

# The *Mus musculus musculus* type Y chromosome predominates in Asian house mice

YUTAKA NISHIOKA\* AND ESTELLE LAMOTHE

Department of Biology, McGill University, Montreal, Quebec, Canada H3A 1B1

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## Summary

Using a mouse Y chromosomal repetitive sequence that differentiates between the *Mus musculus musculus* type Y chromosome and the *M. m. domesticus* type Y chromosome, we studied the Y chromosome in *M. m. molossinus*, *M. m. castaneus* and *M. m.* subspecies specimens recently trapped in Japan, Taiwan and China as well as Asian mice maintained at the Jackson Laboratory and Litton Bionetics. Here we report that the *M. m. musculus* type Y chromosome predominates in Asian house mice and that Japanese mice maintained at some laboratories may not represent typical *M. m. molossinus*.

## 1. Introduction

The isolation of mouse Y chromosomal DNA has been reported by several groups (Bishop *et al.* 1985; Eicher *et al.* 1983; Lamar & Palmer, 1984; Nallaseth & Dewey, 1986; Nallaseth *et al.* 1983; Nishioka & Lamothe, 1986, 1987). These DNA fragments provide powerful molecular tools with which to study the evolution of the mouse Y chromosome as some of them differentiate between the *M. m. musculus* and the *M. m. domesticus* types of Y chromosome (Bishop *et al.* 1985; Nishioka & Lamothe, 1986). It was found that many classical inbred strains have the *M. m. musculus* type Y chromosome (Bishop *et al.* 1985; Lamar & Palmer, 1984; Nishioka, 1987; Nishioka & Lamothe, 1986), despite the fact that the mitochondrial genome and most of the nuclear genomes of classical inbred strains originated from *M. m. domesticus* (Ferris *et al.* 1982; Moriwaki *et al.* 1982, 1985; Yonekawa *et al.* 1980, 1982). Since it is generally accepted that Japanese house mice (*M. m. molossinus*) contributed to the establishment of classical inbred strains (Festing & Lovell, 1981; Keeler, 1931; Potter, 1978), Bishop *et al.* (1985) predicted the presence of the *M. m. musculus* type Y chromosome in *M. m. molossinus*. Indeed *M. m. molossinus* specimens obtained from Litton Bionetics had the *M. m. musculus* type Y chromosome (Nishioka & Lamothe, 1986). We extended the survey to five *M. m. molossinus* specimens recently trapped in Japan and found the *M. m. musculus* type Y chromosome in all of them. In addition, *M. m. castaneus* trapped in Taiwan and

Thailand and two mice (*M. m.* subspecies) trapped in China also had the *M. m. musculus* type Y chromosome. Thus, the *M. m. musculus* type Y chromosome appears to be predominant in Asian house mice. Unexpectedly *M. m. molossinus* strains maintained at the Jackson Laboratory had the *M. m. domesticus* type Y chromosome.

## 2. Materials and methods

Mice: Ibred strains C57BL/6J and SWV (Staats, 1980) were obtained from the Jackson Laboratory (Bar Harbor, Maine) and Dr D. Trasler, McGill University, respectively. *M. m. molossinus* strains, MOLC/Rk, MOLD/Rk and MOLE/Rk were initially provided by Dr F. Biddle, University of Calgary. Subsequently we purchased these strains and MOLF/Ei from the Jackson Laboratory to confirm the observation. We also obtained *M. m. molossinus* specimens from Litton Bionetics (Kensington, Maryland) through Dr M. Potter of the National Cancer Institute. The localities of other Asian mice obtained from Dr Moriwaki, National Institute of Genetics, Japan are indicated in the figure legends.

Filter hybridization: DNAs isolated from liver were digested with Eco RI (Boehringer Mannheim Canada, Dorval, Quebec) and electrophoresed on a 0.8% agarose gel in a buffer consisting of 40 mM Tris (pH 8.0), 80 mM sodium acetate and 1 mM EDTA. DNA fragments were transferred to membrane filters (Gene Screen, New England Nuclear Canada, Lachine, Quebec) by the method of Southern (1975). Filters were pretreated with 50 µg/ml of denatured herring

\* Corresponding author.

