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Sequence of mitochondrial D-loop in laboratory and wild strains of mice of genus *Mus*

Jaroslav FLEGR[1], Jan LUKÁŠ[1] & Tetsu TSUKAMOTO [2]

1) Department of Parasitology, Charles University, Viničná 7, CZ-12844 Praha 2, Czech Republic
2) Department of Forensic Medicine, Tokyo University, Hongo, Bunkyo-ku, Tokyo 113, Japan

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Abstract. A segment of three hundred forty (340) bp. of mtDNA from D-loop region was sequenced in fifteen (15) laboratory strains of mice as well as in seven (7) wild mice of different species, subspecies and geographic origins. The data were compared with another three already published murine D-loop sequences. Eleven of the laboratory strains possessed an identical sequence, in BALB/c, C3H and DBA/2J a single (but different) mutation was detected and NZB differed in seven positions from other strains, being more similar to *M. m. musculus* than to *M. m. domesticus*. The differences between *M. m. domesticus* x *M. m.musculus* was as low as two substitutions (0.6%) while the highest observed interspecies difference in genus *Mus* (*M. m. domesticus* x *M.spicilegus*) was 63 substitutions (19%) which suggest the age of the branching event approximately three million years. The results suggest that the method can be used for biosystematic studies on the wild mice, however, it is not sensitive enough to be used in genealogical studies on strains of laboratory mice.

INTRODUCTION

In spite the fact, that the mice become the universal mammalian model and most of aspects of its biology have been studied extensively, the origin and some details of a genealogical tree of many common laboratory strains are still dim. Also the biosystematics of its natural populations and the evolutionary relationships between the different members of the genus have only recently begun to be explored (Bonhomme et al. 1983, Bonhomme 1986, Moriwaki et al. 1986, Yonekawa et al. 1988). Studies on protein and mtDNA polymorphism have shown that the mitochondrial genome and most of the nuclear genome of the laboratory mouse originated from the European subspecies *M. m. domesticus* (Yonekawa et al. 1980, Bishop et al., 1985, Bonhomme 1986). It was even suggested that the old laboratory strains of inbred mice are descendents from a single female (Ferris et al.1982). The history of breeding of these old strains is more or less known. There are still some open questions concerning of certain parts of their genealogical tree (Klein & Klein 1987). Moreover, there is always a possibility of genetic contamination that can be sometimes rather difficult or impossible to reveal. It would be desirable to get an experimental method for analysis of relationships between different strains of mice. A promising method for this purpose could be an analysis of DNA sequence data.

In this study we focused our attention on D-loop of mtDNA, taking an advantage of current development of PCR technique. We amplified 340 BP long part of a central region of D-loop of mtDNA of 15 laboratory strains of mice and compared its sequence with the sequences obtained from different species and subspecies of wild mice of genus *Mus*. The aim of this study is to evaluate which information on the origin of laboratory strain and on systematic of the genus *Mus*

can be obtained from comparison of this relatively short but highly polymorphic region of mtDNA.

MATERIALS AND METHODS

Organisms

Animals. Laboratory strains of mice A/J, AKR/J, BALB/c, C57BL/6J,C57BL/10J, C3H/HeJ, CBA/J, DBA/1J, DBA/2J, SJL/J, NZB, NZW, C57BR, CF#1 and DDD (Tanaka et al. 1987) were obtained from Animal Center of Medical Faculty, University of Tokyo. The origin of wild mice, *M. musculus domesticus* Schwarz & Schwarz, 1943: Dom-Pgn1 (Canada, Pegion R.), SK/Cam (U.K., Skokholm I), BFM/2 Mpl (France, Montepellier), *M. m.musculus* Linnaeus, 1758: Mus-Njl (Denmark, Northern Jutland), MBT (Bulgaria, Toshevo) was described elsewhere (Nobuhara et al. 1989, Potter 1987). *M.* sp. Har originated from Harran (Sanli Urfa, Eastern Turkey) and *M. spicilegus* Petényi, 1882 from Austria. The D- loop sequences of *Rattus rattus* (Linnaeus, 1758), *R. norvegicus* (Berkenhout, 1769) and *Mus spretus* Lataste, 1883 were obtained from the EMBL database.

