

THE PARENTAGE TEST OF WILD HOUSE MICE BY DNA FINGERPRINTING

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Abstract

This is the first paper about the study of parentage test of wild house mice (*Mus musculus domesticus*) by DNA fingerprinting. Using DNA fingerprinting is able to identify the relationship between wild house mice offspring and their parents. In stead of the similarity coefficients, the comparison of band sharing was recommended to be applied for the parentage test of wild house mice, because even the wild house mice were related to great extent. The mouse probe generated a novel and highly individual specific mouse DNA fingerprint, compared to human probe 33. 6.

Key words Parentage test; DNA fingerprinting; Mouse probe; Wild house mice

Crowcroft (1955) 's classic work on house mice indicated that male mice set up territories, and theorized that territorial males spent so much time defending territories that no-territorial males mated most frequently, though mating frequency was not equal to reproduction success. Levick et al. (1973) also found that there was no correlation between dominance and male reproductive success. On the contrary, Horn (1974) pointed out that the most aggressive strain of males sired 95. 6% of the offsprings. In nature, *Mus* colonies are probably begun by relatively few " founding" individuals, according to the studies of genetic polymorphisms in wild house mice (Selander, 1970). Parimigiani et al. (1982) reported that dominant males are sexually more vigorous and especially more likely to ejaculate when presented with receptive females among house mice. So, it is unknown if there was a positive relation between dominant males and their reproductive success, and the first research step should be to use a precise method

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of parentage test to quantify the relationship of parents and offsprings.

Now, the DNA fingerprinting is the most powerful tools to carry out the parentage test (Burke, 1989). There is evidence that minisatellite probes detect variable complex band patterns in a wide range of vertebrate species, including mice (Jeffreys et al., 1987; Burke, 1989; Kelly et al., 1989). Whether the fingerprinting system will provide sufficient statistical power will, however, depend very much on the precise questions and the parameters of the system (numbers of bands, degrees of band sharing and independence). House mice can clearly withstand a considerable degree of inbreeding (Berry, 1981), i. e., the relationship among mice under natural habitat was of highly related. In particular, it is yet unknown whether relationships will be easily resolved in situations where populations are relatively inbred, and if it is possible to identify the relationship among wild house mice by DNA fingerprinting. In a word, we tried to use DNA fingerprinting to test the parentage of wild house mice, and to answer the questions above to some extent.

MATERIAL AND METHODS

DNA extraction: For mammalian DNA fingerprint studies, high molecular weight DNA is usually prepared from leucocytes, alternatively homogenized tissues (e. g. liver, brain, etc.) may be used. Because many enzyme in liver would damage DNA during the long-time frozen. In this case, the frozen brain was preferred, due to less enzyme in brain. Approximately 50mg of frozen brain from each individual was grounded to a fine powder in liquid nitrogen. The brain cells were lysed in 600 μ l of isotonic buffer (1 \times SET, a buffer consisting of sodium chloride, EDTA and Tris), 15 μ l Proteinase K (10 mg/ml, stored at -20°C) and 7.5 μ l of 25% (w/v) SDS (sodium dodecyl sulphate solution, stored at 37°C), and incubated overnight at 55°C . Addition of Proteinase K was able to inactivate the nucleases released by lysis. Proteins containing the DNA were denatured and removed during a series of extractions with immiscible organic solvents. Here, the DNA was extracted with a series of phenol and phenol/chloroform washes. To the DNA solution was added 500 μ l of buffered phenol (pH 8.0), the phases were mixed for 20–30 minutes then separated by centrifugation for 10 minutes at high speed. Proteins partition was removed to the lower organic phase or precipitate at the soluble interface. The aqueous phase was recovered and additionally extracted phenol/chloroform/isoamyl alcohol (24:23:1, v/v/v) until no further precipitation occurs at the interface. The final traces of phenol were removed by a brief chloroform/isoamyl alcohol (23:1, v/v) extraction. The DNA was then recovered by the addition of 2 \times volumes of cold (-20°C) absolute ethanol, for 30 min at -20°C which caused the precipitation of DNA. DNA pet was collected by centrifugation. Traces of ethanol were removed by drying in incubating box for 2–3 hours and the DNA was dissolved in 150 μ l TE buffer (A buffer consisting of Tris and EDTA), according to the size of pet, by incubating at 55°C overnight. Next morning, using cut tip resuspended DNA by gently pipetting.