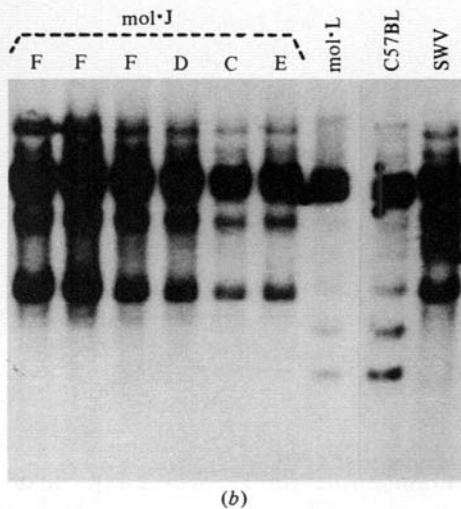
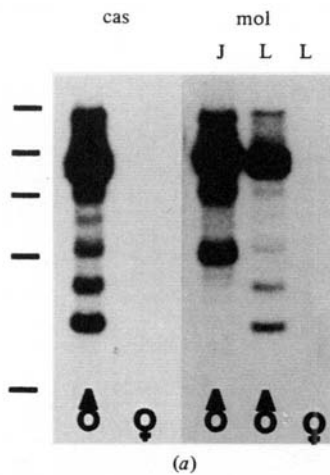


Fig. 1. Southern blot analysis of Asian mice maintained at two laboratories. About 10  $\mu$ g of DNAs were digested with Eco RI and electrophoresed on a 0.8% agarose gel. DNA fragments were transferred to membrane filters and hybridized to nick-translated AC11.

(a) cas: *M. m. castaneus* (Thailand) obtained from Litton Bionetics. mol. J: *M. m. molossinus* (MOLF/Ei) obtained from the Jackson Laboratory. mol. L: *M. m. molossinus* obtained from Litton Bionetics (sample number 19717). Solid lines indicate size markers from top to bottom 23 kb, 9.6 kb, 6.8 kb, 4.3 kb and 2.3 kb.

(b) mol-J: *M. m. molossinus* (C, D, E and F stand for strains MOLC/Rk, MOLD/Rk, MOLE/Rk and MOLF/Ei, respectively). mol-L: *M. m. molossinus* (Litton Bionetics, sample number 19717).

C57BL/6J and SWV are used as controls for the *M. m. musculus* type Y chromosome and the *M. m. domesticus* type Y chromosome, respectively.

sperm DNA in a solution containing 5  $\times$  SSC (1  $\times$  SSC is 0.15 N-NaCl and 0.015 N-Na-citrate), 50% formamide, 1  $\times$  Denhardt's solution (Denhardt, 1966) and 0.1% SDS for 12–16 h at 41  $^{\circ}$ C. Filters were then hybridized to a [ $^{32}$ P]-labelled Y chromosomal DNA

fragment designated as AC11 (Nishioka & Lamothe, 1986) in the same solution for 16 h, washed in 500 ml of 0.1  $\times$  SSC at 50  $^{\circ}$ C and exposed to Kodak XAR-5 films for 8–16 h with Cronex intensifying screens at  $-70^{\circ}$ C.

