THE THIRD ALLELE OF SUPERNATANT ISOCITRATE DEHYDROGENASE OF HOUSE MOUSE, *Id-1^c*, ORIGINATES FROM ASIAN CONTINENT¹⁾

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Electrophoretic survey of *Id-1* alleles in the Japanese wild mouse, *Mus musculus molossinus*, revealed the existence of four alleles, *Id-1^a*, *Id-1^b*, *Id-1^c* and *Id-1^d*. Two alleles, *Id-1^c* and *Id-1^d*, are not present in European and American wild mouse populations, had overall frequencies of .138 and .006, respectively, in the Japanese mouse. The *Id-1^b* allele was most frequent (.782) and *Id-1^a* was relatively rare (.074). Geographically, the northern populations possess mostly *Id-1^b* allele (.985), while *Id-1^a*, *Id-1^b*, and *Id-1^c* alleles are common in the southern regions. This geographical distribution can be explained by the multiple migration hypothesis of the wild mouse into Japan through two southern routes. This explanation is partly supported by the presence of *Id-1^c* allele in the Asian house mice from the Philippines and Thailand.

INTRODUCTION

Three types of isocitrate dehydrogenase isozymes exist in the mouse. Two of them are found mainly in the mitochondrial fraction and one is mainly in the supernatant fraction of tissue homogenates. In the house mouse, genetic variations have so far been known only in the supernatant isocitrate dehydrogenase, *Id-1*. Two alleles, *Id-1^a* and *Id-1^b*, were reported in inbred strains of mice (Henderson 1965; Roderick *et al.* 1971; Staats 1976), and were also observed in the Western Hemisphere wild house mice, though the frequencies of the two alleles were variable among the populations investigated (Ruddle *et al.* 1969; Selander and Yang 1969; Selander *et al.* 1969; Hunt and Selander 1973; Berry and Peters 1977; Britton and Thaler 1978). One female carrying an *Id-1^c* allele has been found in the Hawaiian Islands (Wheeler and Selander 1972). In the Japanese wild mice (*Mus musculus molossinus*), four alleles, *Id-1^a*, *Id-1^b*, *Id-1^c* and *Id-1^d*, were reported preliminarily (Minezawa *et al.* 1976, 1978).

This paper is concerned with the geographical distribution of the four *Id-1* alleles

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in Japanese wild mouse populations. In addition, these alleles in several individuals from the other Asian countries are investigated to trace back the origin of *Id-1^c* alleles.

MATERIALS AND METHODS

A total of 170 mice, 92 females and 78 males, examined in this study were collected at 34 localities in Japan during three years through August, 1974 to September, 1977 (Fig. 1). These localities were divided into 14 geographical regions: e.g., 7 island

Fig. 1. Major regions and places of collection in Japan where wild mice were trapped.

1. Nakashibetsu; 2. Sunagawa; 3. Sapporo; 4. Ooma; 5. Johoji; 6. Tsuruoka; 7. Niigata; 8. Kanazawa; 9. Tokorozawa; 10. Nijigaoka; 11. Gotenba; 12. Fukuroi; 13. Anjo; 14. Nagoya; 15. Ootsu; 16. Wakayama; 17. Tanabe; 18. Okayama; 19. Yanago; 20. Yasugi; 21. Mine; 22. Tokushima; 23. Kochi; 24. Matsuyama; 25. Tsushima; 26. Sasaguri; 27. Hakozaiki; 28. Miyazaki; 29. Makurazaki; 30. Tanegashima; 31. Yakushima; 32. Tokunoshima; 33. Okinoerabushima; 34. Okinawa.

regions (Hokkaido, Shikoku, Kyushu, Tsushima, Oosumi Isls., Amami Isls., and Okinawa) and 7 regions in the Japanese mainland (Tohoku, Hokuriku, Kanto, Tokai, Kinki, San-yo, and San-in). Detailed conditions of the trapping sites were published previously (Minezawa *et al.* 1979). The other Asian mice tested were as follows: one male from Quezon and one female from Batangas, the Philippines; one female and one male from Lahore, Pakistan; one female and one male from Kabul, Afganistan. Wild mice from the Philippines are classified as *Mus musculus castaneus* and those from the latter two countries, as *Mus musculus bactrianus* according to Marshall (1977). In addition, two fancy mice from Thailand were also examined.