DNA restriction: For mice, the ideal restriction enzyme is *Hinf* I (Jeffreys et al., 1985). An aliquot (10 μ l) containing an excess ($>10\mu$ g) of genomic DNA was digested at 37 °C overnight in the presence of 4 mM Spermadine HCL. The extent of the digestion was monitored by a "minigel assay", a small aliquot was electrophoresed through an 0.8% agarose minigel, stained with ethidium bromide and the resulting smear was examined. All samples were quantitatively assayed fluorometrically, and were adjusted to 0.15 μ g/ μ l with 2 \times B. P. B. (Gel loading buffer containing bromophenol blue).

Electrophoresis: DNA fragments were separated by electrophoresis according to size of by molecular sieving through an agarose gel under the influence of an applied electrical field. A 1.0% (w/v) agarose gel was prepared by dissolving by microwaving the appropriate mass of agarose into 375 ml 1 \times TAE buffer (Tris acetate). The agarose solution was cooled to 55 °C and poured into a gel-mould to set. The gel was placed into an electrophoresis tank containing 2.5 litre of 1 \times TAE electrophoresis buffer. The samples and appropriate molecular weight markers were heated to 65 °C for 10 minutes to dissociate non-contiguous fragments, joined by their cohesive termini, generated by restriction, then rapidly quenched on ice. They were micropipetted into the performed sample wells of the gel. The samples were allowed to equilibrate with the electrophoresis buffer for ten minutes prior to commencing electrophoresis. Electrophoresis was necessarily long and slow (64 hours at 40 V) in order to minimize "band smiling".

Blotting: For ease of handling the DNA fragments were transferred from the gel to a solid support matrix by capillary blotting, thus maintaining their relative positions. Southern transfer were used here (In: Molecular Cloning. A Laboratory Manual, 1989, 2nd Ed. Cold Spring Harbor Laboratory Press; Fritsch—Maniatis). Large DNA fragment (>10 kb) retained within the gel were additionally fragmented in site by brief acid hydrolysis by soaking the gel in 0.2 M HCL for 10 minutes. The double stranded DNA was then separated by alkali in 1.5 M NaCl, 0.5M NaOH for 35 minutes, followed by a gel neutralization in 3 M NaCl, 0.5 M Tris pH 8.0 for 45 minutes. The DNA fragments were then transferred to the membrane by blotting. The gel was placed on a wick in contact with a reservoir of the high ionic strength buffer 20 \times SSC (Standard sodium citrate), a nylon membrane was placed onto the gel surface. DNA fragments were eluted from the gel and deposited onto the membrane surface, as the 20 \times SSC was absorbed into paper towels above the filter membrane overnight. Next morning, the membrane was washed in 2 \times SSC and fixed by baking at 80 °C in a vacuum oven.

Prehybridization, Hybridization, Preparation of a Hybridization Probe and autoradiograph: The membrane was hybridized with a radioactively P- labelled human probe RNA 33.6 (Jeffreys et al., 1985) and autoradiographed at -80 °C with two intensifying screens for up 7 days. In addition, both Elliott (1986) and Kelly et al. (1989) have found mouse probes. Under low-stringency hybridization conditions the collapsed subclone of Ms6-hm cross-hybridizes to other unstable loci in the mouse genome to generate

a novel and highly individual specific mouse DNA fingerprint (Kelly et al. , 1989). So, mouse probe was also used in the experiment and compared with human probe. Gels were examined and the position of all the bands noted. The Probe 33. 6 and Mouse Probe were provided by Professor A. Jeffreys.

Parentage testing; Bands which migrated the same distance were assumed to be the same band. Individual tracks were then scored for the presence or absence of each band. A comparison of the bands in individual tracks enabled the calculation of a similarity coefficient for the given each pairs of mice and the band sharing between offspring and putative parents (Wetton et al. , 1989; Burke, 1989; Fang, 1992).

RESULTS

1. Paternal test

Figure 1a illustrated the DNA fingerprinting bands diagram including all the individuals in the wild house mice colony L-1 which 3 offspring mice's mother was known, but their father was unknown. It was found that there were many shared bands among the mice individuals. Even if all shared bands were omitted, the most of similarity coefficients (F) calculated afterwards were much higher than 0. 5 (Table 1), the average value of F between parents and their offspring (Wetton et al. , 1989).

