Genetic Variability within and between Outbred Wistar Strains of Rats

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The allelic frequencies at 6 isozyme loci (Es-1, Es-2, Es-3, Es-4, Es-Si and Amy-1) were examined in 4 outbred Wistar strains and additionally 2 SD strains as controls. From the allelic frequencies, the averages of the heterozygosities in individual strains and the geometric genetic distances between every pair of all possible strain combinations were calculated. The averages of the heterozygosities in 2 SD strains were both intermediate (around 0.2) and the genetic distance between them was rather short. But among the Wistar strains, the averages of the heterozygosities were highly variable and the genetic relationships among them were very variable in their genetic distances. From these results, it was suggested that the outbred Wistar strains were different each other in their genetic constitutions and that no suggestion was obtained to discriminate genetically the Wistar strains from the SD strains.

As one of the most popular strains of rats in Japan "Wistar" strain exists. It is said that the Wistar strain was founded at the Wistar Institute of Biology and Anatomy in USA at the turn of the century [7]. Because of their hardiness and general excellence for laboratory breeding [7], the strain has spread to almost all over the world including Japan and various inbred strains have been established these strains of rats. Twenty-four inbred strains, which were originated from Wistar strain, are listed in "Inbred Strains of Rats" [3].

On the other hand, so-called "Wistar" rats from outbred strains are available for many commercial breeders in Japan. Some Wistar strains seem to be derived from rats introduced from USA before World War II and others were recently obtained from USA. Nevertheless, they are equally dealt with under the same name of

"Wistar", and there seems to be a lot of confusion concerning the name of "Wistar", which probably arises from their different characteristics. It will be easily expected from not only their derivations but also their appearances that there are considerable genetic variations within and between these "Wistar" strains.

In order to confirm the degree of the genetic heterogeneity of individual strains and the genetic diversity among these strains, a survey of alleles at several isozyme loci was performed on four outbred "Wistar" strains and additionally two SD strains for comparative purpose. These strains were selected according to their dissimilarities of their origins. The average of the heterozygosity in each strain, the genetic distances between every pair of these six strains were calculated from their allelic frequencies.

Table 1. The origins and numbers of rats examined.

Strain	Breeder	Origin	No. of Rats		
			♀	♂	Total
Wistar	A	Carwarth Farm, USA (1970)	25	25	50
	B	Institute of Med. Sci., Univ. Tokyo (1968)	24	24	48
	C	Hilltop Lab. Anim., USA (1972)	25	24	49
	D	Natl. Inst. Hygenic Sci., Osaka Branch, Japan (1963 ?)	23	24	47
S D	A	Charles River Breed. Lab., France (1968)	25	25	50
	B	Charles River Breed. Lab., USA (1975)	24	24	48

Materials and Methods

Animals: A total of 194 Wistar rats were purchased from 4 commercial breeders (WA, WB, WC and WD) and additionally 98 Sprague-Dawley (SD) rats were obtained from two breeders (SA and SB) for comparative purpose. The origin, sex and number of the rats in each strain were shown in Table 1. Food (Funabashi Farm, F-2) and tap water were provided ad libitum for 1 to 3 weeks before the animals were killed. The environmental temperature was maintained at 24 ± 2 C and the relative humidity at $60 \pm 10\%$.

Preparation of samples: After the rats were killed by ether inhalation at 8 to 11 weeks of age, small intestines, kidneys and pancreas were removed. The blood samples withdrawn by cardiac puncture in syringes with heparin were centrifuged to obtain plasma. About 0.3 g of small intestine, 0.5 g of kidney and pancrea were homogenized with 3-, 2- and 2-folds of isotonic saline respectively and centrifuged at 12,000 rpm for 20 min at 4 C. All the samples had been stored in a freezer at -20 C for maximum 4 weeks before electrophorases.