Mice were killed by bleeding from the orbital sinus, the kidney or liver was homogenized with an equal volume of distilled water by a disperser. The supernatant was obtained by centrifugation at $11,000\times g$ for 30 min at 0°C.

Electrophoresis was carried out in a horizontal tray using 10-14% hydrolysed starch and Tris-citrate continuous buffer system (pH 7.0), at a constant current of 0.9 mA/cm for 5-6 h. Staining was done at 37°C for 20-30 min with a mixture containing sodium isocitrate 30 mg, NADP 4 mg, Nitro Blue Tetrazolium 4 mg, phenazine methosulphate 2 mg, $MnCl_2$ 198 mg and agarose 200 mg in 24 ml of water (Shaw and Prasad 1970).

RESULTS

Seven phenotypes of *Id-1*, A, AB, B, AC, BC, C and BD (Fig. 2), were observed in the wild population of *Mus musculus molossinus*. Although genetic analysis of the new four phenotypes, AC, BC, C and BD, has not been carried out, it is possible to assume that those seven phenotypes are controlled by four co-dominant alleles, *Id-1^a*, *Id-1^b*, *Id-1^c* and *Id-1^d*. Namely, C seems to be a homozygote of *Id-1^c*, and AC and BC are revealed as heterozygotes of *Id-1^c* with *Id-1^a* or *Id-1^b*. In the same way, BD would be a heterozygote of *Id-1^b* and *Id-1^d*. *Id-1^c* and *Id-1^d* alleles have not been described in the Western Hemisphere populations except for a single Hawaiian female mouse.

The geographical distribution of these *Id-1* phenotypes and the allele frequencies are summarized in Table 1. *Id-1^b* is predominant in Japan and *Id-1^d* is rare. Though

Fig. 2. Diagrams of *Id-1* phenotypes. Electrophoresis was carried out at pH 7.0.

Table 1. Geographical distribution of *Id-1* phenotypes of Japanese wild mouse and their allelic frequencies in 14 regions

Regions	No. of mice	Phenotypes								Allelic frequency			
		A	AB	B	AC	BC	C	BD	a	b	c	d	
Hokkaido	19			18				1		.974			.026
Mainland (Honshu)													
Tohoku	14			14							1.000		
Hokuriku	6			6							1.000		
Kanto	4			4							1.000		
Tokai	17			16				1		.971			.029
Kinki	10			10						1.000			
San-yo	15			15						1.000			
San-in	13		1	12					.038	.962			
Shikoku	7			7						1.000			
Tsushima	11		1	1	5		4		.273	.136		.591	
Kyushu	42	1	10	10	3	13	5		.178	.512		.310	
Oosumi Isls.	3						3				1.000		
Amami Isls.	8			3	4	1			.188	.750		.062	
Okinawa	1					1				.500		.500	
Pooled	170	1	15	117	8	15	12	2	.074	.782	.138		.006

overall frequencies of the four alleles, *Id-1^a*, *Id-1^b*, *Id-1^c*, and *Id-1^d*, are .074, .782, .138, and .006, respectively, regional differences in the distribution of each allele are observed. In the northern and central parts of Japan, such as Hokkaido, the mainland and Shikoku, *Id-1* locus consists of *Id-1^b* allele with the exception of three individuals from Nemuro (Hokkaido), Gotenba (Tokai) and Mine (San-in). Whereas, in the southern parts of Japan, this locus mostly consists of two or three alleles, such as *Id-1^a*, *Id-1^b*, and *Id-1^c*. The frequencies of these alleles are different among the several regions (Fig. 3). Allelic frequencies of *Id-1^c* vary from .591 to .062, from .300 to .700 for *Id-1^b* and from .133 to .250 for *Id-1^a*. In Oosumi Islands, all three mice examined possessed *Id-1^c* allele in a homozygous state.

Intraregional differences in the frequencies of those three alleles were also observed between Hakozaki and Sasaguri in Kyushu, which are separated by only 10 km ($0.05 > P > 0.01$). In the former, the frequency of *Id-1^b* is .700, whereas in the latter it is .437 and so, frequencies of *Id-1^a* and *Id-1^c* are increased in the latter.

