

ANIMAL PARENTAGE TEST AND DNA FINGERPRINTING

FANG Jiming

(Department of Biology, Beijing Normal University, Beijing, 100875)

Abstract

The DNA fingerprinting and its application to parentage test was reviewed in this paper. Compared with other methods of parentage tests, the updated and powerful DNA fingerprinting might be the best, and be able to be used in many species.

Key words Parentage test; DNA fingerprinting

Investigations of kin-biased behaviour and mating choice require precise knowledge of the genetic relatedness of the experimental animals, as do all other phases in the analysis of kin recognition and optimal outbreeding (Fletcher et al., 1987; Barnard, 1990; Fang, 1992). An important question which has not yet been addressed concerns the extent to which relatedness influences social and sexual interactions between animals in the context of their natural population structure and life history. Therefore, it is very necessary to identify the relationship among animal individuals in cage, or enclosure, particular in field.

1. Simple review of methods to identify relationship

Several methods are employed to obtain accurate relatedness data and to mark the animals (Fletcher et al., 1987). A common procedure is to rear animals from known parents under laboratory and/or field conditions. The second method of obtaining information about the genetic relatedness of experimental animals is to observe naturally breeding populations either in captivity or in their natural habitats. The third method is to use electrophoretic and serological techniques for determining maternity and paternity. Hanken et al. (1981) employed six polymorphic loci coding for blood proteins to determine electrophoretically that 78% of the litters of Belding's ground squirrels studied by them were multipaternity. Unfortunately, there are obviously some problems in the three methods when determining relatives among wild animals living in groups (Burke, 1989).

Though some studies on wild animal populations have used morphological or chro-

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mosomal characteristics under simple genetic control (e. g. colour, chromosomal inversions), most have used biochemical polymorphism detected by starch gel electrophoresis. Genetic methods have most often been applied to tests of paternity, since true mothers are often known or assumed. However, precise data have been difficult to obtain, even in those studies using electrophoresis in which many polymorphic genetic systems were available. In electrophoretic studies, the mean probability of detecting the misassignment of a male (the exclusion probability) was typically in the range 0.4—0.7 (Gundel et al., 1981), and so the corresponding probability of non-detection (the inclusive probability $= 1 - \text{exclusion probability}$) was still usually too large to allow biological fathers to be detected with a high level of certainty. There has therefore been a need for methods that produce a much larger exclusion probability. This probability is a function of the number of polymorphic loci examined and the number and frequencies of alleles detected at those loci. The use of protein polymorphisms has proved inadequate because the number of scorable polymorphic loci has usually been insufficient to compensate for the relatively low number of alleles, and low heterozygosity at those loci. It is now apparent that the methods of DNA analysis that have been developed during recent years will often provide an alternative and successful solution to the problem of detecting more genetic variability (Burke, 1989).

Four different classes of variable DNA sequence have been used in the study of mating behaviour: random restriction fragment length polymorphisms (RFLPs); minisatellite DNAs (detected as DNA fingerprints); sex-linked sequences and mitochondrial DNA.

The random RFLP and DNA fingerprinting methods are the most generally applicable, and both have been used first in field studies of birds, because avian erythrocytes are nucleated (unlike those of mammals) and a very small drop of blood therefore provides an adequate quantity of DNA (Burke et al., 1987). The random RFLP method, applied to the detection of non-kin involves the specific detection of polymorphisms at one locus at a time. The alternative approach, DNA fingerprinting, simultaneously detects polymorphisms at multiple loci, and has been applied in particular to the analysis of paternity. Though the two methods detect distinct classes of DNA sequence, they use closely related technology. The locus-specific RFLP and multilocus DNA fingerprinting approaches each have their own advantages and disadvantages. The main advantage of fingerprinting is that the available probes can be applied to many diverse species, whereas at least 80% of random RFLP probes probably derive from non-coding unique sequences which are unlikely to be conserved among species (see details in Burke, 1989). Therefore, genetics method—DNA fingerprinting is the best method of identifying relationship perhaps.

Methods that allow the detection and verification of genetic relationships among organisms studied in the field have long been sought by workers in evolutionary biology. Since its creation in 1985, DNA fingerprinting has already begun to fulfill its promise as

a widely applicable solution to this problem (Burke, 1989).

2. DNA fingerprinting

A "minisatellite" (also known as a hypervariable region, HVR, or a variable number tandem repeat locus, VNTR) is a DNA sequence (usually less than 20 000 base pairs) comprising multiple copies of a short sequence ("tandem repeat unit") of typically less than 65 base pairs. A minisatellite's organization is therefore similar to that of "satellite" DNA, which also consists of multiple repeat sequences but on a much more extensive scale. Satellite DNAs are non-coding and so, presumably, are most minisatellites, though two are known to form parts of coding sequences.

The key advance that led to the development of DNA fingerprinting came when, in the course of an analysis of the human myoglobin gene, Professor Alec Jeffreys and colleagues at Leicester University, England, discovered a new family of mini-satellite sequences that had in common a "core" sequence of about 12 nucleotides (Jeffreys et al., 1985a, 1987b). They showed that those minisatellites that consisted of multiple repeats of this core sequence could be used as probes to detect simultaneously the hypervariable minisatellites at many separate loci. The theoretical probability of the same set of DNA fragments being detected in two humans is so small that every human except identical twins is expected to have a unique pattern, and the pattern of bands obtained on an autoradiograph is therefore described by analogy as a DNA "fingerprint". Two slightly different polycore probes, 33.6 and 33.15, were found which could each be used to obtain distinct fingerprints (Jeffreys et al., 1985b). Therefore, hypervariable tandem-repetitive minisatellite regions of human DNA can be used to generate individual-specific DNA fingerprints, validation studies have demonstrated the reliabilities, and the unparalleled degree of individual specificity (Cawood, 1989).