Amplification, cloning and sequencing of DNA

Primers containing Bgl II restriction site in 5' end, 15308-GAAGATCTGATAGTATAAACATTACTCTG and 15686-GAAGATCTTGGTTTCACGGAGGATGG were used for amplification. The number refers to the position of the 3' base of the primer in the complete mouse mtDNA sequence (Bibb et al., 1981). Less then 1 ng of purified DNA or DNA contained in 1µl of 50 times diluted and 3 minutes boiled blood, was used as a template. The reaction mixture (50µl) contained 50mM KCl, 10mM Tris-Cl pH 8.4, 1.5 mM MgCl2, 0.1mg/ml gelatin, 0.2mM dNTP mix, 250 nM primers and 1 u of Taq polymerase; the amplification was performed in 30 cycles consisting of 1 minute in 96° C, 1 minute in 55° C and 2 minutes in 74° C. The amplified segment was purified by centrifugation through 200µl of Bio-Gel P-60, digested by Bgl II and cloned into Bam HI site of dephosphorylated BlueScript plasmid. Recombinant plasmids were isolated as dsDNA and sequenced in both directions using dideoxiribonucleotide method. Several recombinant plasmids were sequenced from every sample to exclude mutations that occurred artificially during the amplification. Program DNAPARS from system of programs PHYLIP 3.5 (Felsenstein, 1985) was used for a cladistic analysis and a for a construction of cladograms. Phenetic analysis was performed by a program Statistica (UPGMA, Percent disagreement distance).

RESULTS

Fragment of the length 340 nucleotides from 15 laboratory strains of mice was sequenced. Eleven of these sequences were identical with the sequence reported for a laboratory mouse by Bibb et al. (1981). In BALB/c mouse **A** in position 15437 was substituted with **G**, in C3H mouse **A** in position 15614 was substituted with **T**, in DBA/2J mouse **A** in position 15540 was substituted with **C**. In the NZB mouse seven substitutions were detected (Fig. 1). To estimate an intraspecies and interspecies variability, the fragment of D-loop was sequenced in wild mice of seven different subspecies and species (Fig. 1). Two most similar subspecies, *M. m. musculus* and *M. m. domesticus*, differed in 0.6% positions only, while the two most dissimilar species, *M. m. domesticus* and *M. hortulanus*, differed in 19% positions. Within two hundred seventy (270) mutations detected, there were one hundred of the transitions (100) and one hundred sixty transversions (160). Eight (8) single-nucleotide deletions/insertions and two (2) dinucleotide deletions/insertions were also detected.

All polymorphic sites in the sequences were used for cluster analysis using the program Statistica. The percent disagreement distances between sequences were computed and the taxons were clustered by Unweighted Pair Group Method with Arithmetic mean (UPGMA). The tree diagram is shown in the Figure 2.

Taxonomically relevant characters, i.e. nucleotides in positions where at least two individuals share the same mutation and where two outgroup species, *Rattus rattus* and *R. norvegicus*,

