J Forensic Sci, July 2003, Vol. 48, No. 4
Paper ID JFS2002435_484
Available online at: www.astm.org

CASE REPORT

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DNA Fingerprinting of Mosses

ABSTRACT: Our study introduces the use of DNA fingerprinting of clonal plants in combination with phylogenetic and vegetation studies as a prospective forensic tool in criminal investigations. In this homicide case, the bryophyte species found on the suspects were identified as *Brachythecium albicans, Calliergonella lindbergii,* and *Ceratodon purpureus.* Colonies of all three species occurred at the crime site. DNA fingerprinting analyses were conducted for *B. albicans* and *C. lindbergii,* which were expected to reproduce mainly clonally, unlike *C. purpureus,* and included samples found on the suspects and samples collected from the crime site and other locations. It was concluded that *B. albicans* found on the suspects was likely to originate from the crime scene and that the sample of *C. lindbergii* may also have originated from the same site.

KEYWORDS: forensic science, DNA typing, simple sequence repeats, random amplified polymorphic DNA, mosses, *Brachythecium albicans, Calliergonella lindbergii, Ceratodon purpureus*

The case concerned a man in his early 30s who was last time seen alive in September 2001 at a service station and at a café in southern Finland meeting his three former criminal partners. The victim and the three men were seen leaving together by car. A month later the victim's body was discovered in the woods about 5 km from the café. The three suspects were arrested, but no human blood or other key evidence was found during the police investigation. However, small pieces of plant material, which turned out to be bryophytes, were found on the shoes and clothes of the suspects and in the car they had used. We were asked by detectives to determine whether those bryophyte species occur at the crime site, and, if they did, whether DNA could be extracted and matched with bryophyte samples collected from that area.

DNA fingerprinting has been utilized in forensic science since the mid-1980s, when it was discovered that eukaryotic genomes contain a large number of highly polymorphic stretches of DNA (1). The first case in which DNA profiling based on polymorphic DNA was used to solve a crime was to identify a murderer in England in 1986 (2). Soon afterward, DNA profiling of humans, based on samples from blood, hair, bone, saliva, semen, and other body tissues and products, became widely accepted as a forensic tool (3,4). A typical criminal DNA investigation involves samples from the crime site and a suspect, DNA extraction, and the determination of the DNA profile for a set of markers with the help of polymerase chain reaction (PCR). Amplification of the desired parts of the DNA by PCR enables the use of minute amounts of DNA in the analyses. If the DNA profiles do not match exactly, the sample found on the suspects is considered not to have originated from the crime scene. The more genetic markers included in the analysis, the greater the odds for a unique pattern.

Although forensic DNA studies usually concern humans, nonhuman DNA may also be employed. There are cases when specific varieties of plants have been identified (5), and there are methods to identify the species, and even the origin, of suspected illegal plants, such as cannabis or certain mushrooms (6–10). Also, species of animals subject to poaching have been identified using DNA fingerprinting (11–13). However, the use of nonhuman DNA as evidence in a murder is very rare. The known cases include DNA extracted from cat or dog hair (14–16) and from a tree (17). In these cases, the aim has been to show that the animal hair or the plant material found on the suspect originated from an individual animal or plant located at the crime site. An attempt to connect biological material in a murder case with a group of origin (population, colony, variety, or strain), not with an individual, has dealt with the strains of HIV viruses in Louisiana (18,19). Although lawyers had challenged the admissibility of the phylogenetic analysis of the HIV strains, the technique was ruled to be valid and reliable science. Indeed, such a phylogenetic approach to forensic studies may also have considerable potential in cases in which material originating from clonal plants is found in the possession of a suspect.

