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Genetic Relationships between CD-1 Stocks of Mice in Uruguay

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Abstract

Multivariate analysis of mandible measurements was applied to identify and to investigate the genetic relationships between 5 outbred stocks of female mice, Dlv: CD-1, Hg: CD-1, Crj: CD-1, CF-1 and Slc:ddY. (1) Principal component analysis revealed that CF-1 had a relatively large mandible with an obtuse muscular process and low anterior mandibular body, whereas Hg: CD-1 had a smaller mandible and exhibited intermediate values in terms of muscular process shape and anterior height. Crj: CD-1 showed a moderately large mandible and a higher anterior mandibular body. While the mandibles of Dlv: CD-1 and Slc: ddY were comparatively similar to each other in that they were both medium-sized, the former had an acute muscular process projecting posteriorly whereas the latter had a less prominent process. These morphological relationships between the 5 stocks determined by principal component analysis were generally in agreement with Mahalanobis' distance between stocks based on the same mandible measurements. (2) According to the results of discriminant analysis, the frequency of misclassification was 11.4%, or 9/79 head when actual mandible measurements were used in categorization and 10.1%, or 8/79, when relative values, which exclude the size effect, were used. These data indicate that the genetic constitution of the 3 CD-1 stocks examined in this study differed from one another. (3) Based on dendrograms calculated from actual mandible measurements and relative values, Dlv: CD-1 was more closely related to CF-1 or Slc: ddY, which are derived from mice of other origins, than the CD-1 stocks examined in this study. Thus genetic monitoring seems to be essential for outbred stocks as well as for inbred strains.

Discipline: Animal health / Genetic resources

Additional key words: laboratory mice, outbred stock, mandible analysis, principal component analysis, discriminant analysis

Introduction

The CD-1 strain, one of the most useful outbred stocks (closed colonies) of laboratory mice, has been utilized for various testing conditions including those for drugs and biological products. In Uruguay, CD-1 mice have also been introduced to several research institutions by the Charles River Laboratories (USA) and have been maintained and used for the above-mentioned tests. Since the CD-1 mice were introduced separately into each institute and were maintained independently from generation to generation, it is possible that the genetic constitution of each stock may differ from one another. Therefore, investigations of the genetic relationships between the outbred stocks should be carried out.

For inbred strains of laboratory mice or rats, genetic monitoring such as strain identification and estimation of genetic relationships between strains has mainly been performed by the use of biochemical and immunological

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marker genes[7]. These methods, however, are not as effective for outbred stocks such as CD-1.

Mandible analysis proposed by Festing[1] is based on the assumption that the shape of the mandible is a heritable trait. This type of analysis is suitable not only for genetic monitoring of inbred strains but also for that of outbred stocks[2-4].

In light of the above-mentioned advantages of mandible analysis, the present study was undertaken to clarify the genetic relationships among several independently maintained CD-1 stocks.

Materials and methods

1) Animals used

Five stocks of outbred female mice were selected for the present investigation. They belonged to 3 CD-1 stocks (Dlv:CD-1, Hg:CD-1 and Crj:CD-1), in addition to CF-1 and Slc:ddY. The latter 2 were selected as the control stocks. Dlv:CD-1 was maintained at the Direccion de Laboratorios Veterinarios (DI.LA.VE.), CF-1 at the Faculdad de Veterinaria, Univ. de la Republica Oriental del Uruguay, and Hg:CD-1 was bred at the Instituto de Higiene, Faculdad de Medicina of the same university by rotation crossing without inbreeding. They were raised in rooms maintained at temperatures ranging from 20 to 27°C and relative humidity between 50 to 70%, were fed with commercially manufactured food pellets VITA (Vitaron S.A., Montevideo) and received tap water *ad libitum*. SPF mice of the Crj:CD-1 and Slc:ddY stocks were purchased from the Japan Charles River Co., Ltd. (Tokyo) and Japan SLC Co., Ltd. (Hamamatsu), respectively.

The number, age and body weight of the mice examined are shown in Table 1.

2) Preparation of mandible

Each mouse was sacrificed using ether and was decapitated immediately. The head was boiled at 100°C for 10 min, and the skin, muscles and soft tissues were then removed. The mandible was isolated from the head following immersion in a 0.3% papain solution at 37°C for 24 h. The mandible was washed in running water, and dried at room temperature for 12 h.