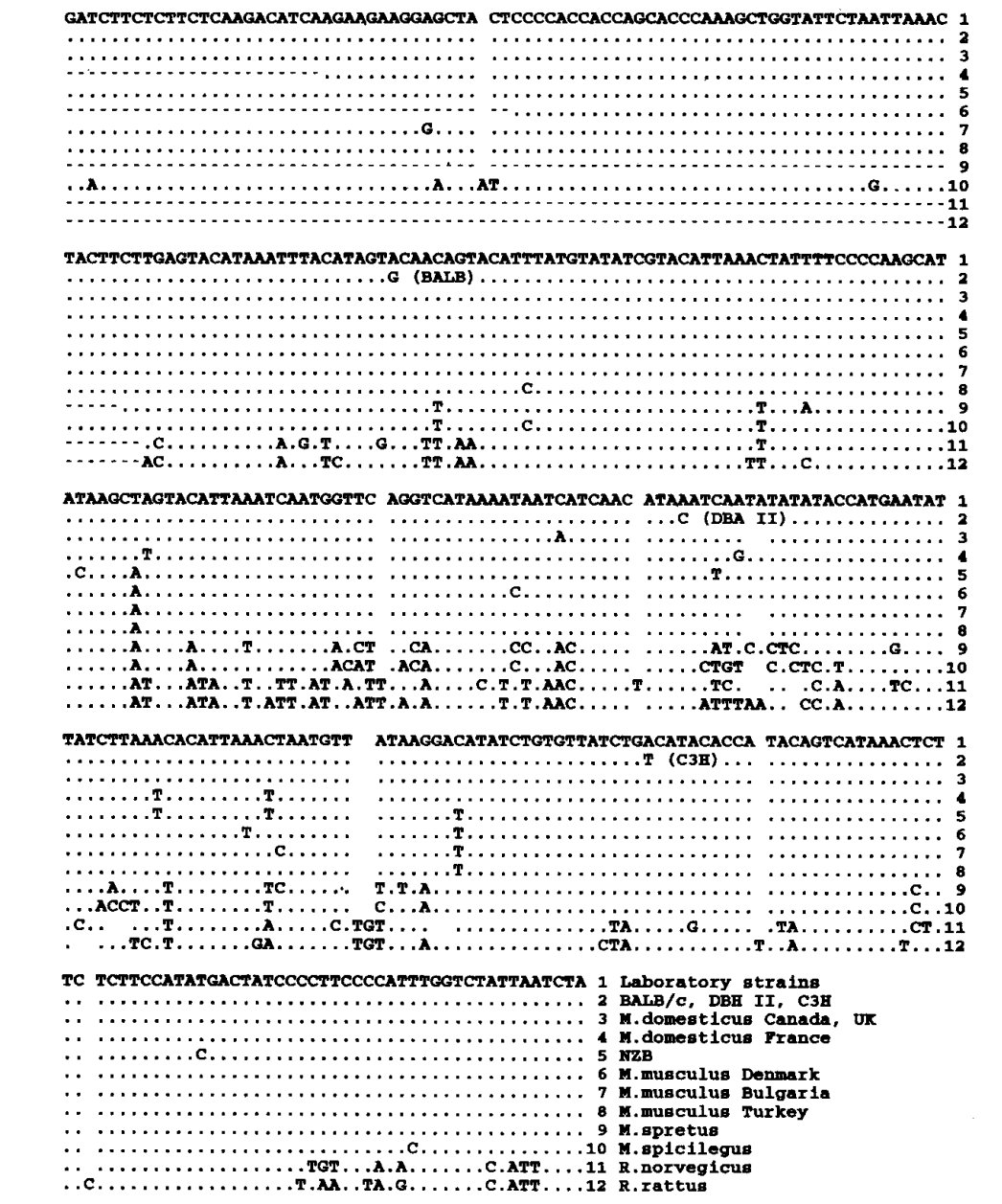

Fig. 1. Sequence of the D-loop fragment of different mice of genus *Mus*. For the list of laboratory strains see Materials and Methods.

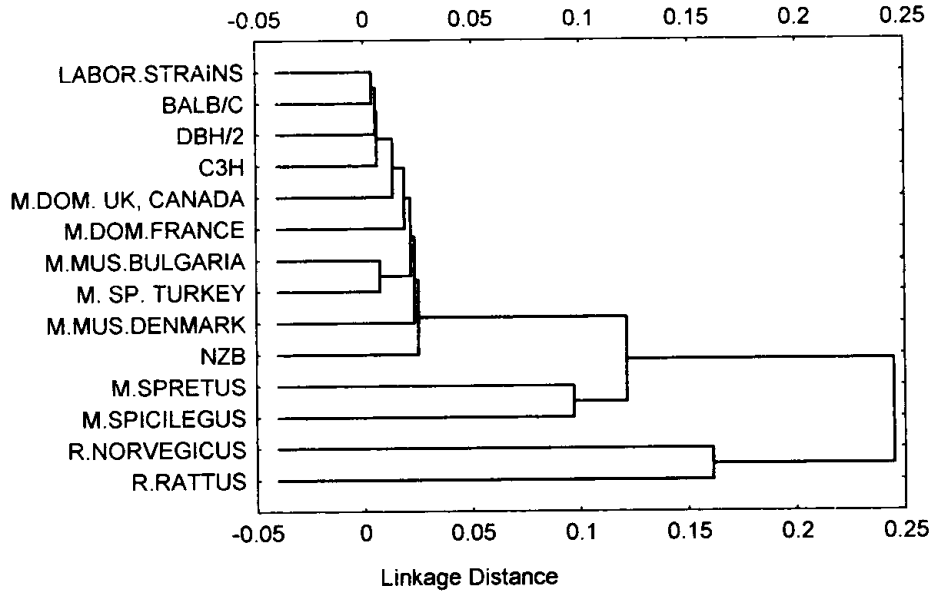

Fig. 2. Phylogenetic tree of genus *Mus* constructed by UPGMA on the basis of sequences of D-loop of mtDNA. For details see Materials and Methods.

have an identical nucleotide, were analyzed using program for cladistic analysis DNAPARS. Set of cladograms differing in minor details were obtained (the results not shown). In general, there was a good agreement between results of UPGMA and those of cladistic analysis as well as between our results and present knowledge on the phylogeny of the genus *Mus* (Bonhomme 1986). According to our results, *Mus spicilegus* branched out before *Mus spretus*. Unexpectedly, the laboratory strain NZB was more closely relative to *Mus musculus musculus* than to *Mus musculus domesticus*.