### 3. Results and discussion

AC11 is a mouse Y chromosomal repetitive sequence containing a Bkm-related sequence (Nishioka & Lamothe, 1986). In this study a portion of the clone deprived of the Bkm-related sequence was used as the molecular probe. When hybridized to male and female DNAs isolated from *M. m. castaneus* and *M. m. molossinus*, male lanes showed significant accumulation of AC11-related sequences (Fig. 1a), as previously observed in DNAs of *M. m. musculus* and *M. m. domesticus* (Nishioka & Lamothe, 1986), and *M. m. castaneus* had the *M. m. musculus* type Y chromosome. Since *M. m. molossinus* is an inter-subspecific hybrid between *M. m. musculus* and *M. m. castaneus* (Yonekawa *et al.* 1986), the presence of the *M. m. musculus* type Y chromosome in *M. m. molossinus* was inferred. Unexpectedly, both types of the Y chromosome were found among *M. m. molossinus* specimens. Four strains, MOLC/Rk, MOLD/Rk, MOLE/Rk and MOLF/Ei, from the Jackson Laboratory had the *M. m. domesticus* type Y chromosome, whereas two specimens from Litton Bionetics had the *M. m. musculus* type Y chromosome (Fig. 1 and 2). We then extended our survey to 5 *M. m. molossinus* specimens recently trapped in Japan, 1 *M. m. castaneus* from Taiwan and 2 *M. m.* subspecies from China and found the *M. m. musculus* types Y chromosome in all of them (Fig. 2).

The Japanese mice (*M. m. molossinus*) maintained at Litton Bionetics were collected by Dr Hamajima of Kyushu University and sent to Dr M. Potter in 1967 from which 4 strains (MOLC/Rk, MOLD/Rk, MOLE/Rk and MOLF/Ei) were developed at the Jackson Laboratory using a single male as the founder (M. Potter, 1978, personal communications from M. Potter and M. Davisson). In view of the fact that *M. m. musculus* and *M. m. molossinus* are genetically closer to one another than either one to *M. m. domesticus* (Bonhomme *et al.* 1984; Ferris *et al.* 1983; Moriwaki *et al.* 1982; Sage, 1981; Yonekawa *et al.* 1986), the presence of the *M. m. domesticus* type Y chromosome in *M. m. molossinus* background should be considered exceptional.

House mice are commensal. They can spread through commercial traffic and introduce new genetic material into aboriginal populations. For example, a specimen with Robertsonian translocation, caught on Ogasawara Island, Japan was shown to be a hybrid between *M. m. domesticus* and *M. m. molossinus* (Moriwaki *et al.* 1984) and *M. m. domesticus* type mitochondrial DNA was found in a specimen caught in Kyushu, Japan (H. Yonekawa, personal communi-