Table 1. Number of mice examined

Stock	No. of mice	Age (month)	Body weight (g)
Dlv:CD-1	15	5–9	32.5–50.5
Hg:CD-1	15	2–3	23.4–30.0
Crj:CD-1	17	2–3	24.2–30.4
CF-1	15	3–9	28.7–50.5
Slc:ddY	17	2–3	29.8–32.5

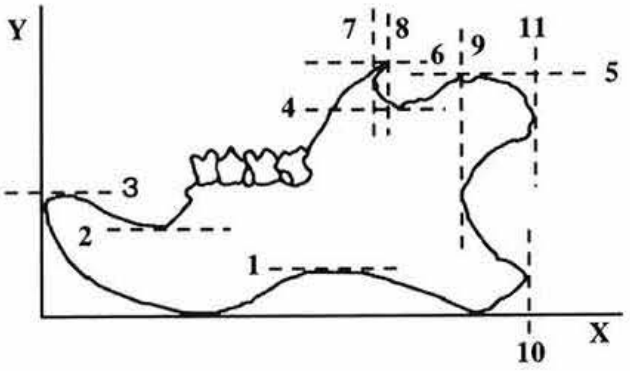

Fig. 1. Mandible measurements

X_1–X_6: Height from X axis to horizontal dotted line at each site.

X_7–X_{11}: Length from Y axis to vertical dotted line at each site.

3) Measurement of mandible

Measurements were performed at 11 sites (Xj, j=1,2,...,11) on the right half of the mandible, as shown in Fig. 1. For example, X_6 is the height from the X-axis to the highest point of the muscular process; X_8 is the length from the Y-axis to the posterior end of the process. The mandible was measured at a magnification of 10 times using a Universal Projector (Nikon V-20A, Nippon Kogaku Co., Ltd., Tokyo) installed at the National Agriculture Research Center (Japan).

4) Statistical analysis

The data obtained were subjected to principal component analysis and discriminant analysis with the Statistical Analysis Systems (SAS)[8] by using MAFFIN (Ministry of Agriculture, Forestry and Fisheries Research Network) in Tsukuba, Japan. A dendrogram was constructed by applying the group-average method to Mahalanobis' distances calculated from the discriminant analysis.

Results

1) Test of normality

In conducting the principal component analysis and discriminant analysis with the 11 variables shown in Fig. 1, it is necessary for these variables to display a multivariate normal distribution. Each variable was then subjected to a test of normality using the Shapiro-Wilk's statistic. The variables presented a normal distribution with the exception of 2 variables, X_2 and X_8. The same test of normality was performed for the relative value of

Table 2. Means and standard deviations for specific variables in outbred stocks

Stock	No. of mice	Variable										
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Dlv:CD-1	15	0.765[1)]	1.920	2.828	4.956	5.656	6.178	8.958	9.585	10.860	12.557	12.868
		0.058[2)]	0.060	0.152	0.085	0.141	0.148	0.266	0.467	0.183	0.225	0.270
Hg:CD-1	15	0.602	1.959	2.964	4.591	5.145	5.680	8.557	8.935	10.282	11.735	12.263
		0.070	0.081	0.140	0.190	0.252	0.210	0.145	0.164	0.186	0.239	0.178
Crj:CD-1	17	0.699	2.081	3.086	4.827	5.431	6.077	8.860	9.368	10.594	12.170	12.542
		0.056	0.080	0.125	0.136	0.129	0.121	0.175	0.283	0.169	0.231	0.197
CF-1	15	0.838	2.099	2.971	5.204	5.939	6.460	8.884	9.281	11.150	12.985	13.204
		0.109	0.086	0.075	0.191	0.232	0.241	0.191	0.223	0.316	0.366	0.336
Slc:ddY	17	0.771	2.043	2.915	5.134	5.711	6.214	8.929	9.339	10.918	12.713	12.783
		0.046	0.082	0.098	0.093	0.131	0.096	0.212	0.256	0.175	0.190	0.213

1): Mean (mm).
2): Standard deviation (mm).

mandible measurement. In this study the relative value was calculated as $Xj/\sum_{j=1}^{11} Xj\ (j=1,2,\ldots,11)$ (measurement for each variable / sum of measurements for 11 variables) in each mouse. Only 2 variables, X_8 and X_{10}, slightly deviated from the normal distribution (data not shown).

2) Morphological characteristics of the mandible in each outbred stock

Table 2 shows the means and standard deviations for the 11 variables. However, deducing the morphological differences between the stocks from Table 2 is very complicated. Between the variables, 45 out of 55 correlations were found to be significant (not shown in the table).