DISCUSSION

Single (but different) mutation was detected in BALB/c, C3H and DBA/2J mice. Positions of these strains within the genealogical tree of laboratory mice (Klein & Klein 1987) suggested, that these mutations either occurred or were fixed in populations of mitochondria independently during the history of breeding of laboratory strains. Alternatively, the results could be explained by the existence of polymorphism in populations of mice in the founder colony or in the population of mitochondria in the founder mice cells.

The sequence of D-loop in NZB mice differed from other laboratory strains in seven positions. This strain was developed in Otaga Medical School mouse colony, New Zealand, from randomly bred mice brought by W. H. Hall in 1930 from Imperial Cancer Research Fund Laboratories at Mill Hill, London (Bieloschowsky & Goodall 1970). It was pointed out by others that the mtDNA of this strain differed from mtDNA of other strains of laboratory mice (Ferris et al. 1983). Our data showed that the sequence of the D-loop fragment of NZB mice was more similar to *M. m. musculus* than to *M. m. domesticus*. This fact suggested that, in contrast to other laboratory strains, mtDNA of NZB strain could originate from *M. m. musculus*, rather than from *M. m. domesticus*. Classical inbred strains of mice are supposed to be derived from *M. m. dome-*

sticus (Ferris et al. 1983). Most of them, however, are in fact recombinants between *domesticus*- and *musculus*-like genomes (Blank et al. 1986). It was shown, for example, that most of these strains contain a Y chromosome from *M. m. musculus* (Bishop et al. 1985). It is supposed, that *M. musculus* genes have been introduced into laboratory mice's genomes by hybridization with wild *M. m. musculus* males. In NZB strain, however, the *M. m. musculus* mouse had to be introduced by female, because of maternal character of mtDNA inheritance. Relatively low intrasubspecies variability was detected in D-loop. In fact, *M. m. domesticus* mice captured in England and Canada had identical sequences of D-loop. We believe, that it can be explained by relatively recent history of colonization of America by house mouse, paralleling those of the modern men. The most distant sequences were those of *M. m. domesticus* and *M. spicilegus*. Nearly 19% of different nucleotides (after correction for multiple substitutions) suggested that these two species might diverge nearly three millions years ago (She et al, 1990).

Most of the detected mutations were substitutions. Ten different deletions/insertions have been also found. The polymorphism in position 15546, the deletion of TA dinucleotide, was detected in some *M. m. musculus*, as well as in some *M. m. domesticus* mice (cf. Fig. 1). It suggested, that the identical mutation occurred either in two different subspecies independently, or the polymorphism in this locus is older than the event of separation of these two taxons. The interspecies vertical transfer of mtDNA polymorphism can occur rather easily, because of an existence of multiple copies of mtDNA in one cell. The amount of polymorphism in 340 bp. long fragment of D-loop of mtDNA was not high enough for genealogical studies at the level of laboratory-mice strains. The fragment covered approximately 1/3 of all D-loop, of the most variable part of mtDNA. It is theoretically possible that the sequencing of longer fragments of mtDNA could reveal additional variability for phylogenetic analysis. The presence of multiple copies of mtDNA in one cell and the possibility of an existence of the polymorphism in mtDNA population, however, could lead to random fixation of the same mutation in unrelated strains of mice. If only limited set of mutations is available for the analysis, such random fixation could dramatically influence on the results of the study. The example of TA-deletion polymorphism in both *M. m. musculus* and *M. m. domesticus* suggested, that such phenomenon should be seriously considered during the analysis of mtDNA data.

The frequency of polymorphic positions in mtDNA in our sample of wild mice was high enough for phylogenetic analysis at the interspecies level. Further study is necessary, to find out the range of variability for mice collected in different parts of theirs geographical areal. The amount of data obtained in single analysis, the easiness of collecting of biological material (blood smears) as well as the possibility of application of this nondestructive method on museums specimens make this method superior in comparison with other biochemical methods used in molecular taxonomy.