Table 1 The similarity coefficients between offsprings and putative parents in the mice colony L-1 which the offspring's father was unknown, their mother was F1 Hybridized by Human Probe 33. 6

Parents No.	Offspring No.			
		M104	M173	M103
Mother	F1	0. 842	0. 737	0. 571
Putative father	M1	0. 952	0. 857	0. 625
	M11	0. 556	0. 667	0. 615
	M12	0. 667	0. 778	0. 769
	M21	0. 778	0. 667	0. 615
	M31	0. 444	0. 444	0. 462
	M32	0. 558	0. 588	0. 667
	M33	0. 632	0. 632	0. 571

M₁ male; F₁ female

On the base of the analysis of band sharing among offspring, their mother and their putative fathers (Figure 1a), it was easy to find that the biological father of the offspring was male No. 1 (M1). And the F values between offspring and M1 and F1, compared to the left males, were higher to certain extent (Table 1).

The result mentioned above was also able to be confirmed with the analysis of DNA fingerprinting hybridized by mouse probe (Figure 1b, c). In the Figure 1c's DNA fingerprinting diagram, a few obvious bands made it easy to identify the father from many putative fathers according to the band positions of offspring and their mother.

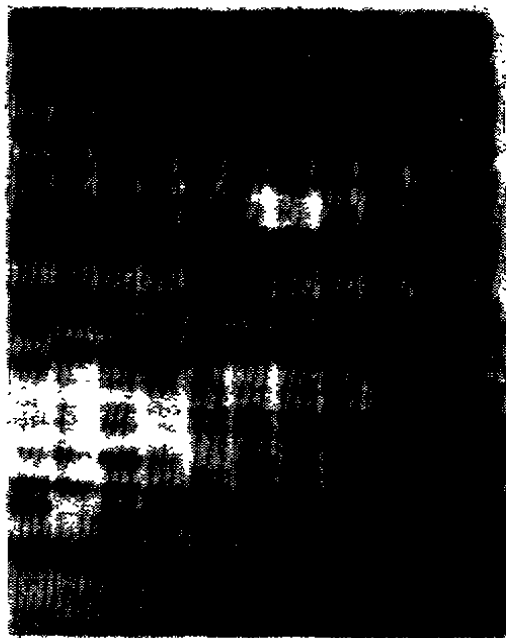


Fig. 1a DNA fingerprinting hybridized by Human Probe 33. 5 in the wild house mice colony L—1 which were composed of 3 offspring whose father was unknown, their mother was F1

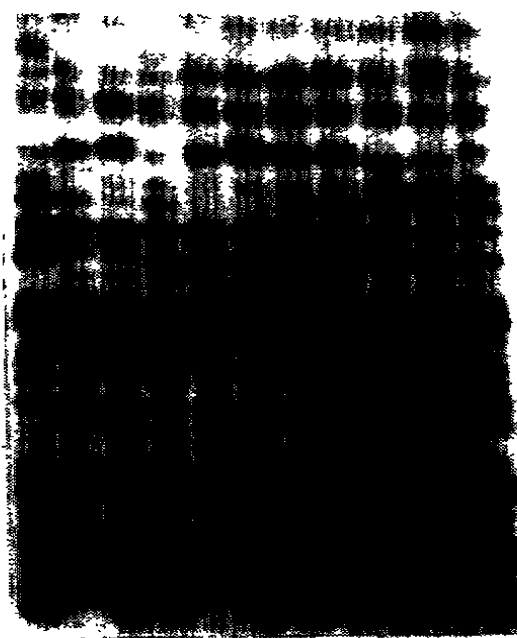


Fig. 1b DNA fingerprinting hybridized by Mouse Probe in the same colony described in Figure 1a

2. Parentage test

The putative parents of 5 offspring in the mice colony L—2 were listed in Figure 2, it was still impossible to identify the biological parent on the base of similarity coefficients (Table 2). According to the analysis of bands sharing, the biological father was male No. 1 (M1), the biological mother was female No. 1 (F1).

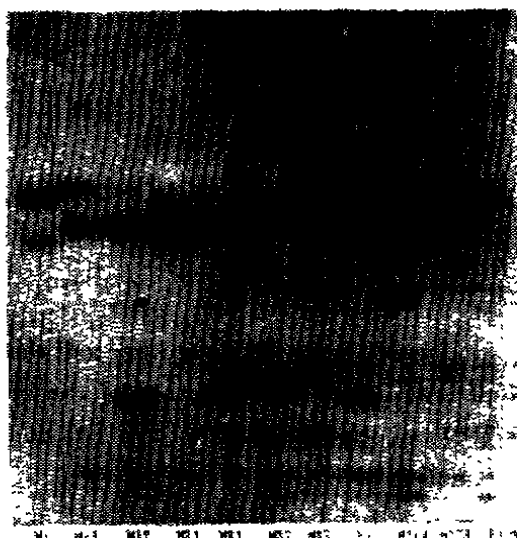


Fig. 1c DNA fingerprinting hybridized by Mouse Probe under low-stringency hybridization condition in the same colony described in Figure 1a