We have now applied for the first time, as far as we know, a phylogenetic approach utilizing clonal plants to provide genetic evidence in a murder case. Populations of clonal plants commonly possess more than one clone, which may be due to several colonization events, somatic mutations, or from recombination due to occasional sexuality in the past. Yet, the amount of genetic variation is often lower than in populations of sexually reproducing plants (20–22), and each clone may be composed of a great number of shoots having an identical genotype. A phylogenetic analysis compares DNA samples from various sources, in this case different shoots of bryophytes representing different colonies, to see how closely they are related. If the phylogenetic approach leads to a complete genotypic match, it is possible to compute an estimate of the probability

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Received 30 Dec. 2002; and in revised form 14 Feb. 2003; accepted 14 Feb. 2003; published 1 May 2003.

of DNA from a randomly chosen member of the species matching the DNA in question. Such a probability can be obtained by multiplying the allele frequencies at multiple loci. If no complete genotypic match is available, we have to rely on genetic distances and phylogenetic trees to assess the meaning of the results.

Materials and Methods

Species Identification and Sample Collecting

Species identification was conducted using dissecting and compound microscopes. A few leaves of each sample were first soaked in ethanol, then in a dilute solution of potassium hydroxide, and finally in water prior to preparing the slides. The identification keys with illustrations (23–25) were used for species identification, and, in addition, reference material from the Helsinki Herbarium (H) collections were studied. Sample B1, collected from the car of the suspects, was identified as *Brachythecium albicans* (Hedw.) Br. (Brachytheciaceae) (23). It is easily recognized even in the field by the pale, silky, string-like branches with imbricate, plicate leaves (24). Sample C1, collected from the trousers of a suspect, was identified as *Calliergonella lindbergii* (Mitt.) Hedenäs (Amblystegiaceae) based on the combination of stem leaf features typical for the species: the stem leaves are oblong-ovate with gradually narrowed acuminate apex, with short and double nerve, and with large and distinct alar cells groups (25). Sample P1, collected from the shoes of a suspect, was identified as *Ceratodon purpureus* (Hedw.) Brid. (Ditrichaceae). A small-size, short leaf cell and a strong leaf nerve are typical for the species (24). Sample P1 was excluded from the genetic analyses because the species is known to commonly reproduce sexually.

In addition to Sample B1, the samples of *B. albicans* included three fresh samples collected from different patches at the crime site (B2–B4) and 16 fresh reference samples (B5–B20) collected from different locations in southern Finland between 7–18 November 2001. Besides Sample C1, the samples of *C. lindbergii* included seven fresh samples collected from different patches at the crime site (C2–C8), five fresh samples collected in southern Finland (C9–C13), and two herbarium samples collected in southern Finland in 1995 (C14) and in central Finland in 2001 (C15). The fresh samples of *C. lindbergii* were collected between 11–18 November 2001.

Genetic Analyses

DNA was extracted using DNeasy Plant Mini Kit (QIAGEN, Inc.). Due to time restrictions, small amounts of DNA available, and complete lack of previous genetic information of the species examined, only such DNA fingerprinting methods were usable that required no former information of the genomes. The fingerprinting method found suitable involved amplification by polymerase chain reaction (PCR) with arbitrary 10-base primers (RAPD) and with 17- or 18-base simple sequence repeat primers (SSR). The SSR primers contained a repeat sequence interrupted at the 3′-end by one or two out-of-phase bases, which act as anchors. The RAPD primers were from Operon Technologies, Inc., and the SSR primers had been assembled by the Nucleic Acid–Protein Service Unit, University of British Columbia. All amplification products were electrophoresed in 1.5% agarose gels and detected by staining with ethidium bromide. All samples of *B. albicans* were screened for two RAPD primers (OPF-01 and OPF-09) and six SSR primers (808, 811, 826, 827, 835, and 848), and the samples of *C. lindbergii* were screened for four RAPD primers (OPF-01, OPF-03, OPF-09, and OPF-10), which all produced polymorphic and reproducible banding patterns (Table 1). Reproducibility of banding patterns was confirmed by comparing duplicate or triplicate reactions for all samples.

Based on the primary data (presence or absence of the polymorphic bands), pairwise genetic distances between samples were calculated using the Dice method of the RAPDistance Package version 1.04 (26). Genetic distances were clustered using the programs KITSCH and DRAWGRAM of PHYLIP (Phylogeny Inference Package, version 3.6a2) (27).