When analyzing the differences between the stocks, these significant correlations should also be considered. In this case, principal component analysis, one method of multivariate analysis, is the most suitable method to compare the mandible size and shape in each strain.

The cumulative contribution from the first (PC1) to the third (PC3) principal components was 91.1%, indicating that the first 3 PCs extracted most of the morphometrical information from the original data.

Table 3 shows the eigenvectors of the 11 variables in each PC. In PC1, because all the coefficients were positive, an increase in the value of any variable caused an increase in PC1. Thus PC1 was suitable as the size factor. In PC2, 2 coefficients, X_2 and X_3, associated with the height of the anterior mandibular body, showed particularly large positive values, whereas the other coefficients showed small absolute values, indicating that in a stock with large PC2 values, mandibles show a higher anterior mandibular body. In PC3, 2 coefficients, X_7 and X_8, associated with the length of the muscular process, showed particularly large positive values, whereas the other coefficients were negative, though their absolute values were small. Thus PC3 extracted the factor for the shape of the muscular process. A stock with a large PC3 can be described as having a sharp muscular process.

Figs. 2 and 3 indicate the position of 5 stocks in planes constructed by PC1 and PC2, PC1 and PC3. From the interpretation of each of the PCs mentioned above, CF-1 had a large PC1 and small PC3 and a large mandible with an obtuse process. Hg:CD-1 had a small

Table 3. Eigenvector of each principal component

Variable	PC		
	1st	2nd	3rd
X_1	0.3239	–0.1355	–0.207
X_2	0.1758	0.5983	–0.310
X_3	0.0092	0.7392	–0.068
X_4	0.3489	–0.0338	–0.160
X_5	0.3470	–0.1400	–0.164
X_6	0.3523	–0.0350	–0.140
X_7	0.2769	0.1713	0.545
X_8	0.2184	0.1073	0.692
X_9	0.3561	–0.0480	–0.033
X_{10}	0.3549	–0.1040	–0.070
X_{11}	0.3473	–0.0360	0.054

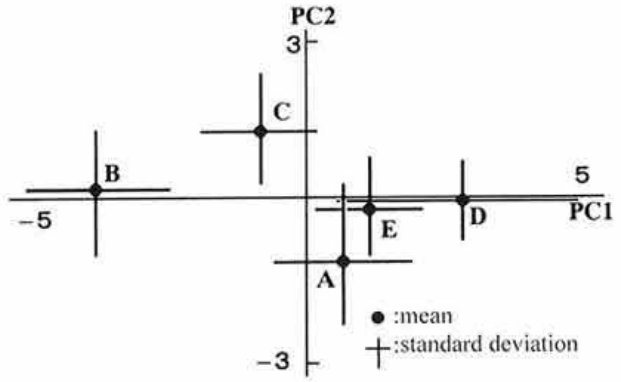

Fig. 2. Position of 5 stocks in the plane constructed by 1st principal component (PC1) and 2nd (PC2)
A: Dlv:CD-1, B: Hg:CD-1, C: Crj:CD-1, D: CF-1, E: Slc:ddY.

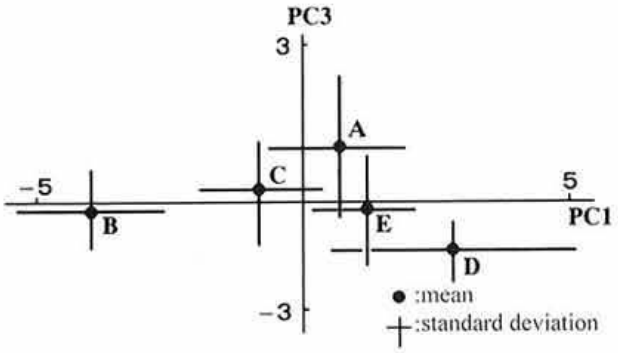

Fig. 3. Position of 5 stocks in the plane constructed by PC1 and PC3
A–E: Same as in Fig. 2.

PC1 and a small-sized mandible. In this stock, the height of the anterior mandibular body and the shape of the muscular process were intermediate among those of the 5 stocks examined. The mandible of Crj:CD-1 shown on the upper left side of Fig. 2 was relatively small with a less prominent process and exhibited the highest anterior body among the 5 stocks. Both Dlv:CD-1 and Slc:ddY had medium-sized mandibles, but the former showed an acute muscular process projecting posteriorly, while the latter showed a less prominent process.