3. Parentage test among offspring from different litters

In some cases, several pregnant females gave birth at the same time and raised their offspring together. In the mice colony H, 12 offspring at same age were affirmed to belong to different litters, six of them were used to identify their relationship (Figure 3 and Table 3). According to band sharing analyses and similarity coefficients, the parents of offspring L07 and L08 were M31 and F2, the parents of offspring L09, L10 and L11 were M65 and F63. Offspring L12 was difficult to identify, because she had a particular band marked by a star in Figure 3 which was

not shared by other mice. She probably was a mutant.

Table 2 The similarity coefficients between offspring mice and putative parents in the mice colony L-2 which 5 offspring's parentage was unknown
Hybridized by Mouse Probe

Parents No.	Offspring No.					
		M01	F02	F03	F04	F05
Putative mother	F1	0.667	0.880	0.741	0.933	0.769
	F2	0.500	0.667	0.609	0.538	0.455
	F22	0.609	0.667	0.769	0.759	0.640
	F32	0.455	0.696	0.560	0.714	0.500
Putative father	M1	0.696	0.750	0.846	0.828	0.720
	M11	0.667	0.636	0.667	0.741	0.522
	M12	0.783	0.667	0.769	0.828	0.720
	M21	0.522	0.583	0.640	0.714	0.667
	M31	0.455	0.696	0.640	0.714	0.667
	M32	0.381	0.455	0.417	0.593	0.522
	M33	0.560	0.692	0.643	0.839	0.667

M₁ male; F₁ female

Table 3 The similarity coefficients between offspring mice and putative parents in the mice population which 6 offspring were composed of different litters and their parentage was unknown
Hybridized by Mouse Probe

Parents No.	Offspring No.						
		M07	F08	F09	F10	F11	F12
Putative mother	F1	0.734	0.778	0.526	0.526	0.571	0.727
	F2	0.800	0.842	0.600	0.700	0.533	0.700
	F63	0.632	0.556	0.526	0.526	0.429	0.545
Putative father	M31	0.783	0.727	0.700	0.609	0.556	0.846
	M61	0.800	0.737	0.700	0.700	0.533	0.696
	M62	0.800	0.842	0.600	0.700	0.533	0.609
	M63	0.818	0.762	0.727	0.636	0.471	0.720
	M65	0.636	0.571	0.727	0.818	0.471	0.720
	M66	0.556	0.588	0.444	0.333	0.308	0.571
	M67	0.632	0.556	0.737	0.737	0.286	0.727

M₁ male; F₁ female

DISCUSSION

It is very important and necessary to determine the kin relationship, while studying the effect of kinship on animal kin discrimination, mating choice, and other social behaviours (Fletcher et al., 1987; Barnard, 1990). Normally, the maternal relationship was easy to be defined in both laboratory and field studies, but paternity test was more difficult (see results above, Fang 1994).

As predicted, human probes can detect multiple variable DNA fragments in a wide range of vertebrate DNAs with varying degrees of success depending on the species examined (Jeffreys et al., 1987; Burke, 1989). In particular, DNA fingerprints can be obtained from mouse DNA using human minisatellite probes. However, the mouse

probe generated a novel and highly individual specific mouse DNA fingerprint, compared with human probe 33. 6 (Figure 1a, b, c).

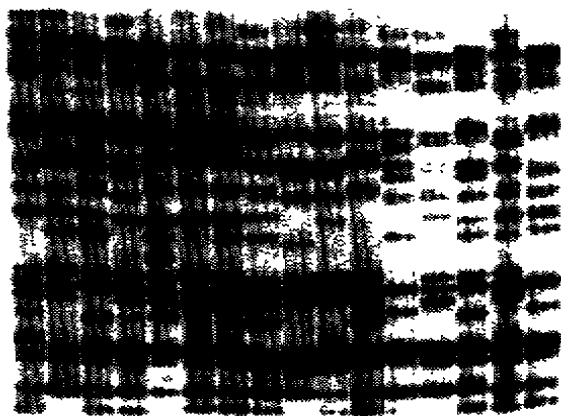


Fig. 2 DNA fingerprinting hybridized by Mouse Probe in the mice colony L-2 which were composed of 5 offspring whose parentage was unknown

