Forensic botany: Usability of bryophyte material in forensic studies

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Abstract

Two experiments were performed to test the relevance of bryophyte (Plantae, Bryophyta) material for forensic studies. The first experiment was conducted to reveal if, and how well, plant fragments attach to footwear in general. In the test, 16 persons walked outdoors wearing rubber boots or hiking boots. After 24 h of use outdoors the boots were carefully cleaned, and all plant fragments were collected. Afterwards, all plant material was examined to identify the species. In the second experiment, fresh material of nine bryophyte species was kept in a shed in adverse conditions for 18 months, after which DNA was extracted and subjected to genotyping to test the quality of the material.

Both experiments give support for the usability of bryophyte material in forensic studies. The bryophyte fragments become attached to shoes, where they remain even after the wearer walks on a dry road for several hours. Bryophyte DNA stays intact, allowing DNA profiling after lengthy periods following detachment from the original plant source. Based on these experiments, and considering the fact that many bryophytes are clonal plants, we propose that bryophytes are among the most usable plants to provide botanical evidence for forensic investigations.

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1. Introduction

Forensic science has utilized different DNA fingerprinting methods since the mid-1980s, when it was discovered that eukaryotic genomes contain a large number of highly polymorphic sites [1]. Soon after DNA profiling had first been used to identify a murderer in England in 1986 [2], DNA profiling of humans based on blood, hair, bone, saliva, semen and other body tissues and products became a widely accepted and utilized forensic tool [3,4].

Although forensic DNA studies usually concern humans, non-human DNA may also be employed. Animal DNA has been examined to identify species subject to poaching, e.g., [5–7]. There are also homicide cases, in which DNA extracted from cat or dog hair has been used as evidence [8,9]. However, molecular forensic botany is still an under-utilized field of investigations [10]. Most common plant applications involve the identification of specific plant varieties [11–13], and the identification of suspected illegal plants, such as cannabis and certain mushrooms [14–16]. Korpelainen and Virtanen [17] have reported a homicide investigation in which the fingerprinting of bryophytes was used to provide evidence in a murder case. Bryophyte material found on the suspect’s car and clothes were successfully linked with bryophyte patches located at the crime scene.

Recently, efforts to develop better and more reliable methods to accurately utilize botanical evidence have been initiated, mainly the development of microsatellite marker sets for a number of common bryophyte species to allow precise population genetic analyses and clone identification (Korpelainen et al., in preparation) and a DNA sequence-based identification system for grass species to determine the taxon of unknown grass samples [18]. Bryophytes are convenient for forensic investigations as many of them are clonal and common, their fragments can easily become attached to, e.g., shoes and clothes, and their DNA can be analyzed quite long after the plant has been fragmented. Correspondingly, grasses are plants frequently encountered by humans and many of them are clonal.

Clonal plant colonies usually consist of only one genotype, which can be identified with DNA markers and used as a piece of evidence in court when trying to link a suspect to a crime scene. In the homicide case reported by Korpelainen and Virtanen [17], small shoot particles of one of the studied
bryophyte species found in the suspect’s car, apparently transferred via shoes or clothes, clearly originated from the crime site in a forest, and another piece of bryophyte material found on the suspect’s clothing was likely to have originated from the same site.

Botanical evidence can be a useful tool for criminal investigations [17,10]. However, we lack the data on how well plants, and different groups among them, attach to clothes or footwear. Besides, it is not adequately known how long DNA stays intact in fragmented plant material when detached from the original source. In the present study, we aimed to test two aspects related to the use of bryophyte material as a forensic tool: (1) How likely it is to recover plant material, specifically bryophytes, from shoes after a person has been walking in a forest, and (2) How well does bryophyte material, when exposed to adverse environment, maintain its DNA intact enough to allow DNA profiling.

2. Materials and methods

2.1. Description of bryophytes

Bryophytes are small plants that are typically 1–10 cm tall, but the species sizes range from being microscopic to over a meter. They commonly grow close together forming mats or patches on, e.g., ground, rock and wood. In the nature, bryophytes occur in all environments except in the sea. They are rootless plants having stems with leaves, the stems ranging from single- to multi-branched. The structure of stems is quite fragile, and the shoots or fragments of the plants can quite easily break off. Occasionally, the fragments form new plants, and the fragmentation is actually a means of vegetative reproduction. Because of the morphology and common occurrence of bryophytes in the nature in many areas, and the fact that many species grow on ground, the bryophyte shoots were expected to become attached to shoes and to be found on footwear in this experiment.

2.2. Experiment 1: do bryophyte shoots get caught on shoes?

Footwear from 16 persons participating in a field exercise were cleaned with a heavy brush. The shoes were either hiking boots (14 pairs) or rubber boots (2 pairs). After 24 h of trekking in a forest and semi-urban environment (the last 4 h spent on gravel or paved roads), shoes were examined and sampled for all attached plant material. Afterwards, samples were studied under a light microscope and the taxa were identified (Table 1) when possible.

All collected bryophyte material, including both fresh and dry specimens, were examined. Dried plants recovered their form on moistening. A study of leaf shape and cells was necessary for identification. For that, leaves were dissected from the stem and a slide for microscoping examination was prepared. The bryophytes were identified using a light microscope, e.g., Leitz 513 594 with magnification from 10 (for studying leaves) to 40 (for studying cells) times. The identification of species was based on keys used in bryophyte floras (e.g., [19]).