The other Asian house mice also possess *Id-1^a*, *Id-1^b* and *Id-1^c* alleles (Table 2). The wild mice from the Philippines, probably *M. m. castaneus*, show *Id-1A* and *Id-1C* phenotypes and the fancy mice from Thailand have only *Id-1C*, whereas the wild mice from Lahore and Kabul, *M. m. bactrianus*, exhibit only *Id-1B*.

Fig. 3. The distribution of *Id-1* alleles in the southern regions of Japan. The radius of the circles is roughly in proportion to the number of animals examined in each region. Open area in the circle represents the frequency of *Id-1^a* allele, shaded area *Id-1^b* and dark area *Id-1^c*.

Table 2. Phenotypes of isocitrate dehydrogenase (*Id-1*) of house mouse from four Asian countries

Country	No. of mice	No. of phenotype		
		A	B	C
Philippines (wild mouse)	2	1		1
Pakistan (wild mouse)	2		2	
Afganistan (wild mouse)	2		2	
Thailand (fancy mouse)	2			2

DISCUSSION

Seven electrophoretic patterns controlled by four alleles at *Id-1* were observed in the Japanese wild mouse. Three of them A, AB and B, seem to be identical with *Id-1* phenotypes already described for the wild mouse of North America and Europe besides laboratory mice, whereas, the other four patterns have not been reported. Based on this finding, we designated two new alleles, *Id-1^c* and *Id-1^d*.

Geographic distribution of Id-1 alleles in Japan and possibility of their multiple introduction

The distribution of *Id-1* alleles shows remarkable regional differences. *Id-1^b* is present in the whole area of Japan except for a few regions. The frequency of each allele, however, varies between the localities. For instance, *Id-1^a* and *Id-1^c* alleles are confined to southern parts of Japan. This distribution pattern is somewhat similar to that of *Hbb* alleles; that is, *Hbb^d* is found in abundance in the southern peripheral regions of Japan (Minezawa *et al.* 1979). The localization of *Id-1^c* allele in Kyushu and the existence of this allele in *M. m. castaneus* from the Philippines may suggest that the multiple migrations of wild mice from outside this country had occurred at least twice as well as in the case of *Hbb* (Minezawa *et al.* 1978, 1979). The first immigrants possessed only *Id-1^b* allele and the second ones *Id-1^a* and *Id-1^c* besides *Id-1^b*. Following the estimation of a divergence time between *M. m. molossinus* and *M. m. domesticus*, which was calculated at around 1.5×10^6 years by Nei's genetic distance (Moriwaki *et al.* 1979), the first population of Japanese mouse seems to have migrated by either one of the two land bridges which existed between the Asian Continent and Hokkaido or Northern Kyushu during the Late Pleistocene (Minato *et al.* 1965). The distribution pattern of *Id-1^a* and *Id-1^c* in Japan seems to indicate a recent invasion of the second population through the following routes: Korea→Tsushima→Kyushu and/or Formosa→South east archipelago→Kyushu. *Id-1^d* allele has been observed only in northern Japan which probably suggests a recent occurrence of mutation on this population.

Distribution of Id-1 polymorphism and relationship among Mus musculus subspecies

Generally speaking, in Western Hemisphere mice the common *Id-1* alleles are *Id-1^a* and *Id-1^b*, though the frequencies of these two alleles differ among various populations

of species, *Mus musculus* (Ruddle *et al.* 1969; Selander and Yang 1969; Selander *et al.* 1969; Hunt and Selander 1973; Berry and Peters 1977; Britton and Thaler 1978). In the Japanese populations, *Id-1^c* allele is also common besides the above two alleles. This difference suggests an early divergence between the Asian and West European forms. Marshall (1977, 1978) classified the former as castaneus type by having a convex-edged zygomatic plate and the latter as domesticus type by having a straight-edged zygomatic plate. This is further supported by the presence of *Id-1^c* in *Mus musculus castaneus* from the Philippines and Thailand (Nishimura 1977) and fancy mice from Thailand. In connection with this, the Hawaiian *Mus musculus* population would have been constructed partially with the contribution of Asian subspecies, because of the existence of *Id-1^c* (Wheeler and Selander 1972). In *Mus musculus bactrianus* from Lahore and Kabul, we observed only *Id-1^b*, but the sample size is too small to exclude the possible existence of *Id-1^a* and *Id-1^c* alleles. Based on the finding that *M. m. bactrianus* from Iran shows various zygomatic shapes, the possibility of hybridization between Asian and West European house mice was suggested (Marshall 1978). *Hbb^p* allele, characteristic to Asian mice, was found in *M. m. bactrianus* (Minezawa *et al.* 1978). Although the border line between Asian and Western Hemisphere mice is still obscure and interminglings between them may have occurred, it can be assumed that existence of *Id-1^c* allele is limited to East Asian house mice, at least, castaneus group including *M. m. molossinus*.