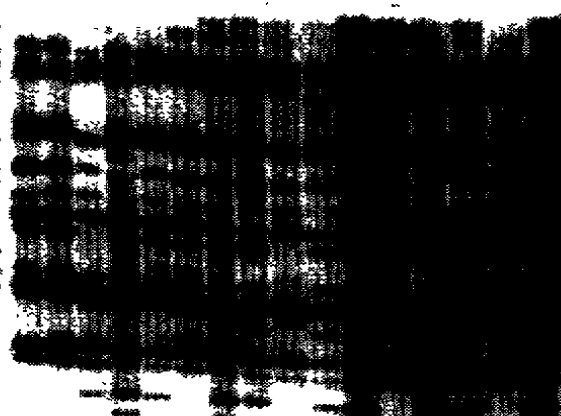


Fig. 3 DNA fingerprinting hybridized by Mouse Probe in the mice colony H which were composed of 6 mice from different litters and their parentage was unknown

DNA fingerprinting is certainly a powerful method for both paternity and maternity testing, but the similarity coefficients method had some trouble in the parentage test of the mice. The proportion of bands shared by the wild house mice was on average much higher than their coefficient of relatedness (i. e. $F=0.5$), it was very difficult to distinguish who the offsprings' biological parents were among putative parents by similarity coefficients, because even unrelated pairs of individuals will share many bands (usually 10—30%, Burke, 1989). If scoring all bands in the 3 colonies, the average bands per individual was 18. 95 (SD=2. 73, $N=43$); if omitting band shared by all individuals (i. e. the invariant bands), the average bands per individual was 10. 44 (SD=2. 42, $N=43$); and the similarity coefficients for both band scorings were significantly correlated ($r=0.89$, $P<0.001$). The results thus provided some evidence that even wild house mice might be inbred and related to great extent. Jeffreys et al. (1987) revealed that there was a high degree of variability among inbred strains in the housed mice, large numbers of minisatellite fragments (less or equal than 10) were found to be co-inherited and therefore, closely linked, however, in all of these species (human, dog, cat, mouse, house sparrow and dunnoek) except mouse, the bands were found to be predominantly independent. So, it was obvious that the comparison of band sharing perhaps was better than the similarity coefficients. In stead of the similarity coefficients, the comparison of band sharing, the simple and easy method, was recommended to be applied for the parentage test of wild house mice.

Jeffreys et al. (1987) also revealed that the resulting DNA "fingerprints" vary substantially between inbred strains but relatively little within an inbred strain, i. e., the overall patterns are specific to each inbred strain, though minor variations exist within all strains examined. Therefore, it might be impossible to test the parentage within the

inbred strain of laboratory mice. However, the wild house mouse DNA fingerprints were noticeably more complex (presumably reflecting heterozygosity at these variable loci), compared with the inbred strains mice (see Figure 1a, b, c, 2 and 3; Jeffreys et al. 1987). This perhaps could be the base of parentage test of wild house mice by using DNA fingerprinting.

Although the similarity coefficients were not an ideal method of parentage test in house mice, a rule "the more related, the higher the similarity coefficients" was still right. This could be proved by the result that the similarity coefficient of biological parents was significantly higher than that of non-biological parents (T-test, $t=4.12$, $df=127$, $P<0.001$). This might be all right for the similarity coefficient of both bands of offspring (see above), though the similarity coefficients when all bands were scored were definitely higher than the similarity coefficients when invariant bands were omitted (see also Everitt et al., 1991). Therefore, the similarity coefficient method should be for reference in the parentage test.

To sum up, identifying the relationship between offspring and their parents in the wild house mice was possible and had several choices sometimes. The better way to solve it might be to use different researching methods and to compare the results altogether.

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272-280

中文摘要

⑥

用 DNA 指纹图谱方法进行野生小家鼠的双亲判别

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本文以在英格兰捕获的野生小家鼠为研究材料, 尝试用 DNA 指纹图谱方法判别动物后代的双亲。从冷冻鼠脑组织中提取的 DNA, 经限制性酶 Hinf-I 的酶切、凝胶电泳、尼龙膜吸附、与 P^{32} 标记的人或鼠 RNA 探针杂交、放射自显影, 最后得到 DNA 指纹图谱。图谱分析表明: 野生小家鼠间的亲缘关系很近, 个体间的相似性系数较高, 不易判别后代的双亲, 建议采用简便而又准确的条带比较法, 取代相似性系数法。鼠探针与鼠 DNA 杂交所得到的图谱与人探针 33-6 的相比, 个体的特异性更强。

关键词 双亲判别; DNA 指纹图谱; 鼠探针; 野生小家鼠