations (not shown in Table 1). In addition, a close correlation was found between variables, so that differences between colonies should not be discussed without considering this correlation. In this case, principal component analysis was useful in analyzing the multivariable data with correlation.

In principal component analysis, the eigenvalue and its contribution in each principal component (PC) were obtained and shown in Table 2. The cumulative contribution from 1st (PC1) to 3rd (PC3) was 92.3%, so that the first three principal components extract most of the morphological information from the original data. In other words, most of the morphological characteristics were interpreted by these PCs. Table 3 shows the eigenvector of 12 variables classified as PC1, PC2 and PC3. Eigenvectors are coefficients to be multiplied by variables and the coefficients suggest morphological meanings extracted by PCs. In PC1 in Table 3, all coefficients were positive and they were particularly large for X₇ to X₁₂ (length of mandible), so that PC1 means to extract the size factor of the mandible, espe-

Fig. 3. Mandibles of 9 colonies. A mandible is selected randomly from each colony, so that they do not show the representative shape of the colony.

Table 2. Eigenvalue and its contribution in each principal component (PC)

PC	Eigenvalue	Contribution ratio	Cumulative contribution ratio
1st	6.880	0.574	
2nd	3.602	0.300	0.874
3rd	0.593	0.049	0.923

Table 3. Eigenvector of each principal component

Variable	Principal component		
	1st	2nd	3rd
X ₁	0.2645	-0.2197	-0.5905
X ₂	0.2737	-0.2266	-0.5454
X ₃	0.2941	-0.3079	0.0262
X ₄	0.2652	-0.3468	0.0750
X ₅	0.1948	-0.4170	0.3236
X ₆	0.2135	-0.3597	0.4254
X ₇	0.3062	0.2698	0.1450
X ₈	0.3280	0.2353	0.0922
X ₉	0.2836	0.3354	-0.0653
X ₁₀	0.3415	0.2141	0.1175
X ₁₁	0.3112	0.2794	-0.0370
X ₁₂	0.3457	0.1322	0.1137

cially its length. A colony with a large PC1 is therefore considered to have large mandibles.

In PC2, the coefficients from X1 to X6 (height of mandible) were negative and their absolute values were large in X4 to X6 (height of condyloid and angular processes at posterior part of mandible). The coefficients from X7 to X12 (length of mandible) were all positive. This suggests that PC2 extracts the proportion factor of the mandible. In other words, when PC2 values are large, the mandibles are considered to be short in height, especially in the height of posterior processes, compared to the length.

The contribution ratio of the eigenvalue of PC3 was only 4.9% (Table 2), and the coefficients of eigenvectors from X1 and X2 (height of anterior part of mandible) were negative and absolute values were large. The eigenvectors of X5 and X6 (height of posterior processes) were positive and large. Judging from the values, a colony exhibiting a large PC3 value has mandibles that have a short anterior part of the mandible and high posterior processes.

Figure 4 shows the positions of 9 colonies in the PC1-PC2 plane. According to the abovementioned interpretation of PCs, the mandible of colony A was the largest in size and that of colony O was the smallest.

Fig. 4. Scatter diagram for 9 colonies of rabbits constructed by PC1 and PC2. Numerals in parenthesis are the sum of PC1 and PC2 contribution ratios.

Fig. 5. Scatter diagram for 9 colonies of rabbits constructed by PC1 and PC3. Numerals in parenthesis are the sum of PC1 and PC3 contribution ratios.

Table 4. Results of classification analysis by means of discriminant analysis

Colony	Assigned colony									Total	discriminant rate (%)
	A	E	I	K	L	O	R	F5	F8		
A	10									10	100
E		9					1			10	90
I			9	1						10	90
K				7	2		1			10	70
L	1			1	8			1	2	13	61.5
O						14				14	100
R							20			20	100
F5					1		1	7	1	10	70
F8					2				8	10	80

Colonies E and R were the smallest next to O in the size of mandibles. In both colonies the mandibles were similar in shape, had relatively high posterior processes, and were stocky in shape as a whole. Mandibles of colonies I, K and L were intermediate types between those of A and O, and they were similar to one another morphometrically, having short posterior processes, so that the shape was slender as a whole. The position of F5 in the PC1-PC2 plane was almost the same as in its parent colonies. In F8, however, the position on the PC1-PC2 diagram was larger in PC2, with shorter posterior processes.

Figure 5 shows the PC1-PC3 values for 9 colonies. The variation in PC3 between colonies was very small, but in F5 and F8 the values were slightly larger. In other words, this means the mandible anterior height is less. The mandibles of F5 and F8 were of medium size and the height of the mandible was short in both anterior and posterior parts.

2. Discriminant analysis—Analysis of genetic uniformity in colony when compared with others—

Table 4 shows the results of discriminant analysis of

9 colonies. For example, of the mandible in colony E, nine of ten mandibles were correctly classified in colony E, but one mandible was wrongly classified in colony R. In colonies A, O and R, all mandible examined were classified correctly, so that these colonies were regarded as genetically uniform. In the E and I colonies, nine of ten mandibles were classified correctly, and the colonies were also regarded as almost genetically uniform. On the other hand, three of ten mandibles in colony K and five of 13 in colony L were wrongly classified. These colonies seem to be not uniform genetically. While in developing colonies of JWNS, three of ten mandibles in F5 and two of ten in F8 were wrongly classified.