3) Stock identification using mandible measurements

The results of principal component analysis revealed differences in the size and shape of the mandible between the stocks. Therefore, stock identification appeared to be possible by discriminant analysis using the mandible measurements in this study.

Table 4 presents the results of identification of the 5 outbred stocks by this analysis. For example, one Dlv:CD-1 mouse was erroneously identified as a Slc;ddY. Within the CF-1 and Slc:ddY stocks, nearly all of the mice were classified correctly. The rate of misidentification was 11.4%, or 9/79 head, when the actual mandibular measurements were used. Table 4 also presents the results of discriminant analysis using relative values which enabled to eliminate the mandible size effect as a factor. Within the CF-1 stock, all of the mice were classified correctly. This stock was therefore considered to be genetically uniform. Moreover, because 16 out of 17 Slc:ddY mice were classified correctly, this stock was also considered to be nearly genetically uniform. The rate of misidentification was 10.1%, or 8/79.

4) Genetic relationships between stocks

Fig. 4 shows the relationships among the 5 stocks based on the Mahalanobis' distances calculated from the mandible measurements. When actual mandibular measurements were analyzed, a dendrogram was constructed with 2 large clusters (Hg:CD-1 and Crj:CD-1) and (Dlv:CD-1, Slc:ddY and CF-1) as shown in Fig. 4(a). The Mahalanobis' distances between the Hg:CD-1 and CF-1 stocks and between the Hg:CD-1 and Slc:ddY stocks were comparatively long. On the other hand, distances between the Hg:CD-1 and Crj:CD-1 stocks and between the CF-1 and Slc:ddY stocks were relatively short. Although the origin of Dlv:CD-1 was the same as that of the Hg:CD-1 and Crj:CD-1 stocks, their distances were comparatively long. These relationships between stocks generally paralleled the relationships seen on the planes PC1 and PC2, PC1 and PC3 (Figs. 2 and 3). These results indicate that the Mahalanobis' distance between stocks is clearly related to the morphological differences in mandibles. A dendrogram calculated from the relative values showed that the clusters corresponded to the distances derived from actual mandibular measurements (Fig. 4(r)).

Discussion

Although it may sometimes be difficult, outbred stock (non-inbred closed colony) laboratory animals must be maintained so as to preserve a uniform genetic constitution from generation to generation. Festing[2] reported a case in which mice considered to belong to the same stock by 2 breeders differed genetically from one another. Groen and Lagerwerf[5] surveyed weekly genic heterogeneity at 12 loci encoding biochemical polymorphisms in an outbred stock of Swiss:SE mice. They found 6 loci showing a 2-allelic variation within each of the 3 stock units (pavillions) and weekly fluctuations of the allele frequencies for 2 loci. Hayakawa[6]

Table 4. Results of classification analysis using discriminant analysis (relative value)

Stock	Type of data[1)]	No. of mice	Assigned stock					Probability[2)]
			A	B	C	D	E	Mean±SD[3)]
Dlv:CD-1(A)	a	15	13	0	1	0	1	0.831±0.258
	r		13	0	1	1	0	0.771±0.290
Hg:CD-1(B)	a	15	0	13	2	0	0	0.875±0.249
	r		0	13	2	0	0	0.805±0.301
Crj:CD-1(C)	a	17	1	1	15	0	0	0.870±0.227
	r		1	2	14	0	0	0.812±0.283
CF-1(D)	a	15	0	0	0	14	1	0.909±0.175
	r		0	0	0	15	0	0.858±0.187
Slc:ddY(E)	a	17	1	0	0	1	15	0.873±0.189
	r		1	0	0	0	16	0.836±0.186

1): a, Actual measurement of mandible, Xj.

r, Relative value, $Xj/\sum_{j=1}^{11} Xj$ (j=1,2,...,11), see text.

2): Probability of assignment to the proper stock.

3): Standard deviation.

purchased ICR (CD-1) mice twice from the same breeder on 2 different instances at separate intervals and investigated the genetic variability at 8 loci. It was found that the allelic frequency at the C3-1 locus differed between samples from the same colony. These reports indicate that genetic variability of outbred mice may differ within the same stock even when designated by the same name and supplied by the same breeder. The CD-1 mice, which originated from ICR mice, have been introduced into research institutions around the world. In Uruguay, they are also maintained independently at several institutions and are used for various tests.