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LITERATURE CITED

- Berry, R. J., and J. Peters, 1977 Heterogeneous heterozygosities in *Mus musculus* populations. Proc. R. Soc. Lond. B. **197**: 485-503.
- Britton, J., and L. Thaler, 1978 Evidence for the presence of two sympatric species of mice (genus *Mus* L.) in Southern France based on biochemical genetics. Biochem. Genet. **16**: 213-225.
- Henderson, N. S., 1965 Isozymes of isocitrate dehydrogenase: subunit structure and intracellular location. Exp. Zool. **158**: 263-274.
- Hunt, W. G., and R. K. Selander, 1973 Biochemical genetics of hybridization in European house mice. Heredity **31**: 11-33.
- Marshall, J. T., Jr., 1977 A synopsis of Asian species of *Mus* (Rodentia, Muridae). Bull. Amer. Mus. Nat. Hist. **158**: 175-220.
- Marshall, J. T., 1978 Brief review of European house mice. In "Origin of Inbred Mice" (H. C. Morse III, ed.) pp. 511-517. Academic Press, New York.

- Minato, M., M. Gorai, and M. Hunahashi, 1965 "Geologic Development of Japanese Islands" pp. 494. Tsukiji Shokan, Tokyo.
- Minezawa, M., K. Moriwaki, and K. Kondo, 1976 Studies on protein polymorphism of Japanese wild mouse, *Mus musculus molossinus*. Ann. Rep. Natl. Inst. Genet. (Japan) **26**: 23-25.
- Minezawa, M., K. Moriwaki, and K. Kondo, 1978 Allelic constitution of *Hbb* loci in Asian house mouse subspecies. Ann. Rep. Natl. Inst. Genet. (Japan) **28**: 32-33.
- Minezawa, M., K. Moriwaki, and K. Kondo, 1979 Geographical distribution of *Hbb^p* allele in the Japanese wild mouse, *Mus musculus molossinus*. Japan. J. Genetics **54**: 165-173.
- Moriwaki, K., T. Shiroishi, M. Minezawa, T. Aotsuka, and K. Kondo, 1979 Frequency distribution of histocompatibility-2 antigenic specificities in the Japanese wild mouse genetically remote from the European subspecies. J. Immunogenet. **6**: 99-113.
- Nishimura, M., 1977 Studies on biochemical marker genes of 26 inbred mouse strains. Proceedings for the 12th Annual Meeting of JEARA at Nishinomiya. pp. 96.
- Roderick, T. H., F. H. Ruddle, V. M. Chapman, and T. B. Shows, 1971 Biochemical polymorphism in feral and inbred mice (*Mus musculus*). Biochem. Genet. **5**: 457-466.
- Ruddle F. H., T. H. Roderick, T. B. Shows, P. G. Weigl, R. K. Chipman, and P. K. Anderson, 1969 Measurement of genetic heterogeneity by means of enzyme polymorphisms. J. Hered. **60**: 321-322.
- Selander, R. K., and S. Y. Yang, 1969 Protein polymorphism and genic heterozygosity in a wild population of the house mouse (*Mus musculus*). Genetics **63**: 653-667.
- Selander, R. K., W. G. Hunt, and S. Y. Yang, 1969 Protein polymorphism and genic heterozygosity in two European subspecies of the house mouse. Evolution **23**: 379-390.
- Shaw, C. R., and R. Prasad, 1970 Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet. **4**: 297-320.
- Staats, J., 1976 Standardized nomenclature for inbred strains of mice: Sixth listing. Cancer Res. **36**: 4333-4377.
- Wheeler, L. L., and R. K. Selander, 1972 Genetic variation in population of the house mouse, *Mus musculus*, in the Hawaiian Islands. Studies in Genetics VII. Univ. Texas Publ. **7213**: 269-296.