Discussion

In their study of inbred mouse strains, Wayne and O'Brien [14] reported that there was an insignificant correlation ($r=0.24$) between genetic distances and morphological distances, and that the structural gene and morphometric variation in mandible traits were not coupled. On the other hand, Festing and Roderick [4] argued that the low correlation probably arose because their sample included only a single pair of closely related substrains and because they failed to correct for nonlinearity between morphometric and single-locus measurement scales, and if these were corrected, then the correlation would increase to 0.63. Furthermore, they found that there was a highly significant correlation ($r=0.72$) when 12 inbred mouse strains including an inbred strain from the wild type and pairs of closely related substrains were used.

Goto *et al.* [5] applied multivariate analysis (principal component analysis and discriminant analysis) to the mandible measurements of 5 strains of rabbits (3 inbred strains and 2 closed colonies), and showed that the mandible analysis is useful not only for strain identification, but also for estimation of genetic relationships.

The shape and size of mandibles are quantitative traits controlled by polygenes [1, 2, 11]. Generally these traits have been considered to be affected by the age of the animal and the quality of food given [8]. In laboratory mice more than 7 weeks old, however, the mandibles were hardly affected by food or breeding conditions, if they were reared in an ordinary proce-

dure for laboratory animals [3, 12].

The rabbits examined in the present study were reared under different breeding conditions depending on the colony. Judging from the fact that the rabbits are bred normally and produce young in any colony, breeding conditions in colonies seems to hardly affect the growth of the mandible. As the rabbits examined were adult females over 10 months old, the results of discriminant analysis and the relationship between colonies were considered to reveal genetic differences.

On PC1-PC2 plane (Fig. 4), colonies A and O occupied contrasting positions. Colony A has been selected for large type JW rabbits at the Nlb since 1948, while O has been selected for small rabbits at the Nippon Institute for Biological Science since 1964.

In F5 and F8 of JWNS, PC2 and PC3 values were larger than those of their parent colonies I, L and K (Figs. 4 and 5). The F8 value tended to be larger than F5. This suggests that the developing strain (JWNS) were different from parent colonies in mandible morphology, becoming short in mandible height with the advance of generations. Colony R is divergent one from E, so that both mandibles were similar morphometrically, being small in size and with short process height.

The discriminant analysis revealed that colonies A, O and R are perfectly classified. In colonies E and I, correct classification rates were 90%. These A, O, R, E and I colonies were considered to be established as strains.

In F5 of JWNS, three of ten mandibles were wrongly classified as F8, L and R, and in F8 two of ten were classified as parent colony L. Originally the discriminant rate of colony L was the lowest (61.5%) among the colonies examined, carrying the genetic variability within the colony. Therefore, one of F5 and two of F8 might be classified as belonging to colony L.

As mentioned above, the JWNS have been bred since 1989 for selective breeding to develop a new strain that simplifies blood collection, reproduces well and is of medium body size. As a result of mandible analysis of F5 and F8 in developing generations, the JWNS is considered to be almost established as a strain of medium-sized JW rabbits in the 8th generation.

The present study was supported by the Working Group for Rabbits, the Japanese Society of Laboratory Animals (JSLA).

References

1. Atchley, W.R., Plummer, A.A., and Riska, B. 1985. Genetics of mandible form in the mouse. *Genetics* 111: 555-577.
2. Bailey, D.W. 1985. Genes that affect the shape of the murine mandible. Congenic strain analysis. *J. Hered.* 76: 107-114.
3. Festing, M.F.W. 1973. A multivariable analysis of subline divergence in the shape of the mandible in C57BL/Gr mice. *Genet. Res. Camb.* 21: 121-132.
4. Festing, M.F.W. and Roderick, T.H. 1989. Correlation between genetic distances based on single loci and on skeletal morphology in inbred mice. *Genet. Res. Camb.* 53: 45-54.
5. Goto, N., Watanabe, K., Umezawa, H., and Kuramasu, S. 1987. Morphometrical observations on the mandible of five strains of rabbits and strain identification using mandible measurements. *Lab. Anim.* 21: 188-194.
6. Inayoshi, D. 1952. On Japanese rabbits. *Bull. Exp. Anim.* 1: 34-35 (in Japanese).
7. Ishibashi, M. and Sakuma, Y. 1988. History and present status of rabbit. pp. 2-16. In: *Rabbit—Reproductive Physiology and Experimental Procedure—* (Sakuma, Y. ed.), Kindai Shuppan, Tokyo (in Japanese).
8. Ito, T., Morishima, A., Hokao, R., Agematu, Y., Takizawa, R., and Goto, N. 1989. Effect of the food on the development of the mandible in rat. *Jpn. J. Vet. Inform.* 22: 25-30 (in Japanese).
9. Japanese Society of Laboratory Animals. 1988. Strain identification using mandible measurements in Japanese White rabbits. *Exp. Anim.* 37: 493-500 (in Japanese).
10. Kinukawa, Y. 1949. *Jituyou Youto-hou* (How to Breed Rabbit), 5th ed., pp. 1-15. Chikyu Shuppan, Tokyo (in Japanese).
11. Leamy, L. 1974. Heritability of osteometric traits in a randombred population of mice. *J. Hered.* 65: 109-120.
12. Lovell, D.P., Totman, P., and Johnson, F.M. 1984. Variation in the shape of the mouse mandible. 1. Effect of age and sex on the results obtained from the discriminant functions used for the genetic monitoring. *Genet. Res. Camb.* 43: 65-73.
13. Suzuki, K. 1972. Rabbit. pp. 77-80. In: *Textbook of Laboratory Animal Science, Part II* (Tajima, Y. ed.), Asakura Shoten, Tokyo (in Japanese).
14. Wayne, R.K. and O'Brien, S.J. 1986. Empirical demonstration that structural gene and morphometric variation of mandible traits are uncoupled between mouse strains. *J. Mammal.* 67: 441-449.