Results and Discussion

The bryophyte species found on the suspects were identified as *Brachythecium albicans, Calliergonella lindbergii,* and *Ceratodon purpureus.* When visiting the crime scene we found colonies of all the three species near the spot where the body of the victim had been lying. The first two taxa, which were expected to reproduce mainly clonally, were selected for further studies. However, we excluded *C. purpureus,* which is known to commonly reproduce sexually. Sexual reproduction would possibly result in considerable genetic variation even within colonies, which would make such a taxon less suitable for DNA fingerprinting analyses. *B. albicans* was found to grow in three patches and *C. lindbergii* in six patches at the crime scene along a path in the woods (Fig. 1). There were no other suitable habitats for the taxa nearby. Although these bryophytes do not commonly grow together, the patch closest to the body was a mixed one.

We conducted DNA fingerprinting analyses for *B. albicans* and *C. lindbergii*. The analyses involving RAPD and SSR primers resulted in 20 polymorphic markers in *B. albicans* and 15 polymorphic markers in *C. lindbergii* (see Table 1). Despite its mainly clonal propagation, *B. albicans* was found to possess a fair amount

TABLE 1—*Oligonucleotides used as arbitrary primers and their sequences, and the sizes of polymorphic bands used as genetic markers.*

Species	Primer	Sequence (5′ to 3′)	Sizes of Polymorphic Bands (bp)
Brachythecium albicans	OPF-01	ACGGATCCTG	800, 900, 1150, 1400
	OPF-09	CCAAGCTTCC	950
	808	AGAGAGAGAGAGAGAGC	460, 520, 560, 850
	811	GAGAGAGAGAGAGAGAC	600, 670, 700
	826	ACACACACACACACACC	700, 730
	827	ACACACACACACACACG	1000, 1050, 1100
	835	AGAGAGAGAGAGAGAGYC	1100
	848	CACACACACACACACARC	1150, 1250
Calliergonella lindbergii	OPF-01	ACGGATCCTG	650, 680, 780, 800, 1500
	OPF-03	CCTGATCACC	550, 620, 950, 1200, 1250
	OPF-09	CCAAGCTTCC	400, 580, 600
	OPF-10	GGAAGCTTGG	620, 730

of genetic variation, and we did not detect a complete match for the sample of *B. albicans* found from the suspects (B1) and for the three samples (B2–B4) collected at the crime site. However, Sample B1 differed by only one marker out of 20 from Samples B2 and B3, which were collected at the crime site near the body (see Fig. 1) and expressed an indistinguishable fingerprinting profile. B1 differed by four markers from the third crime site sample of *B. albicans* (B4) located at a distance of 10 m from Sample B2, and it differed from the reference samples of *B. albicans* (B5–B20) by three to eleven markers. The results of the cluster analysis are shown in Fig. 2*a*. Since snowfall prevented the collection of more samples and, at the same time, the criminal investigation had a tight schedule, we did not have a chance to conduct further collecting and analysis of *B. albicans* inhabiting the crime site, which could have potentially led to the discovery of a complete genetic match. Yet, it is likely that the Sample B1 found from the car of the suspects originates from the crime site.

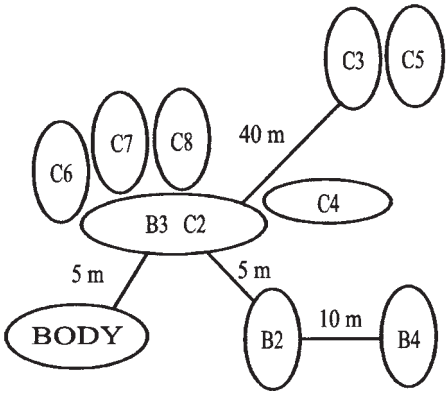

FIG. 1—*The locations of the patches and samples of Brachythecium albicans (samples B2–B4) and Calliergonella lindbergii (C2–C8) at the crime site.*