Electrophorases: The thawed samples were absorbed in 5mm $\times$ 5mm filter paper (Toyo Filter, No. 2) for esterases and 1mm $\times$ 8mm for amylase which were applied to the gels. For esterases, electrophorases

were performed with a 11.5% horizontal starch gel, using the EBT buffer system described by Boyer *et al* [1] (0.001 M EDTA, 0.025 M boric acid, 0.045 M tris, pH: 8.6) at the constant voltage of 10 V/cm for 17-19 hrs at 4 C. The gels were sliced in a thickness of 3 mm and stained with Fast Blue RR in 0.08 M tris-HCl buffer (pH; 7.0) containing α -naphthyl acetate as a substrate. For pancreatic α -amylase, electrophorases were performed with thin layer agarose gel on a glass, using the veronal buffer system by Watanabe and Tomita [10] (0.01 M veronal, 0.05 M sodium veronal) at the constant current of 1 mA/cm for 2 hrs at 4 C and visualization of the amylase band was performed by I_2 -KI solution.

Genetic analyses: Nomenclatures of esterase loci and their alleles were followed by Serov [8], Womack [11, 12 and 13] and Gasser *et al* [4] and those of amylase by Mizuno and Suzuki [5].

Allelic frequencies at Es-1, Es-3 and Es-Si loci were calculated under the assumption of being in Hardy-Weinberg equilibrium [2], because alleles of Es-1^b, Es-3^c and Es-Si⁻ were null alleles and it was difficult to distinguish their heterozygotes from their counterpart's homozygotes by the densities of the bands.

The degree of genetic heterogeneity of individual strains was quantified by the average of heterozygosity at six loci exa-

mined in each strain ($H = 1 - \sum_i q_i^2$, where q_i was the frequency of i -th allele at a locus and the average was over six loci examined). From the allelic frequency data obtained, the geometric genetic distances were calculated between every pair of the strains by the following formula:

$$\overline{D}_{jk} = \left[\sum_i (q_{ij} - q_{ik})^2 \right]^{1/2}$$

where q_{ij} and q_{ik} were the frequencies of the i -th allele at a locus in the j -th and k -th strains respectively and the average was over the six loci examined.

From the matrices of the genetic distance values, a dendrogram was drawn by adopting the weighed-pair-group method of clustering in numerical taxonomy [9].

Results

In order to detect genetic differences among the Wistar and SD strains of rats, six isozyme loci, which were polymorphic among nine inbred strains of rats examined by the authors [15], were selected in this study.

During this study, two new alleles were found. One was Es-3^d, the band of which migrated faster than that of Es-3^a and was tentatively designated as Es-3^d (Fig. 1). The other was Es-4^c which had two faster migrating bands than the two bands of Es-4^a as shown in Figure 2 and was tentatively designated as Es-4^c.

Since in the allelic frequencies sex difference was not founded, the data of both sexes were pooled. The allelic frequencies at these six loci in 4 Wistar and 2 SD strains were shown in Table 2. All the loci examined were polymorphic among these strains with 4 alleles at Es-3 locus, 3 alleles at Es-2 and Es-4 and 2 alleles at remaining 3 loci. Alleles of Es-2^c, Es-3^b, Es-4^c and Amy-1^b were very rare ones (less than 9%) among these strains.

At the all loci in the WB strain the alleles were completely fixed to only one allele as far as examined. On the contrary

Fig. 1. Starch gel zymogram of rat small intestine esterase (Es-3). Channel 1 is d/d, channel 2 d/d+a/a, channel 3, 4 a/a, channel 5 a/a+b/b, channel 6, 7 a/b and channel 8 b/b.

Fig. 2. Starch gel zymogram of rat kidney esterase (Es-4). Channel 1 is a/a, channels 2, 3 b/b, channel 4 c/c and channel 5 b/c.

Table 2. The allelic frequencies at five esterase and a amylase loci and the averages of the heterozygosities (H) in Wistar and SD strains of rats.