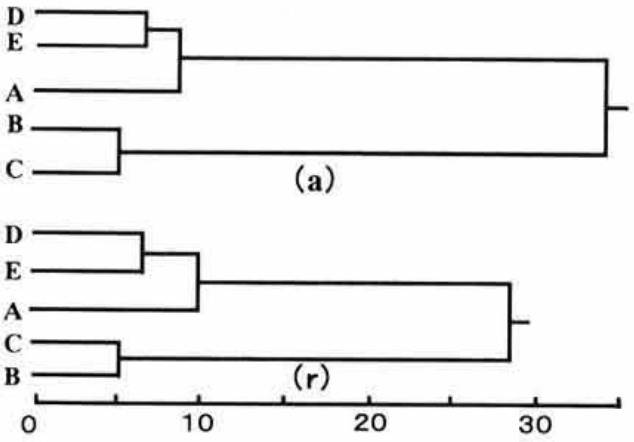

Fig. 4. Dendrograms of 5 stocks of outbred mice based on mandible measurements

Scale refers to the Mahalanobis' distance.

(a): Actual measurement of mandible, Xj.

(r): Relative value, $Xj/\sum_{j=1}^{11} Xj$ (j=1,2,...,11), see text.

A–E: Same as in Fig. 2.

The results of principal component analysis revealed differences in the size and shape of mandibles between stocks. Based on these differences, identification of the stocks was found to be possible by discriminant analysis using mandible measurements. Although the size and shape of the mandible are heritable traits, the size is also affected by the age as well as environmental factors including food quality. In the present investigation, the age of the mice ranged from 2 to 9 months and the quality of the food pellets used varied between Uruguay and Japan. As indicated in Table 4, the results of discriminant analysis showed some similarities between cases in which actual mandibular measurements were used and those in which relative values were used. Considering the effects of age and the environment on mandible size, however, it may be preferable to use relative values which eliminate these factors.

Although several mice in each CD-1 stock were misclassified when discriminant analysis was used, the CD-1 stocks could be distinguished from one another at rates of 2/15 in Dlv:CD-1, 2/15 in Hg:CD-1 and 3/17 in Crj:CD-1. These data suggest that the genetic constitution of the CD-1 stocks differed from one another. These

genetic differences between the CD-1 stocks may be attributed to the following factors:

(1) The original stocks of mice at each institute may have initially differed genetically at the time of purchase.

(2) Each stock had changed and exhibited a different genetic constitution due to independent breeding since the purchase.

As shown in Fig. 4, it is evident that the Mahalanobis' distances were the shortest between Hg:CD-1 and Crj:CD-1 which are derived from mice of other origins and were long between these stocks and the CF-1 and Slc:ddY stocks. Dlv:CD-1 is genetically more closely related to Slc:ddY or CF-1, which are derived from mice of other origins, than the CD-1 stocks examined in this study. Reintroduction of CD-1 mice to DI.LA.VE. is therefore recommended.

The results obtained in the present study suggest that genetic monitoring is essential for outbred stocks in each institution as well as for inbred strains.

References

1) Festing, M. F. W. (1972): Mouse strain identification. *Nature*, **238**, 351–352.
2) Festing, M. F. W. (1974): Genetic reliability of commercially-bred laboratory mice. *Lab. Anim.*, **8**, 265–270.
3) Fukuta, K. et al. (1996): Analysis of mandible of newly developing strain of Japanese White rabbit (Nlb:JWNS). *Exp. Anim.*, **45**, 361–368.
4) Goto, N. et al. (1987): Morphometrical observations on the mandible of five strains of rabbits and strain identification using mandible measurements. *Lab. Anim.*, **21**, 188–194.
5) Groen, A. & Lagerwerf, A. J. (1979): Genic heterogeneity and genetic monitoring of mouse outbred stocks. *Lab. Anim.*, **13**, 81–85.
6) Hayakawa, J., Koizumi, T. & Natsuume-Sakai, S. (1980): Constancy of genetic variability in mice from non-inbred closed colonies. *Lab. Anim.*, **14**, 233–236.
7) Hoffman, H. A. et al. (1980): Genetic quality control of laboratory animals with emphasis on genetic monitoring. *In* Proc. 7th ICLAS Symp. Gustav Fischer Verlag, Stuttgart, New York, 307–317.
8) Statistical Analysis Systems Institute (1988): SAS/STAT user's Guide. Release 6.03 Edition, Cary, NC.