2.3. Experiment 2: does bryophyte DNA stay intact in shoots when stored in a highly fluctuating environment?

The persistence of DNA from nine bryophyte species (Table 2, one sample from each species) was studied by leaving fresh, moist plant material in paper bags in an unheated shed in southern Finland for 18 months, beginning September 2003. During the period, the temperature varied between +25 °C and –28 °C. Afterwards, DNA was extracted using Qiagen DNeasy® Plant Mini Kit. Microsatellite PCR reactions were performed for one or three microsatellite loci per sample to test the quality of the DNA. Regular PCR amplification and successful genotyping were considered to be a sign of satisfactory DNA quality. Genotyping was performed using fluorescently labelled primer pairs developed for the bryophyte species in question (Korpelainen et al., unpublished). Amplifications were performed in 5 μl reactions, containing 0.5 μl DNA (about 5 ng, quantified visually from a gel), 2.75 μl ddH2O, 0.5 μl 10× reaction buffer including 1.5 mM MgCl2, 0.1 μl dNTP mix (10 mM), 0.15 μl DyNAzyme® II DNA Polymerase (2 U/μl) and 0.5 μl of both primers (5 pmol/μl). For genotyping, PCR–products were first diluted with ddH2O. After that, 2 μl of each sample was pipetted into a plate with 96 wells, and 8 μl of standard (MegaBACE ET400-R) diluted with ddH2O (1:31) was added. The plates were then run with the MegaBACE™ 1000 DNA Analysis System.

3. Results

It was discovered that many of the shoes studied contained some amount of plant material despite the fact that the persons participating in the study spent the last 4 h trekking on gravel or bitum-surfaced roads. Bryophytes were found on 9 out of 32 shoes (28%), altogether 22 shoots representing 5 species (Table 1). Additionally, a few pine or spruce needles, and small pieces of grass and unidentifiable vascular plant material were found on eleven shoes (34%). Most of the plant particles were attached to the seams or laces or other upper parts of the shoes.

Bryophyte DNA extracted from samples stored for 18 months in a fluctuating environment proved to work well in the Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Microsatellite loci</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aulacomnium palustre, Bog Groove-moss</td>
<td>AUPA-1</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>AUPA-5</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>AUPA-7</td>
<td>84</td>
</tr>
<tr>
<td>Brachythecium reflexum, Reflexed Feather-moss</td>
<td>BRRE-5</td>
<td>124</td>
</tr>
<tr>
<td>Plagiochila asplenoides, Greater Feather-wort</td>
<td>PLAS-4</td>
<td>64</td>
</tr>
<tr>
<td>Pleurozium schreberi, Red-stemmed Feather-moss</td>
<td>PLSC-134</td>
<td>81</td>
</tr>
<tr>
<td>Polytrichum juniperinum, Juniper Haircap</td>
<td>POIU-1</td>
<td>98</td>
</tr>
<tr>
<td>Rhytidioalplus squarrosum, Springy Turf-moss</td>
<td>RHSQ-4</td>
<td>77</td>
</tr>
<tr>
<td>Rhytidioalplus triquetrus, Big Shaggy-moss</td>
<td>RHTR-1</td>
<td>77</td>
</tr>
<tr>
<td>Rhizomnium punctatum, Dotted Thyme-moss</td>
<td>RHPU-1</td>
<td>124</td>
</tr>
<tr>
<td>Sphagnum girgensohni, Girgensohn’s Bog-moss</td>
<td>SPGI-1</td>
<td>104</td>
</tr>
</tbody>
</table>

Alleles detected (the sizes of the amplification product as bp) are shown.
microsatellite analysis conducted to test the quality of DNA and the success of genotyping. All samples listed in Table 2 gave an easily scorable result even though some of the samples were partly moulded at the time of DNA extraction.

4. Conclusion

The present study demonstrates that plant material can be found from the footwear of people who walk outdoors. Especially when strolling in forest or semi-urban areas, bryophyte shoots are frequent among the attached plant material. The second experiment testing the quality and usability of bryophyte DNA after exposure to adverse and variable conditions included nine species, which were all found to maintain their DNA intact enough to allow successful genetic fingerprinting. This demonstrates that plant material can be valuable even when it cannot be processed fresh.

The 9 bryophyte species tested to discover how well their DNA stays preserved for an extended time in varying conditions belong to a group of 21 globally common bryophyte species (Appendix 1), for which we have developed sets of microsatellite markers to allow genetic profiling (Korpelainen et al., unpublished). In addition, four of the five bryophyte species found attached to the footwear in the field experiment are included in the above-mentioned list of taxa.

We propose that in the future, genetic profiling of clonal plants in combination with phylogenetic and vegetation studies could prove to be a useful tool for forensic investigations, specifically in cases when no human DNA is available. Pieces of plants can become attached to shoes, clothing and car tires. Furthermore, since at least bryophyte material stays intact for a long time, it can be used in forensic applications even after extended periods of time. Therefore, plant fragments found on suspects or victims should not be ignored as evidence. Such plant material may be a link to connect a suspect with a crime scene [17], or reveal whether a death is due to suicide, accident or homicide [10]. Even a small, insignificant-looking piece of plant material may turn out to reveal important information for the court.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.forsciint.2006.11.012.

References