Locus	Allele	Allelic frequency					
		WA	WB	WC	WD	SA	SB
Es-1	a	0.49	1.00	0.03	0.47	1.00	1.00
	b	0.51	0	0.97	0.53	0	0
Es-2	a	0.76	1.00	0.06	0.69	0.69	0.67
	c	0	0	0	0	0.09	0
	d	0.24	0	0.94	0.31	0.22	0.33
Es-3	a	0.43	0	0.02	0.25	0.13	0.24
	b	0.01	0	0	0.04	0	0
	c	0	0	0.20	0.29	0.66	0.15
	d	0.56	1.00	0.78	0.43	0.20	0.62
Es-4	a	0	0	0	0.04	0.18	0
	b	1.00	1.00	1.00	0.90	0.82	1.00
	c	0	0	0	0.05	0	0
Es-si	+	0.23	0	1.00	0.53	0	0
	-	0.77	1.00	0	0.47	1.00	1.00
Amy	a	0.92	1.00	1.00	0.98	1.00	0.94
	b	0.08	0	0	0.02	0	0.06
H		0.31	0.00	0.09	0.38	0.21	0.18

Table 3. Matrices of the geometric genetic distances between every pair of the Wistar and the SD strains of rats.

	WB	WC	WD	SA	SB
WA	0.3512	0.5597	0.1879	0.3887	0.2409
WB		0.7350	0.4626	0.2816	0.1717
WC			0.4599	0.7912	0.6683
WD				0.3792	0.3260
SA					0.1935

at the all loci in the WD strain alleles were segregated into 2 to 4 different alleles. The most frequent allele at each locus was consistent at 3 loci (Es-3, Es-4 and Amy-1) among the Wistar strains, but at 5 loci (except Es-3) between the SD strains.

The averages of the heterozygosities calculated for these strains were varied from 0.00 (WB) to 0.38 (WD) as shown in the bottom line of the Table 2. Although 2 SD strains had similar values (around 0.2), among the Wistar strains these values

were varied for rather wide range.

The geometric genetic distances between every pair of the all strain combinations were shown in Table 3. The lowest value of the genetic distances was found between WB and SB ($D=0.1717$) and followed by the values between WA and WD ($D=0.1879$) and between SA and SB ($D=0.1935$). The highest values was found between WC and SA ($D=0.7912$). Excluding the values of the strain combinations with WC, the average values of the distances were relatively low (0.2983 ± 0.0313), whereas the average values of the distances among the 5 strain combination with WC were very high (0.6428 ± 0.0598). From this it is clear that the WC strain had consistently high values of the genetic distance with the combinations with other strains. From these facts, it will be expected that the WC strain was genetically diversified from other strains. The distances among the strain combinations within the Wistar strains were very variable and any indic-

ations to separate the SD strains from the Wistar strains on a genetical basis were not found as far as the six loci examined.

Discussion

Since the alleles of Es-1^b, Es-3^c and Es-Si⁻ are null alleles and it was difficult to distinguish their heterozygotes from their counterpart's homozygotes by the densities of the bands, allelic frequencies at these loci were calculated by the assumption of being at Hardy Weinberg equilibrium. But the assumption might be valid under the condition that the strain had been maintained for sufficiently long time by a continuous random breeding system and also with sufficiently large population size. Otherwise some biases may exist in the calculated allelic frequency. In this study, as the breeding system at each breeder was unable to be confirmed, such biases were ignored.

Two new alleles found in this study were tentatively designated as Es-3^d and Es-4^c. And confirming the allelism at each locus by crossing with other alleles at the locus and also to confirm the expression of the heterozygote with other alleles will be done in near future.

Since only six loci, which were polymorphic among nine inbred strains of rats, were examined and monomorphic loci among the inbred strains were omitted, the averages of the heterozygosities (H) were apparently overestimated compared with the so-called mean heterozygosity ($\bar{H}$). But the values will be able to be used as a relative one of the heterozygosity within the scope of the strains examined. It is not surprising that the calculated averages of the heterozygosities in almost all strain were not zero, since they had not been maintained as inbred strains. But the fact that the average of the heterozygosity of WB was zero may suggest that the strain might be submitted to close inbreeding such as sib-mating earlier in order to be established as an inbred strain.