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REFERENCES

BIBB M. J., VAN ETTEN R. A., WRIGHT C. T., WALBERG M. W. & CLAYTON D. A. 1981: Sequence and gene organization of mouse mitochondrial DNA. *Cell* **26**: 167-180.

BIELOSCHOWSKY M. & GOODALL C. M. 1970: Origin of inbred NZ mouse strains. *Canc. Res.* **30**: 834-836.

BISHOP C.E., BARON B., BONHOMME F. & HATAT D. 1985: Most classical Mus musculus domesticus laboratory mouse strains carry a Mus musculus musculus Y chromosome. *Nature* **315**: 70-72.

BLANK R. D., CAMPBELL G. R. & EUSTACHIO P. D. 1986: Possible derivation of the laboratory mouse genome from multiple wild mus species. *Genetics* **114**: 1257-1269.

BONHOMME F., CATALAN J., BRITTON-DAVIDIAN J., CHAPMAN V.M., MORIWAKI K., NEVO E. & THALER L. 1983: Biochemical diversity and evolution of the genus mus. *Biochem. Genet.* **22**: 275-303.

BONHOMME F. 1986: Evolutionary relationships in the genus Mus. *Curr. Top. Microbiol. Immunol.* **127**: 19-34.

FELSENSTEIN J. 1985: Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783-791.

FERRIS S. D., SAGE R. D. & WILSON A. C. 1982: Evidence from mtDNA sequences that common laboratory strains of inbred mice are descended from single female. *Nature* **295**: 163-165.

FERRIS S. D., SAGE R. D., PRAGER E. M., RITTE U. & WILSON A. C. 1983: Mitochondrial DNA evolution in mice. *Genetics* **105**: 681-721.

KLEIN J. & KLEIN D. 1987: Mouse inbred and Congenic strains. *Methods Enzymol.* **150**: 163-196.

MARSHALL J. T. 1986: Systematics of genus Mus. *Curr. Top. Microbiol. Immunol.* **127**: 12-18.

MORIWAKI K., MIYASHITA N., SUZUKI H., KURIHARA Y. & YONEKAWA H. 1986: Genetic features of major geographical isolates of Mus musculus. *Curr. Top. Microbiol. Immunol.* **127**: 55-61.

NOBUHARA H., KUIDA K., FURUTANI M., SHIROISHI T., MORIWAKI K., YANAGI Y. & TADA T. 1989: Polymorphism of T-cell receptor genes among laboratory and wild mice: diverse origins of laboratory mice. *Immunogenetics* **30**: 405-413.

POTTER M. 1986: Listing of Stock and Strains of Mice in the Genus Mus Derived from the Feral State. *Curr. Top. Microbiol. Immunol.* **127**: 373-395.

SHE J. X., BONHOMME F., BOURSOT P., THALER L. & CATZEFLIS F. 1990: Molecular phylogenies in the genus Mus: comparative analysis of protein electrophoresis, DNA hybridization, and mt DNA RFLP. *Biol. J. Linn. Soc.* **41**: 83-103.

TANAKA S., ESAKI K., KATO H., MATSUZAWA A., SUDA K. & YAMANAUCHI K. 1987: Inbred strains of mice maintained at the Institute of Medical Science University of Tokyo. *Jap. J. Exp. Med.* **57**: 241-245.

THALER L. 1986: Origin and evolution of mice: An appraisal of evidence and morphological traits. *Curr. Top. Microbiol. Immunol.* **127**: 3-11.

YONEKAWA H., MORIWAKI K., GOTOH O., WATANABE J., HAYASHI J.I., MAYASHITA N., PATRAS M. L. & TAGASHIRA Y. 1980: Relationship between laboratory mice and the subspecies Mus musculus domesticus based on restriction endonuclease cleavage patterns of mitochondrial DNA. *Jpn. J. Genet.* **55**: 289-296.

YONEKAWA H., MORIWAKI K., GOTOH O., MIYASHITA N., MATSUSHIMA Y., CHO W. S., ZHEN X.-L. & TAGASHIRA Y. 1988: Hybrid origin of Japanese mice ŕMus musculus molossinusŕ: evidence from restriction analysis of mitochondrial DNA. *Mol. Biol. Evol.* **5**: 63-78.