Each band in an individual DNA fingerprint, except the occasional mutant, can be found in either or both parents' DNA fingerprints, suggesting that DNA fingerprint bands descend from one generation to the next. Similarity coefficient (F) can be used to estimate similarity of DNA fingerprints between two individuals (Wetton et al., 1989). F between parent and offspring, or between siblings is around 0.5. F between half-siblings is about 0.25.

$$F = 2N_{ab} / (N_a + N_b)$$

where N_a and N_b are the number of bands present in individuals "a" and "b", respectively; and N_{ab} is the number of bands shared by both.

One of the first population studies to use DNA fingerprinting was concerned with the relationship of mating and parental care behaviours to paternity in the dunnoek (Burke et al., 1989). Now, the parentage tests of many species, e. g. dog, cat, mouse, house sparrow, dunnoek, and human (Jeffreys et al., 1985, 1987; Burke et al., 1989; Wetton et al., 1989; Everitt et al., 1991), have been identified by DNA fingerprinting.

3. Paternity test

Measures of a male's reproductive performance obtained by counting offspring in nests may be too low if a male's extraband copulations are successful, or too high if he is a victim of this behaviour by other males. It is necessary to measure the offsprings' paternity. Until recently, the only component of fitness of different male phenotypes and behaviours, that it has been possible to compare, is the number of observed matings achieved by each male, but this may be misleading as the number of successful fertilizations may be substantially different. In such cases, it is necessary to find a method to be able to identify the biological fathers of individual offspring.

Apart from individual identification for forensic analysis, the identification of identical twins, monitoring bone marrow transplants and studies of tumours, DNA fingerprinting has so far mainly been applied to the analysis of paternity (Burke et al., 1989; Brookfield, 1989) even, on occasion, in the absence of a sample from the father (partial paternal fingerprints can be inferred from mother-offspring comparisons, and the paternity of siblings compared, see Jeffreys et al., 1985a, b). Brookfield (1989) gave one of methods for assessing the probabilities of DNA fingerprints as a means of establishing the paternity of a disputed offspring. Paternity analysis is the simplest when the assumption can be made that the mother is correctly identified, and this can first be tested (Burke et al., 1987; Jeffreys et al., 1985b).

There is evidence that minisatellite probes detect variable complex band patterns in a wide range of vertebrate species (Burke et al., 1987; Jeffreys et al., 1987a). 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). In particular, it is yet unknown whether relationships will be easily resolved in situations where dispersal is low and populations are relatively inbred (Burke et al., 1989;).

The following paragraph illustrated the methods of paternal test by DNA fingerprint. In a paternity test, digested DNAs from the mother, offspring and putative fathers are processed side by side. The pattern of bands revealed in the offspring's DNA is compared with that in the mother's DNA. All bands that match in position and relative intensity are, or could be, maternal in origin. Thus all of the remaining bands in the DNA fingerprint of the offspring must have been inherited from the biological father. If all of these bands are present in the DNA fingerprint of the alleged father, this is evidence of paternity. In a small proportion of analyses, a band appears in the DNA fingerprinting of a offspring that does not match any band in either mother or father. In established pedigrees, this must arise through mutation. The frequency with which mutation produces unmatched bands has been determined by observation (Jeffreys et al., 1985b).

4. Parentage test

In natural condition or laboratory, usually, it is easier to identify offspring's mother according to body changing of pregnant female and care of young. Sometimes, it is very difficult to identify the mother, e. g. when several females gives birth at the same time and raise their offspring together.

Parentage test may be more difficult than paternal test (see above). When analysing DNA fingerprint profiles, mismatches between offspring and their putative parents can be ascertained by the following simple criteria: (1) comparison of band matching between each putative parent and offspring; (2) similarity coefficients between the putative parents and offspring was strikingly low; (3) the probability of parentage is small based on the analysis methods described by Brookfield (1989).

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中文摘要

动物的双亲判别与 DNA 指纹图谱

房继明

(北京师范大学生物系, 100875)

在亲缘识别和婚配选择等动物行为学研究中, 需要知道动物之间的亲缘关系。通过分析以往的各种双亲判别方法, 如蛋白电泳和血清学技术等, 其中 DNA 指纹图谱法被认为是目前最好的。这种方法已在许多种动物 (狗、猫、小家鼠、家燕、鹳雀、天鹅等) 和人的研究中得到应用。DNA 指纹图谱方法的基本原理是这样, 两个动物的 DNA 片断具有完全相同的碱基排序的情形从理论上讲其可能性极小, 所以每个个体 (除同卵双生子外) 的 DNA 经提取、酶切、凝胶电泳、RNA 或 DNA 探针杂交和放射性自显影后所形成的条带分布应该是有区别的、具有个体的特异性, 这些条带就是 DNA 指纹图谱。动物个体 DNA 指纹图谱中的每一条带, 除了偶然发生的基因突变, 都可以从父母双方、或父方、或母方找到, 据此, 本文介绍了如何使用 DNA 指纹图谱进行包括父方和母方亲代判别的方法, 如条带比较法和相似性系数法。

关键词 DNA 指纹图谱; 亲代判别

动物