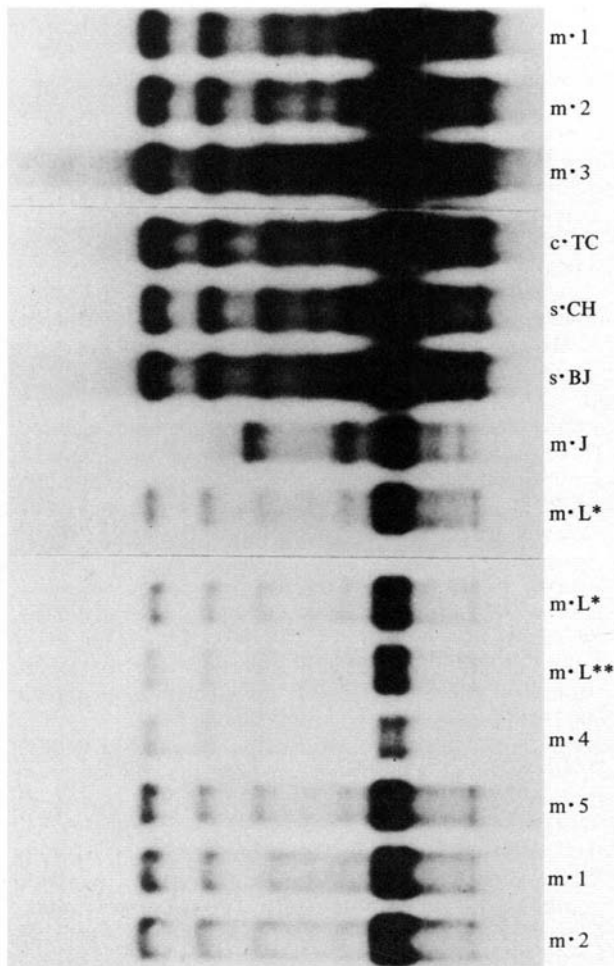


Fig. 2. Southern blot analysis of Asian wild mice. DNAs digested with Eco RI were electrophoresed on a 0.8% agarose gel, transferred to a membrane filter and hybridized to nick-translated AC11. m·J.: *M. m. molossinus* strain MOLF/Ei from the Jackson Laboratory. m·L.: *M. m. molossinus* from Litton Bionetics. \* Sample number 19717. \*\* Sample number 20597. The localities of other Asian mice are as follows: m·1 (*M. m. molossinus*), Izumi, Japan; m·2 (*M. m. molossinus*), Mishima, Japan; m·3 (*M. m. molossinus*), Anjo, Japan; m·4 (*M. m. molossinus*), Nagoya, Japan; m·5 (*M. m. molossinus*), Chiba, Japan; c·TC (*M. m. castaneus*), Taichung, Taiwan; s·CH (*M. m. subspecies*), Changchun, China; s·BJ (*M. m. subspecies*), Beijing, China.

cation). SF/CamEi was established from mice caught in a coal mine in California (Staats, 1980), but has the *M. m. musculus* type Y chromosome which was probably introduced from an Asian mouse brought to California (Nishioka, 1987). Therefore, a likely origin of the *M. m. domesticus* type Y chromosome in the *M. m. molossinus* strains is a *M. m. domesticus* male brought to Kyushu, although the possibility is not completely excluded that the introduction might have occurred in the U.S.A. In this context it is relevant to point out that SK/CamEi which was established from mice caught on Skokholm island, off Pembrokeshire (Staats, 1980) has probably been mixed with SF/Cam as originally suggested by Ferris *et al.* (1982). Subsequently the *M. m. musculus* type Y chromosome

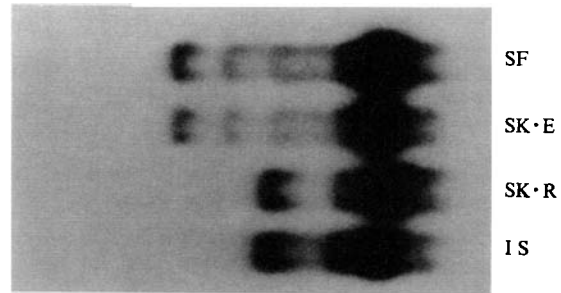


Fig. 3. Southern blot analysis of inbred strains. DNAs digested with Eco RI were electrophoresed on a 0.8% agarose gel, transferred to a membrane filter and hybridized to nick-translated AC11. SF; SF/CamEi; SK-E; SK/CamEi; SK-R; SK/CamRk; IS; IS/CamEi.

was found in SK/CamEi, despite its geographical origin (Nishioka, 1987). The presence of the *M. m. domesticus* type Y chromosome in another SK/Cam strain (SK/CamRk) further supports the above possibility (Fig. 3). In addition, Fig. 3 shows the presence of the *M. m. domesticus* type Y chromosome in IS/CamEi which was established from a cross between *M. m. praetextus* (*M. m. domesticus*) caught in Israel and a *M. m. musculus* composite stock (Staats, 1980).

This report is an extension of our previous survey that classified 39 inbred strains into two categories; strains with the *M. m. musculus* type Y chromosome and those with the *M. m. domesticus* type Y chromosome (Nishioka, 1987). It is clear that the *M. m. musculus* type Y chromosome predominates in Asia, while in America and Western Europe the representative Y chromosome is *M. m. domesticus* type. However, because of their commensalism with human beings, foreign house mice are introduced into certain local populations at an apparently high frequency. Our study demonstrated that DNA probes such as AC11 are useful molecular tools not only for population studies but also for confirmation of the origins of established inbred strains.

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