The genetic distance used in this study was to be originally applied to natural population. But in the laboratory population, isolation among strains is very strict, immigration of genes from other population is negligible and fitness or adaptation to environmental climate is out of thinking, but only they would be submitted to artificial selections for fecundity. So this genetic parameter adopted in this study is a little far from the original meaning, but it could be used as a parameter to indicate comparatively the genetical relationship restricted only among the strains examined in this study.

The five esterase loci out of six isozyme loci examined in this study had been reported to belong to the same linkage group of LG V [4,13 and 14]. It will be desirable to examine about the more loci on the many other chromosomes. In the previous paper [15] the authors reported that transferrin, lactate dehydrogenase, isocitrate dehydrogenase, glutamate oxalate transaminase and glucuronidase, which are polymorphic in the laboratory mice, were not polymorphic among nine inbred strains of rats, and also Mizuno and Suzuki [6] reported almost the same results. These facts may indicate that the rats are genetically less variable than the mice probably due to the narrow origin of the laboratory rats. Nevertheless, it will be required to find out other polymorphic loci over more linkage groups of the rats in order to examine more precisely the genetic relationships among laboratory strains of rats.

In order to clearly show the genetic relationships among these strains, a dendrogram was drawn (Fig. 3) according to the genetic distances calculated between every pair of the all strain combinations. From the dendrogram three clusters of strains would be divided: the first one consisted of the strains of SA, SB and WB, the second one of WA and WD and the last one of WC. The WC strain seems to be very diverse from other five strains in its genetic constitution. The SB strain

Fig. 3. A dendrogram by the geometric genetic distances among the Wistar and the SD strains of rats.

was first clustered with WB and then SA was introduced into the cluster. But the relationship between SA and SB was very close and this will be explained by the fact that they were derived from a common breeder in spite of the different branches. Apart from the WC strain, the remaining five strains seem not to be so diverse each other. As regards distinction between the Wistar strains and the SD strains, no reason to discriminate them was found.

As a conclusion, it will be suggested that the name of the “Wistar” strain indicated only the ancestral origin of the strain during sixty or seventy years but there are nothing to characterize genetic-

ally the name of the “Wistar” strains, because the genetical differences among the Wistar strains are not likely to be so small to separate them from the SD strains. And also it will be advisable to the users of the outbred “Wistar” strain that it will be necessary to use the Wistar rats obtained from the same breeder for a series of experiment and to describe definitely the substrain’s symbol in the manuscripts in order to expect repeatable results in any animal experiments.

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クローズドコロニー Wistar 系統内および 系統間の遺伝的変異

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4 系統のクローズドコロニー Wistar 系ラット、及び対照として 2 系統のクローズドコロニー SD 系ラットについて、5 個の *esterase* 遺伝子座 (Es-1, Es-2, Es-3, Es-4, Es-Si) 及び 1 個の *amylase* 遺伝子座 (Amy-1) における遺伝子頻度を調査した。得られた遺伝子の頻度から、各系統のヘテロ性 (heterozygosity) の平均値

(H) 及びすべての 2 系統の組合せ間の幾何学的遺伝的距離 (geometric genetic distance: D) を算出した。2 系統の SD 系では H は共に中間的値 (約 0.2) でほぼ等しく、両者間の D は小さかった。しかし 4 系統の Wistar 系間では、H は系統によって差が著しく、(0.00~0.38) D から見た各系統間の遺伝的関係にも大きな変異が見ら

れた。これらの結果から、クローズドコロニー Wistar 系は各系統内のヘテロ性にも大きな差があり、又系統間の遺伝的關係にも大きな変異のあることが判明した。又同時に、これら 6 個の遺伝子座における遺伝子の調査結果からすると、これら Wistar 系を SD 系から遺伝的に区別する根拠は何も見出されなかった。

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