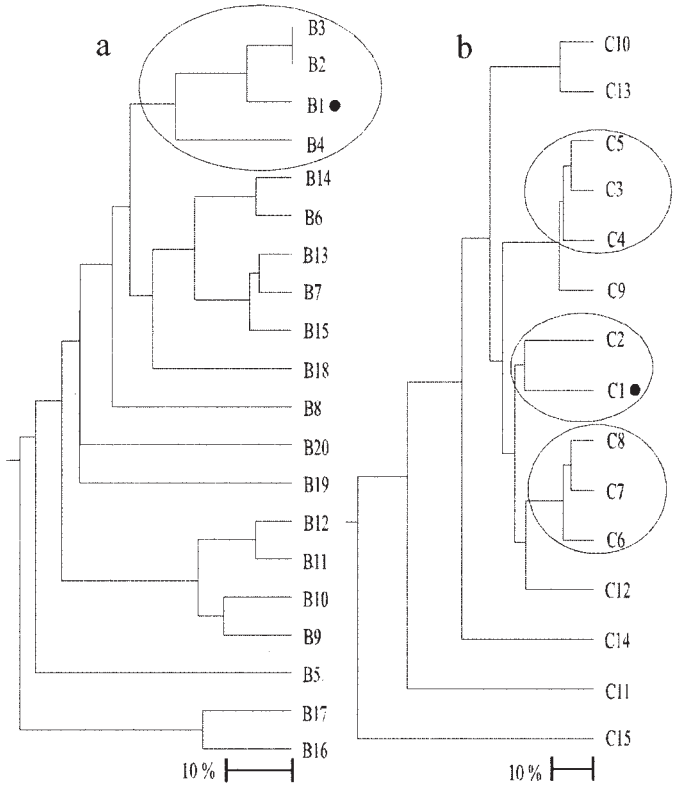

FIG. 2—*Cluster analysis of genetic distances*: (a) *Brachythecium albicans*; (b) *Calliergonella lindbergii. Sample B1 was found from the car used by the suspects and Sample C1 from the trousers of a suspect. Samples B1 and C1 and the samples collected from the crime site are circled.*

The samples of *C. lindbergii* were analyzed using only four primers since it was soon discovered that the species contained a considerable amount of genetic variation even among the seven samples collected from the crime site, which was apparently due to the presence of sexual reproduction in addition to vegetative propagation. The pairs of samples collected at the crime site differed by one to six out of 15 markers. The sample found on one suspect (C1) differed from the crime site samples (C2–C8) by three to seven markers, and it differed from the other samples (C9–C15) by four to nine markers. In the whole dataset, the closest match for Sample C1 was with Sample C2 collected at the crime site. The results of the cluster analysis are shown in Fig. 2*b*. The genetic analysis indicates that Sample C1 may originate from the crime site and most likely from the patch closest to the body (see Fig. 1). However, this evidence is very weak due to the pattern of genetic variation found in *C. lindbergii*.

In the end, through our forensic study involving traces of bryophytes, we were able to come to the conclusion that the three species of bryophytes found on the suspects are found at the crime scene, and, based on the genetic analyses, the sample of *B. albicans* is likely to have originated from the crime site and the sample of *C. lindbergii* may have originated from the crime site. Furthermore, our study on bryophytes demonstrates the application of DNA fingerprinting of clonal plants in combination with phylogenetic and vegetation studies as a potential and noteworthy tool for criminal investigations. Clonal plants, e.g., many bryophytes and grasses, are common, and parts of them can easily become attached to shoes, clothing, and car tires. Such plants should not be ignored as evidence, since even a tiny, insignificant-looking piece of plant material may turn out to be important proof for the prosecution.

Acknowledgments

We thank K. Syrjänen and T. Ulvinen for providing specimens for our study, T. Koponen for kindly helping with the identification of the species, A. Kattan for technical assistance, and K. Tankersley for help in preparing the manuscript.

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